A multielement trace mineral injection improves liver copper and selenium concentrations and manganese superoxide dismutase activity in beef steers[1]

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ABSTRACT: Trace minerals (TM) are vital to health and growth of livestock, but low dietary concentrations and dietary antagonists may reduce mineral status and feeder cattle TM status is usually unknown at arrival. The objective of this study was to examine the effect of TM status on response to mineral injection in beef cattle. Forty steers were equally assigned to diets for an 84-d depletion period: control (CON; supplemental Cu, Mn, Se, and Zn) or deficient (DEF; no supplemental Cu, Mn, Se, or Zn plus Fe and Mo as TM antagonists). Lesser liver Cu and Se concentrations (79.0 ± 11.60 and 1.66 ± 0.080 mg/kg DM, respectively) in DEF steers compared with CON steers (228.8 ± 11.60 and 2.41 ± 0.080 mg/kg DM, respectively) on d 71 of depletion indicated mild deficiencies of these TM ($P < 0.001$). On d 1 of the 85-d repletion period, 10 steers within each dietary treatment were injected with sterilized saline (SAL) or Multimin90 (MM), containing 15, 10, 5, and 60 mg/mL of Cu, Mn, Se, and Zn, respectively, at a dose of 1 mL/68 kg BW. All steers were fed the same repletion diet supplemented with Cu, Mn, Se, and Zn to meet or exceed NRC recommendations. Blood was collected on d 0 and 1, and blood and liver biopsies were collected on d 8, 15, 29, 57, and 85 postinjection. Red blood cell lysate manganese-superoxide dismutase activity was greater in MM ($P = 0.02$), suggesting incorporation of injectable TM into a biological process. The increase in liver Se in response to MM was greater in CON vs. DEF ($P = 0.02$), suggesting TM from injection were used rather than stored in DEF steers. Liver Se and Cu ($P < 0.05$) were elevated through at least d 30 by MM. Dietary TM deficiency decreased neutrophil bacteria killing ability and increased myeloperoxidase (MPO) degranulation ($P < 0.04$) as measured on d 0, 1, 13, and 14 during the repletion period while injection had no impact. Within CON animals, total MPO was greater in animals that received TM injection, but injection did not affect MPO within DEF steers ($P = 0.007$). Overall, TM from an injectable mineral were used differently between TM adequate and mildly deficient steers.

Key words: feedlot cattle, injectable mineral, shipping, trace minerals, mineral status

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INTRODUCTION

Trace minerals (**TM**) are vital for the health and growth of livestock, supporting multiple biochemical processes in the body (Underwood and Suttle, 1999). Trace minerals are commonly supplemented in the diet of domestic livestock; however, variability in soil and plants may lead to low ingredient TM concentrations and bioavailability (Smart et al., 1981). Additionally, dietary antagonists and formation of insoluble complexes (Spears, 1996) may decrease absorption and contribute to poor TM status in ruminants. Cattle with poor TM status have decreased growth performance (Spears and Kegley, 2002) and decreased efficiency of growth (Malcolm-Callis et al., 2000; Salyer et al., 2004) while Cu and Se deficiency specifically may impair neutrophil function and antioxidant enzyme activity (Boyne and Arthur, 1981; Ward and Spears, 1997). Rarely is the TM status of cattle arriving at a feedlot known, so preventative measures to manage risk associated with poor TM status may be beneficial. Supplemental TM delivered through injection bypass the gastrointestinal tract and dietary antagonists and have been shown to improve liver Cu and Se concentrations through at least 15 d postinjection (Pogge et al., 2012) and health and performance in beef cattle (Richeson

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and Kegley, 2011). However, the impact of TM injection on the overall TM status and biomarkers of TM in deficient animals compared with adequate animals has not been evaluated. The objective of this study was to examine the effect of TM status (adequate or mildly deficient) on the response to TM injection of beef feedlot steers.

MATERIALS AND METHODS

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (log number 3-11-7099-B).

Depletion Period

This experiment was conducted at the Iowa State University Beef Nutrition Research Center in Ames, IA. Forty yearling Angus crossbred steers were purchased from a sale barn in Iowa and were stratified by initial body weight (309 ± 14 kg) and assigned randomly to 1 of 2 treatments (n = 20 per treatment). Treatments included control (**CON**), a corn silage-based diet supplemented with 10 mg Cu/kg diet DM, 20 mg Mn/kg DM, 0.1 mg Se/kg DM, and 30 mg Zn/kg DM, or deficient (**DEF**), a corn silage-based diet not supplemented with Cu, Mn, Se, or Zn but supplemented with 300 mg Fe/kg DM and 5 mg Mo/kg DM as dietary TM antagonists for 89 d. Dietary composition can be found in Table 1. Steers were housed in pens of 6 or 7 head by dietary treatment and had ad libitum access to water and feed and were fed once daily at approximately 0800 h. Steer feed intake was recorded daily on an individual basis using the Iowa State University Beef Nutrition Farm's feed intake management system. Steers were fitted with electronic identification tags, which allowed for feed disappearance by a single steer to be monitored. Liver biopsy samples were taken on d 71 of the depletion period to establish TM status before shipping and start of the repletion period using the method reported by Engle and Spears (2000). Fifty-milliliter jugular blood samples were collected on d 69 and 70 from all steers before feeding, each into syringes containing acid citrate dextrose as an anticoagulant for evaluation of neutrophil function before shipping. Steers were weighed on d 83 and 84 at the end of the depletion period and were maintained on the same dietary treatments until d 91.

Shipping Period

Steers were loaded onto a tractor trailer on d 88 (1030 h) and shipped for 20 h to simulate shipping, a common stressor in the beef cattle industry, and to evaluate the effect of dietary TM deficiency on shipping response. Each steer had 1.10 m^2 of space in the trailer. Steers were received back at the Beef Nutrition Research Center on d 89 (0630 h) into the same pens and were allowed to rest for 24 h with ad libitum access to feed and water.

Table 1. Composition of the control (CON) and trace mineral deficient (DEF) diets during depletion period and diet composition during the repletion period

	Depletion		
	CON	DEF	Repletion[1]
Ingredient	% of diet DM		
Corn silage	50	50	15
Corn dried distillers grains with solubles	20.64	20.57	23.02
Dry rolled corn	18	18	50
Soyhull pellets	10	10	10
Limestone	0.9	0.9	1.51
Salt	0.31	0.31	0.31
Vitamin A premix[2]	0.11	0.11	0.11
Rumensin90[3]	0.01	0.01	0.01
Trace mineral premix[4]	0.001	0.001	0.001
CON premix[5]	0.035	–	0.035
DEF premix[6]	–	0.101	–
Analyzed[7]	mg/kg of diet DM		
Cu	13.8	4.1	12.7
Mn	43.9	25.7	32.1
Zn	56.1	33.9	62.3

[1]All cattle received the same diet during the repletion phase.

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

[3]Provided 200 300 mg·steer^{-1}·d^{-1} of the ionophore monensin; donated by Elanco (Greenfield, IN).

[4]Provided per kilogram of diet DM: 0.5 mg I (calcium iodate) and 0.1 mg Co (cobalt carbonate).

[5]Provided per kilogram of diet DM: 10 mg Cu (copper sulfate), 20 mg Mn (manganese sulfate), 0.1 mg Se (sodium selenite), and 30 mg Zn (zinc sulfate).

[6]Provided per kilogram of diet: 300 mg Fe (iron sulfate) and 5 mg Mo (sodium molybdate).

[7]Analyzed mineral values reflect diet total, which includes supplemental mineral.

Repletion Period

Steers were weighed on d 90 and 91, and on d 91 steers were blocked by liver mineral status within depletion period diet and randomly assigned to receive an injection of sterilized saline (**SAL**) or Multimin90 (**MM**; Multimin USA, Fort Collins, CO) at a dose of 1 mL/68 kg BW, resulting in 10 steers per treatment group. The MM injection 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). On Days 90 and 91, 50 mL jugular blood samples from all steers were collected into syringes containing acid citrate dextrose as an anticoagulant for evaluation of neutrophil function after shipping and again on d 13 and 14 for evaluation of the effect of injec-

tion on neutrophil function. Jugular blood samples were collected into vacuum tubes designed for TM analysis (potassium EDTA; Becton Dickenson, Rutherford, NJ) immediately before injection (time 0) and on d 1 (24 h postinjection), and both blood and liver biopsy samples were taken on d 8, 15, 29, 57, and 85 postinjection. Steers were fed a common finishing diet supplemented with 10 mg Cu, 20 mg Mn, 0.1 mg Se, and 30 mg Zn/kg dietary DM for the 85 d repletion period (Table 1).

Tissue Analysis

Blood and liver samples were transported on ice back to the laboratory and blood samples were centrifuged at 1,200 × *g* for 10 min at 4°C. Plasma was removed and stored at –80°C until analysis. Liver samples were dried in a forced air oven at 70°C and digested using trace metal grade nitric acid before mineral analysis (CEMS MARSXpress, Matthews, NC) as described by Richter et al. (2012). Liver and plasma analysis for Cu, Mn, Se, and Zn was determined using inductively coupled plasma mass spectroscopy (PerkinElmer, Waltham, MA) as previously described (Pogge et al., 2012). Red blood cell lysate (**RBCL**) was prepared from packed red blood cells using the method from Pogge et al. (2012) and stored at –80°C until analysis. Red blood cell lysate glutathione peroxidase (**GPx**) activity and RBCL manganese-superoxide dismutase (**Mn-SOD**) activity were analyzed using commercial kits (Cayman Chemical, Ann Arbor, MI; catalog numbers 706002 and 709001, respectively). One unit of GPx activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to oxidized nicotinaminde adenine dinucleotide phosphate (NADP+) per minute at 25°C. One unit of Mn-SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Hemoglobin (**Hb**) concentration of the RBCL was determined as described by Hansen et al. (2010) and Mn-SOD and GPx activities were expressed per unit of Hb.

Neutrophil Isolation and Function

Isolation of polymorphonuclear leukocytes (**PMN**) and preparations of PMN suspensions for use in the assays was completed using the method of Roth and Kaeberle (1981) and all cell preparations were standardized to 5.0×10^7 cells/mL. All PMN function assays were conducted in 96-well flat bottom plates. Extracellularly released myeloperoxidase (**MPO**) following stimulation was expressed as a percentage of total MPO content inside unstimulated PMN using the method of Palić et al. (2005). Total MPO content was determined by lysing PMN in 1 cell preparation with cetyltrimethylammonium bromide solution (0.02% in water) and measuring optical density (**OD**). The percentage of MPO released from PMN was determined for each calf using the following formula: % exocytosis = [OD of stimulated (or Hanks balanced salt solution treated) PMN]/(OD of lysed PMN) × 100. Cytochrome C reduction (Roth et al., 2001) was used to detect extracellular superoxide anion. Neutrophil bacteria killing was evaluated using one of the most common bovine respiratory disease syndrome causing bacteria, *Pasteurella multocida*, isolated at Iowa State University. Viable bacterial numbers were determined by the degree of converting 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) to water-soluble formazan as previously described with modifications (Stevens and Olsen, 1993; Tunney et al., 2004). Optical density was determined using a plate reader at 2 wavelengths (OD = V1 – V2; V1 = 450 and V2 = 650 nm). The OD generated by live bacteria was determined by subtracting mean OD of background wells from mean OD of test wells of each sample. The number of viable bacteria remaining was calculated based on a standard curve obtained from the OD of standard bacterial wells.

Statistical Analysis

Initial liver TM concentration from the depletion period was analyzed using the MIXED procedure of SAS 9.2 (SAS Inst. Inc, Cary, NC) as a randomized complete block design including the fixed effect of treatment (CON and DEF) and the random effect of steer. As steers were individually fed in this trial, steer was the experimental unit ($n = 20$ per diet). Blood and liver TM concentrations and blood biomarkers from the repletion period were analyzed as repeated measures using the MIXED procedure of SAS as a 2 × 2 factorial including the fixed effects of previous diet (CON or DEF) and injection (SAL or MM) and the random effect of steer. Steer was the experimental unit ($n = 10$ per diet × injection treatment). Pre-injection mineral concentrations and biomarker values were used as covariates in analyses using the AR(1) covariance structure, selected based on lowest Akaike information criterion. Neutrophil function data from pre-injection sampling time points were analyzed as repeated measures, preshipping and postshipping. Data were checked for normalcy and homogeneity of variance and transformed when necessary, but only back-transformed data are reported. Outliers were determined using Cook's D statistic and removed. Data reported are least-squared means ± SEM. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10.

Table 2. Effect of trace mineral supplementation (control [CON]) or deprivation (deficient [DEF]) during the depletion period and shipping on neutrophil function[1]

	Preshipping[2]		Postshipping[3]			*P*-value[4]		
Variable	CON $n = 20$	DEF $n = 20$	CON $n = 20$	DEF $n = 20$	SEM	D	S	D × S
Cytochrome C reduction, U[5]	1.95	3.28	3.55	3.65	0.092	<0.001	<0.001	<0.001
MPO degranulation, %	51.57	52.55	42.00	46.42	1.49	0.10	<0.001	0.22
Total MPO, OD	1.81	1.62	1.96	1.92	0.045	0.02	<0.001	0.04
Pasteurella multocida killing, %	53.20	51.00	41.29	42.66	1.877	0.86	<0.001	0.16

[1]Data are means and pooled SEM; MPO = myeloperoxidase; OD = optical density.

[2]Preshipping data are from samples taken on d 69 and 70 of the experiment (19 and 18 d before shipping, respectively).

[3]Postshipping data are from samples taken on d 90 and 91 of the experiment (1 and 2 d postshipping, respectively).

[4]Main effects in statistical analysis were diet (D), shipping (S), and their interaction (D × S).

[5]One unit of cytochrome C reduction is defined as n*M* of extracellular superoxide anion released per 10^6 cells.

RESULTS

Depletion Period Liver Trace Mineral Concentrations

Raw liver Cu and Se concentrations in DEF steers (79.0 ± 11.60 and 1.66 ± 0.080 mg/kg DM, respectively) were lesser than CON steers (228.8 ± 11.60 and 2.41 ± 0.080 mg/kg DM, respectively) after 71 d on the depletion diets ($P < 0.001$). Raw liver Cu concentrations among DEF cattle ranged from 24 to 335 mg/kg, suggesting that Cu status varied widely among these steers in response to a Mo-supplemented diet. Liver Mn tended to be greater ($P = 0.08$) in CON steers (9.37 ± 0.261 mg/kg DM) than DEF steers (8.71 ± 0.261 mg/kg DM). There were no differences initially between dietary treatments in liver Zn ($P > 0.10$).

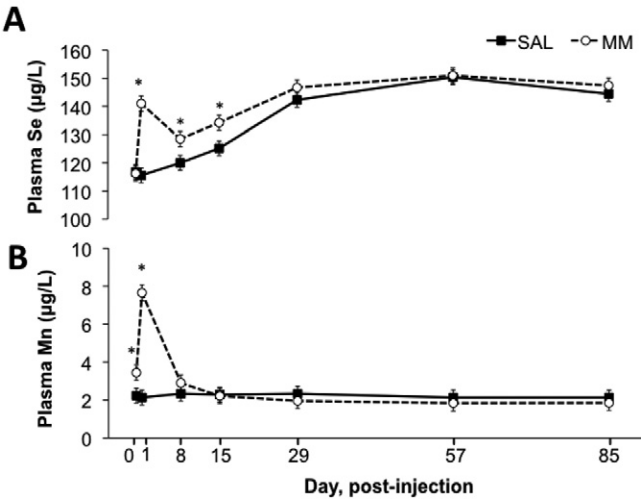

Figure 1. Effect of saline (SAL) or trace mineral injection (Multimin90 [MM]) on plasma Se (A) and Mn concentrations (B); values are means ± SEM, $n = 20$ per injection treatment; injection ($P < 0.01$) and injection × time interaction ($P < 0.01$). Asterisks (*) denote differences ($P < 0.05$) between treatments within day of experiment.

Depletion and Shipping Period Neutrophil Function

Cytochrome C reduction was greater in DEF animals than CON animals before shipping ($P < 0.001$) but was not different after shipping ($P > 0.20$; Table 2). Total MPO followed the opposite trend where CON animals were greater ($P = 0.02$) before shipping but not different from the DEF animals after shipping ($P = 0.36$). Myeloperoxidase degranulation tended to be greater in DEF ($P = 0.10$), but bacteria killing ability was not different when compared with CON animals ($P > 0.20$).

Repletion Period Plasma and Red Blood Cell Lysate Trace Mineral Concentrations, Enzyme Activities, and Neutrophil Function

There was an initial increase in plasma Mn concentrations in response to the injection on d 1 ($P < 0.01$; Fig. 1), and concentrations returned to baseline by d 8. Plasma Se concentrations were elevated in MM steers on d 1 and remained elevated through d 15 postinjection ($P < 0.01$). Both plasma Cu and Zn concentrations were variable throughout the entire experimental period, with no consistent trends (Table 3), but there were diet × day and injection × day interactions ($P < 0.05$) for plasma Zn, driven by differences on d 1, where DEF steers had greater plasma Zn concentrations than CON and SAL steers had greater plasma Zn than MM steers, mainly driven by the low Zn concentration in DEF + MM steers (Fig. 2) Plasma Zn individual pairwise comparisons between treatments on all other days were nonsignificant ($P > 0.20$). There was no effect of diet or injection on RBCL GPx ($P > 0.20$). Manganese-SOD activity, measured in RBCL over the repletion period, was greater in steers treated with MM compared with steers receiving saline ($P = 0.02$; Fig. 3).

Thirteen days postinjection, cytochrome C reduction and bacteria killing were higher in neutrophils isolated from CON steers ($P < 0.04$; Table 3) compared with DEF steers while neutrophil function was not

Table 3. Repletion period plasma trace mineral concentrations, red blood cell lysate enzyme activities, and neutrophil function as affected by previous trace mineral supplementation (control [CON]) or deficiency (DEF) during the depletion period and saline (SAL) or trace mineral injection (Multimin90 [MM])[1]

	CON		DEF			*P*-values[2]		
Variable	SAL *n* = 10	MM *n* = 10	SAL *n* = 10	MM *n* = 10	SEM	D	I	D × I
Plasma[3,4]								
Cu,[5,6] mg/L	1.10	1.07	1.10	1.13	0.020	0.12	0.99	0.21
Mn,[7] µg/L	2.30	3.07	2.17	3.17	0.243	0.94	<0.001	0.64
Se,[7] µg/L	129.9	137.4	131.5	138.4	1.74	0.47	<0.001	0.88
Zn,[8] mg/L	0.96	0.93	0.97	0.97	0.026	0.36	0.54	0.59
Red blood cell lysate[3,4]								
GPx,[6,9] U × 10^3/g Hb	103.5	105.6	104.1	102.9	1.29	0.42	0.71	0.20
Neutrophil function[10]								
Cytochrome C reduction, U[11]	3.44	3.49	3.27	3.16	0.064	<0.001	0.69	0.21
MPO degranulation, %	42.03	43.57	47.24	45.30	1.574	0.03	0.90	0.27
Total MPO, OD	2.04	2.16	2.16	2.09	0.034	0.45	0.36	0.007
Pasteurella multocida killing, %	52.09	54.68	48.35	47.84	2.152	0.04	0.68	0.53

[1]Data are means and pooled SEM based on repeated measures; GPx = glutathione peroxidase; Hb = hemoglobin; MPO = myeloperoxidase; OD = optical density.

[2]Main effects for statistical analysis included diet (D) and injection (I) and their interaction (D × I).

[3]Day 0 values were used as covariates in statistical analyses.

[4] Diet × injection × day: $P > 0.10$.

[5]Data were natural log transformed for analysis and least-square means and SEM were back-transformed for presentation in this table.

[6]Diet × day: $P > 0.10$; injection × day: $P > 0.10$.

[7]Diet × day: $P > 0.10$; injection × day: $P < 0.01$.

[8]Diet × day: $P < 0.05$; injection × day: $P < 0.05$.

[9]One unit of GPx activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol reduced nicotinamide adenine dinucleotide phosphate (NADPH) to oxidized nicotinaminde adenine dinucleotide phosphate (NADP+) per minute at 25°C. Units are reported as U × 10^3 (for example 100 U × 10^3/g Hb = 100,000 U/g Hb.)

[10]All interactions involving day: $P > 0.10$.

[11]One unit of cytochrome C reduction is defined as n*M* of extracellular superoxide anion released per 10^6 cells.

impacted by injection. Steers previously on the DEF diet had greater degranulation than CON animals ($P = 0.03$). There was an interaction between diet and injection in MPO concentrations ($P = 0.007$) where within CON animals, total MPO was greater in animals that received TM injection, but injection did not affect MPO within DEF steers.

Repletion Period Liver Trace Mineral Concentration

Steers receiving MM had greater liver Cu and Se concentrations than SAL steers through at least d 29 ($P < 0.05$; Fig. 4). There was an interaction between diet and injection for liver Cu, where the magnitude of the increase in Cu in response to injection was greater in CON vs. DEF ($P = 0.02$) steers. Liver Zn concentrations were greatest in DEF animals throughout the 90-d repletion period ($P < 0.01$; Table 4), but TM injection had no effect on liver Zn ($P > 0.20$). For liver Cu, Mn, and Se there was a significant diet × day interaction, where DEF steers, regardless of injection treatment, had higher liver Cu, Mn, and Se concentrations than CON animals on d 57 and 85 ($P < 0.01$).

DISCUSSION

While the focus of this manuscript is TM status, performance of these cattle throughout the study was evaluated and is reported elsewhere (Genther and Hansen, 2012). After 71 d consuming a diet containing 5 mg Mo and 300 mg Fe/kg DM and no supplemental Cu, DEF steers in this experiment had lower initial liver Cu than

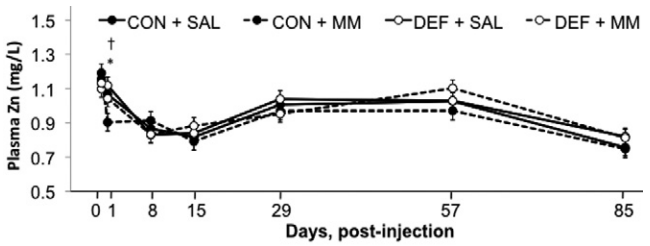

Figure 2. Effect of supplemental trace minerals (control [CON]) or deprivation (deficient [DEF]) during the depletion period and saline (SAL) or trace mineral injection (Multimin90 [MM]) on plasma Zn concentrations; values are means ± SEM, $n = 10$ within each treatment × diet combination. There was a diet × day interaction ($P = 0.01$) and injection × day interaction ($P < 0.05$); asterisks (*) denote differences ($P < 0.05$) between injections and daggers (†) denote differences ($P < 0.10$) between depletion diets, within day of the experiment.

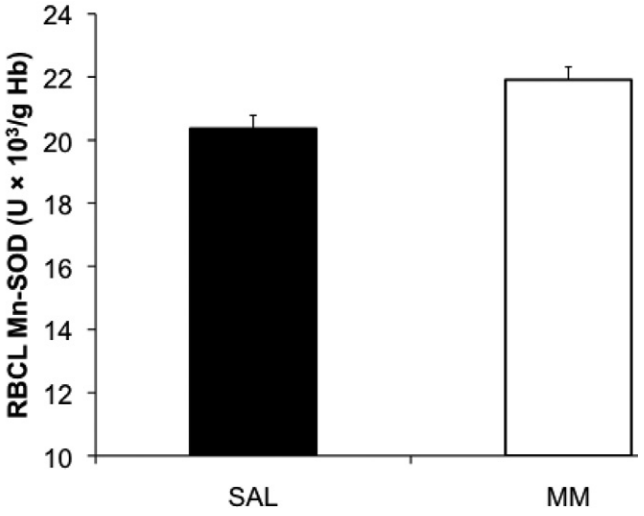

Figure 3. Effect of saline (SAL) or trace mineral injection (Multimin90 [MM]) on red blood cell lysate (RBCL) manganese-superoxide dismutase (Mn-SOD) activity; values are means ± SEM, $n = 20$ per injection treatment; injection ($P = 0.01$). Hb = hemoglobin.

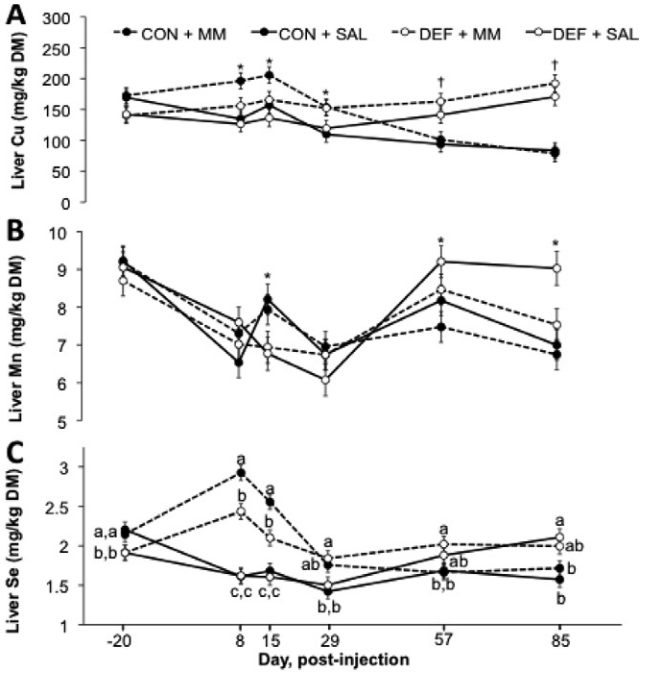

Figure 4. Effect of supplemental trace minerals (control [CON]) or deprivation (deficient [DEF]) during the depletion period and saline (SAL) or trace mineral injection (Multimin90 [MM]) on liver Cu (A), Mn (B), and Se concentrations (C); values are means ± SEM, $n = 10$ within each treatment × diet combination. Within liver Cu there was a diet × day interaction ($P < 0.05$) and injection × day interaction ($P < 0.05$); asterisks (*) denote differences ($P < 0.05$) between injections and daggers (†) denote differences ($P < 0.10$) between depletion diets, within day of the experiment. Within liver Mn there was a diet × day interaction ($P < 0.001$); asterisks (*) denote differences ($P < 0.05$) between depletion diets. Within liver Se there was a diet × injection × day interaction ($P = 0.02$); lowercase letters that differ denote differences ($P < 0.05$) between diet and injection within day.

CON steers. The liver Cu concentration in DEF steers (79.0 ± 11.60 mg Cu/kg DM) would be classified as marginally deficient according to Kincaid (2000). Mills (1987) suggests that cattle with liver Cu concentrations between 100 and 150 mg/kg DM have adequate Cu status, and deficiency is reached once liver Cu concentrations fall below 30 mg/kg DM. In the present study raw liver Cu concentrations ranged from 24 to 335 mg/kg DM indicating that Cu status varied widely among steers.

Kincaid (2000) suggests that an animal with liver Se concentrations between 0.6 and 1.25 mg/kg DM would be classified as marginally deficient. Animals on the DEF diet did have lower liver Se concentrations than CON animals before injection although the concentrations would not be considered deficient. Similarly, in cows fed no supplemental Se for approximately 2 mo, liver Se concentrations were lesser than cows supplemented with Se (Gunter et al., 2003) but were not within the deficient range as defined by Kincaid (2000). Feeding Fe and Mo as dietary antagonists sharply decreases liver Cu in beef heifers (Bailey et al., 2001) further demonstrating the antagonistic affect that Fe and Mo have on body Cu stores. Copper can form insoluble complexes with Mo and S and interact with Fe (Spears, 1996), minerals that are commonly found in feedlot diets at various concentrations. A limitation of our study is the unknown potential impact of the initial supplementation of Fe and Mo to the DEF steers during the depletion period.

The relatively small difference between liver Mn concentrations and lack of differences in liver Zn in DEF and CON steers at the end of the depletion period was not unexpected, as tissue Mn and Zn concentrations are not always reflective of actual TM status (Kincaid, 2000). As defined by Kincaid (2000) the steers in this experiment had adequate Zn status, as liver Zn concentrations fall well within the adequate range of 25 to 200 mg/kg DM. Surprisingly, liver Mn concentrations from both the DEF and CON steers were within the marginally deficient range as defined by Kincaid (2000), between 7 and 15 mg Mn/kg diet DM. However, in another study using beef heifers, supplementation with 50 mg Mn/kg DM (total dietary Mn = 65.8 mg/kg DM) for 196 d only resulted in liver concentrations of 9.4 mg Mn/kg DM at the end of the experiment (Hansen et al., 2006). These data further support the statement by Kincaid (2000) that liver Mn concentrations often do not reflect dietary Mn concentrations.

Between the pre-injection liver biopsy sample (21 d before the start of the repletion period) and the sample taken at d 8, liver Mn concentrations significantly decreased and liver Cu and Se concentrations were numerically decreased in both treatments. Two days before the beginning of the repletion period, animals were shipped for 20 h around Iowa to simulate shipping stress, as would be typical in a production setting. As mentioned previously, stress increases the requirements for many TM including most enzyme cofactors and antioxidants,

Table 4. Repletion period liver trace mineral concentrations as affected by trace mineral supplementation (control [CON]) or deficiency (DEF) during the depletion period and saline (SAL) or trace mineral injection (Multimin90 [MM]) on liver trace mineral concentrations[1]

	CON		DEF			*P*-value[2]		
	SAL	MM	SAL	MM		D	I	D × I
Variable	*n* = 10	*n* = 10	*n* = 10	*n* = 10	SEM			
Liver, mg/kg DM								
Cu[3]	124.6	151.3	139.3	161.6	8.96	0.33	0.001	0.76
Mn[4, 5]	7.64	7.59	7.98	7.57	0.207	0.45	0.26	0.39
Se[4, 6]	1.70	2.13	1.77	2.05	0.060	0.99	<0.001	0.16
Zn[3]	66.9	70.7	79.5	78.7	2.01	<0.001	0.46	0.26

[1]Data are means and pooled SEM based on repeated measures.

[2]Main effects for statistical analysis included diet (D) and injection (I) and their interaction (D × I).

[3]Diet × day: $P < 0.01$; injection × day: $P < 0.01$.

[4]Diet × day: $P > 0.10$; injection × day: $P > 0.10$.

[5]Diet × injection × day: $P > 0.10$.

[6]Diet × injection × day: $P = 0.02$.

and stressed cattle have lesser concentrations of B vitamins, vitamin A, Zn, Cu, Cr, vitamin E, and ascorbic acid (Schaefer et al., 2001). Furthermore, steers subjected to simulated stress by adrenocorticotropic hormone injection and feed and water restriction had negative Cu and Zn retention (Nockels et al., 1993). Additional excretion or need during shipping might explain the decrease in liver TM from –21 d to 8 d postinjection.

Because the TM status of cattle at arrival to a feedyard is unknown, it is important to determine what differences in response to an injectable TM may occur in cattle with either adequate or deficient status. Trace mineral injection improved liver Cu and Se concentrations through at least d 29 when compared to SAL steers. Previous research has shown that TM injection is an effective way to increase liver Cu and Se concentrations in TM adequate steers through 15 d postinjection (Pogge et al., 2012) although differences in liver Cu and Se concentrations between TM injected and saline injected steers may have lasted longer than the 15 d sampling period in that experiment. In the present study, the increase in liver Se concentration in response to TM injection was greater in CON steers when compared with the DEF steers, and a similar numerical trend was identified in liver Cu. This suggests that steers not receiving supplemental TM may have rapidly directed some of the additional Cu and Se provided through TM injection to places were where it was needed immediately, potentially to improve activities of Cu- and Se-dependent enzymes, rather than increase liver stores. Similarly, Owen and Hazelrig (1968) reported that ^{64}Cu administered intravenously to Cu-deficient or Cu-loaded rats was more evenly distributed throughout the blood, plasma, and liver in Cu-deficient rats whereas Cu-loaded rats stored more of the labeled Cu in the liver. Injectable TM more rapidly increased Cu and Se stores in cattle compared with those receiving saline and supplemental Cu and Se in the diet in the present study. It was not until d 57 that DEF + SAL steers had greater liver Cu and Se when compared with liver concentrations at the beginning of the repletion period.

Shipping and receiving into a new location is also a time of significant disease challenge, making optimal immune function vital (Duff and Galyean, 2007). After the depletion period, as previously mentioned, cattle on this study had lesser stores of Cu and Se, before shipping. This is of note as previous research has shown that while Cu and Se depletion in cattle has no impact on the ability of neutrophils to phagocytize bacteria, these TM deficiencies instead negatively impact neutrophil killing ability (Boyne and Arthur, 1981) by decreasing the respiratory burst (Cerone et al., 1998) and SOD and GPx activities in neutrophils (Arthur and Boyne, 1985). Myeloperoxidase is an enzyme required to produce the oxidative killing burst, and decreased total MPO is reflective of impaired neutrophil status and killing ability (Burg and Pillinger, 2001). In the present study, steers receiving a TM deficient diet for 89 d had lower total MPO and greater MPO degranulation, an indicator that MPO is found outside of the neutrophil, indicating fragility of the cells, common to inflammatory disorders (Lacy, 2006). Superoxide anion is important within the neutrophil to destroy microbes (Burg and Pillinger, 2001) but externally can cause oxidative tissue damage. Cytochrome C reduction, a measurement of extracellular release of superoxide anion, was also greater in DEF animals, suggesting that activity of superoxide dismutase (the enzyme that reduces the superoxide anion to hydrogen peroxide) may have been lessened in DEF compared with CON steers. These reductions in function suggest that adequate TM nutrition is necessary to support neutrophil function.

On d 90 and 91, immediately postshipping, there was no difference in either cytochrome C reduction or total MPO concentration between diets. These results are conflicting with results from the preshipping samples; however, function may have been compromised due to dehydration during the last 2 sampling points, as visual evaluation of samples suggested that packed cell volume was elevated and animals may have been experiencing heat stress due to high temperatures.

Early in the repletion period (d 13 and 14), greater bacteria killing ability and lesser MPO degranulation in neutrophils isolated from CON steers suggest that TM supplementation during the depletion period still had a positive impact neutrophil function although TM injection did not. These repletion samples were initially taken to evaluate the impact of injection on neutrophil function; however, although circulating neutrophils have a relatively short half-life in the body, it takes approximately 12 d for neutrophils to mature in the bone marrow (Orr et al., 2007). These samples may have been taken too soon after injection to reflect any changes that TM injection may elicit, or stress from heat or dehydration may have impacted the results. Trace minerals play supportive roles in neutrophil function, and optimal TM nutrition may help feedlot cattle overcome disease challenges more quickly.

Manganese SOD is a Mn-containing antioxidant enzyme found in the mitochondria that catalyzes the conversion of the superoxide radical to less reactive hydrogen peroxide (Weisiger and Fridovich, 1973). Trace mineral injection increased RBCL Mn-SOD activity over the repletion period in this study. Currently there are no good biomarkers of Mn status available in cattle (Herdt and Hoff, 2011); however, as Mn-SOD activity reflected Mn supplementation, in this case by injection and by dietary intake in other animal studies, it has potential to be used as a biomarker of Mn status. The activity of Mn-SOD in the leg muscle of broiler chickens was increased by dietary Mn supplementation up to 300 mg/kg diet DM (Lu et al., 2006). Increasing dietary Mn also increased Mn-SOD activity and Mn concentration in heart tissue of rats (Payter, 1980). The biological relevance of the increase in Mn-SOD activity due to injectable mineral is unclear, as limited research on Mn-SOD has been conducted in cattle; however, the increase in activity indicates that the minerals provided from a TM injection were incorporated into a biological process. Manganese concentration in the liver was not different due to dietary or injection treatment; however, due to the metabolism of Mn, dietary consumption of Mn is not always reflected in liver Mn concentration (Kincaid, 2000).

Unlike Mn-SOD activity, activity of Se-dependent GPx was not affected by diet or injection in this study. This is in contrast to previous research with an injectable mineral, where RBCL GPx activity was elevated by injection over the 15 d sampling period (Pogge et al., 2012). During this study, heat stress during the 4 wk of the repletion period may have negated treatment effects, as all steers had similar GPx activities. Stress, such as heat stress or shipping stress, can increase production of free radicals (Ganaie et al., 2013) so adequate antioxidant enzyme activity is critical.

In this study, DEF steers had greater liver Zn concentrations than CON steers. Similarly, heifer calves fed a diet without supplemental Zn had greater liver Zn after 21 d than calves supplemented with Zn (Engle et al., 1997). Zinc is a very difficult TM to evaluate partially because Zn is a vital component of over 300 metalloenzymes (McCall et al., 2000), so body distribution is difficult to track, leading to a lack of dependable biomarkers (Hambridge, 2003; Lowe et al., 2009). Injection of TM did not have an effect on liver Zn concentrations. This is in contrast to the results found by Pogge et al. (2012), where liver Zn concentrations, based on repeated measures, were increased in TM injected calves, although this may be due to the abbreviated sampling period that ended 15 d postinjection.

There was no difference in any plasma measures between animals previously on the CON or DEF diets when measured at d 0. This was not surprising, as TM homeostasis tightly regulates circulating TM concentrations, so plasma TM concentrations are not ideal measurements to evaluate TM status (Miller, 1975). Plasma concentrations may be good indicators of clinical deficiency but do not reflect moderate deficiency (Kincaid, 2000) as found in the DEF steers in this study. However, a lack of proper biomarkers means that plasma concentrations continue to be used to evaluate TM status. Pogge et al. (2012) found that plasma Cu, Mn, Se, and Zn were elevated 8 to 10 h postinjection, but all values returned to baseline before 24 h, with the exception of Se, which remained elevated through 24 h postinjection. However, in our study, steers that received a TM injection had elevated plasma Se through at least d 15, as plasma Se concentrations were not different between injected and noninjected animals by d 29 postinjection. Plasma Se is unique as a status indicator when compared to other plasma minerals, as it has been shown that plasma Se is reflective of Se intake (Herdt and Hoff, 2011) so plasma Se is a relatively good biomarker.

Between 29 and 57 d postinjection liver concentrations of Cu, Mn, and Se demonstrated an interesting crossover, where steers that were previously on the CON diet had decreasing concentrations of liver Cu, Mn, and Se after 29 d whereas concentrations stayed relatively stable in DEF animals. Ahola et al. (2005) also found that liver Cu and Mn concentrations were numeri-

cally decreased at the end of the finishing period when compared with the end of the growing period in feedlot cattle, regardless of TM supplementation program. This may be due to increasing TM excretion by the CON animals during the finishing period or lower intestinal TM absorption. Alternately, the DEF steers may have increased TM absorption or decreased TM excretion, having lacked adequate TM in the diet previously. However, additional research into the dynamics of TM status and requirements in finishing cattle is warranted.

In conclusion, diets lacking supplemental Cu, Mn, Se, and Zn and supplemented with the antagonists Fe and Mo significantly decrease liver Cu and Se concentrations. In animals with differential TM status, TM delivered through injection appear to be uniquely utilized while throughout the finishing period the dynamics of TM storage in the liver are also different between animals with different initial status. Trace minerals from injection appeared to be incorporated into a biological process, increasing Mn-SOD activity, which may hold potential to be used as a Mn biomarker. Overall, TM injection more rapidly increases TM status than does adequate dietary TM supplementation alone.

LITERATURE CITED

Ahola, J. K., L. R. Sharpe, K. L. Dorton, P. D. Burns, T. L. Stanton, and T. E. Engle. 2005. Effects of lifetime copper, zinc, and manganese supplementation and source on performance, mineral status, immunity, and carcass characteristics of feedlot cattle. Prof. Anim. Sci. 21:305–317.

Arthur, J. R., and E. Boyne. 1985. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Sci. 36:1569–1575.

Bailey, J. D., R. P. Ansotegui, J. A. Paterson, C. K. Swenson, and A. B. Johnson. 2001. Effects of supplementing combinations of inorganic and complexed copper on performance and liver mineral status of beef heifers consuming antagonists. J. Anim. Sci. 79:2926–2934.

Boyne, R., and J. R. Arthur. 1981. Effects of selenium and copper deficiency on neutrophil function in cattle. J. Comp. Pathol. 91:271–276.

Burg, N. D., and M. H. Pillinger. 2001. The neutrophil: Function and regulation in innate and humoral immunity. Clin. Immunol. 99:7–17.

Cerone, S., A. Sansinanea, S. Streitenberger, C. Garcia, and N. Auza. 1998. Bovine neutrophil functionality in molybdenum-induced copper deficiency. Nutr. Res. 18:557–566.

Duff, G. C., and M. L. Galyean. 2007. Recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85:823–840.

Engle, T. E., C. F. Nockels, C. V. Kimberling, D. L. Weaber, and A. B. Johnson. 1997. Zinc repletion with organic or inorganic forms of zinc and protein turnover in marginally zinc-deficient calves. J. Anim. Sci. 75:3074–3081.

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451.

Ganaie, A. H., G. Shanker, N. A. Bumla, R. S. Ghasura, N. A. Mir, S. A. Wani, and G. B. Dudhatra. 2013. Biochemical and physiological changes during thermal stress in bovines. J. Vet. Sci. Technol. 4:126.

Genther, O. N., and S. L. Hansen. 2012. Investigation of the impact of mineral status and use of an injectable mineral on beef cattle performance. Iowa State University Animal Industry Report. 2012. A. S. Leaflet R2693. www.ans.iastate.edu/report/air/2012pdf/R2693.pdf. Accessed Jan 23, 2013.

Gunter, S. A., P. A. Beck, and J. M. Phillips. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J. Anim. Sci. 81:856–864.

Hambridge, M. 2003. Biomarkers of trace mineral intake and status. J. Nutr. 133:948S–955S.

Hansen, S. L., M. S. Ashwell, A. J. Moeser, R. S. Fry, M. D. Knutson, and J. W. Spears. 2010. High dietary iron reduces transporters involved in iron and manganese metabolism and increased intestinal permeability in calves. J. Dairy Sci. 93:656–665.

Hansen, S. L., J. W. Spears, K. E. Lloyd, and C. S. Whisnant. 2006. Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese. J. Anim. Sci. 84:3375–3380.

Herdt, T. H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Vet. Clin. North Am. Food Anim. Pract. 27:255–283.

Kincaid, R. L. 2000. Assessment of trace mineral status of ruminants: A review. J. Anim. Sci. 77:1–10.

Lacy, P. 2006. Mechanisms of degranulation in neutrophils. Allergy Asthma Clin. Immunol. 2:98–108.

Lowe, N. M., K. Fekete, and T. Decsi. 2009. Methods of assessment of zinc status in humans: A systematic review. Am. J. Clin. Nutr. 89(Suppl.):2040S–2051S.

Lu, L., C. Ji, X. G. Luo, B. Liu, and S. X. Yu. 2006. The effect of supplemental manganese in broiler diets on abdominal fat deposition and meat quality. Anim. Feed Sci. Technol. 129:49–59.

Malcolm-Callis, K. J., G. C. Duff, S. A. Gunter, E. B. Kegley, and D. A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. J. Anim. Sci. 78:2801–2808.

McCall, K. A., C. Huang, and C. A. Fierke. 2000. Function and mechanism of zinc metalloenzymes. J. Nutr. 130:1437S–1446S.

Miller, W. J. 1975. New concepts and developments in metabolism and homeostasis of inorganic elements in dairy cattle. A review. J. Dairy Sci. 58:1549–1560.

Mills, C. F. 1987. Biochemical and physiological indicators of mineral status in animals: Copper, cobalt and zinc. J. Anim. Sci. 65:1702–1711.

Nockels, C. F., J. DeBonis, and J. Torrent. 1993. Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. J. Anim. Sci. 71:2539–2545.

Orr, Y., D. P. Wilson, J. M. Taylor, P. G. Bannon, C. Geezy, M. P. Davenport, and L. Kritharides. 2007. A kinetic model of bone marrow neutrophil production that characterizes late phenotypic maturation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R1707–R1716.

Owen, C. A., and J. B. Hazelrig. 1968. Copper deficiency and copper toxicity in the rat. Am. J. Phys. 215:334–338.

Palić, D., C. B. Andreasen, B. W. Menzel, and J. A. Roth. 2005. A rapid direct assay to measure degranulation of primary granules in neutrophils from kidney of fathead minnow (Pimephales promelas Rafinesque, 1820). Fish Shellfish Immunol. 19:217–227.

Payter, D. I. 1980. Changes in activity of the manganese superoxide dismutase enzyme in tissues of the rat with changes in dietary manganese. J. Nutr. 110:437–447.

Pogge, D. J., E. L. Richter, M. E. Drewnoski, and S. L. Hansen. 2012. Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J. Anim. Sci. 90:2692–2698.

Richeson, J. T., and E. B. Kegley. 2011. Effect of supplemental trace minerals from injection on health and performance of highly stressed, newly received beef heifers. Prof. Anim. Sci. 27:461–466.

Richter, E. L., M. E. Drewnoski, and S. L. Hansen. 2012. Effects of increased dietary sulfur on beef steer mineral status, performance, and meat fatty acid composition. J. Anim. Sci. 90:3945–3953.

Roth, J. A., D. E. Frank, P. Weighner, and M. Weighner. 2001. Enhancement of neutrophil function by ultrafiltered bovine whey. J. Dairy Sci. 84:824–829.

Roth, J. A., and M. L. Kaeberle. 1981. Isolation of neutrophils and eosinophils from the peripheral blood of cattle and comparison of their functional activities. J. Immunol. Methods 45:153–164.

Salyer, G. B., M. L. Galyean, P. J. Defoor, G. A. Nunnery, C. H. Parsons, and J. D. Rivera. 2004. Effects of copper and zinc source on performance and humoral immune response of newly received, lightweight beef heifers. J. Anim. Sci. 82:2467–2473.

Schaefer, A. L., P. L. Dubeski, J. L. Aalhus, and A. K. W. Tong. 2001. Role of nutrition in reducing antemortem stress and meat quality aberrations. J. Anim. Sci. 79:E91–E101.

Smart, M. E., J. Gudmundson, and D. A. Christensen. 1981. Trace mineral deficiencies in cattle: A review. Can. Vet. J. 22:372–376.

Spears, J. W. 1996. Organic trace minerals in ruminant nutrition. Anim. Sci. Feed Technol. 58:151–163.

Spears, J. W., and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747–2752.

Stevens, M. G., and S. C. Olsen. 1993. Comparative analysis of using MTT and XTT in colorimetric assays for quantitating bovine neutrophil bactericidal activity. J. Immunol. Methods 157:225–231.

Tunney, M. M., G. Ramage, T. R. Field, T. F. Moriarty, and D. G. Storey. 2004. Rapid colorimetric assay for antimicrobial susceptibility testing of Pseudomonas aeruginosa. Antimicrob. Agents Chemother. 48:1879–1881.

Underwood, E. J., and N. F. Suttle. 1999. Mineral nutrition of livestock. 3rd ed. CABI Publishing, New York.

Ward, J. D., and J. W. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75:3057–3065.

Weisiger, R. A., and I. Fridovich. 1973. Superoxide dismutase. J. Biol. Chem. 248:3582–3592.