

NUTRITION: *Original Research*

# Comparison of multiple single-use, pulse-dose trace mineral products provided as injectable, oral drench, oral paste, or bolus on circulating and liver trace mineral concentrations of beef steers

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## ABSTRACT

**Objective:** The objective was to determine effects of various trace mineral products on steer plasma and liver trace mineral concentrations.

**Materials and Methods:** Fifty-six trace mineral adequate Angus-cross steers ( $303 \pm 15.2$  kg;  $n = 8$  per treatment) were sorted by BW and administered treatments on d 0: injectable saline (CON), injectable Multi-min90 (ITM), Mineral Max Drench (MMD), Mineral Max Paste (MMP), Starting Fluid Drench (SFD), Se365 bolus (Se365), or Reloader250 bolus (Rel250). Steers received a common diet (silage-based diet d 0–49; corn-based diet d 50–122), and individual feed disappearance was recorded. Plasma (0, 8, 24, and 48 h) and liver (–7, 2, 15, 29, 49, 65, 91, and 120 d) were analyzed for Cu, Mn, Se, and Zn.

**Results and Discussion:** Plasma Zn, Mn, and Se concentrations were affected by treatment  $\times$  time ( $P = 0.001$ ); steers given ITM had greater concentrations through 8 h for Zn and 24 h for Mn and Se versus other treatments. Liver Se concentration was greater in ITM versus other treatments through d 15, but Rel250 was greater than ITM and MMP on d 91 and greater than CON, MMD, MMP, and SFD on d 120 (treatment  $\times$  time;  $P \leq 0.001$ ). Liver Mn, Zn, and Cu were affected by time ( $P \leq 0.001$ ), where liver Mn concentrations were least on d 2 and increased over time but liver Zn concentrations were greatest on d 2 and least on d 29 to 120.

**Implications and Applications:** Single-use, pulse-dose products increased circulating trace minerals most quickly as an injection (increasing plasma Mn, Se, Zn) compared with other treatments, whereas liver Se concentrations were increased by injection (through d 29) and Rel250 (by d 91).

**Key words:** copper, manganese, selenium, zinc, cattle

## INTRODUCTION

Trace minerals are essential for supporting growth and health of cattle. Producers provide supplements in the form of free-choice trace mineralized salt or in the TMR, but these supplementation strategies can still lead to the deficiency of one or more important trace minerals. These deficiencies can be caused by a multitude of factors including dietary antagonisms of trace minerals within the gastrointestinal tract and variability in trace mineral intake among cattle. It is increasingly common for producers to give cattle single-use, pulse-dose products containing trace minerals to improve trace mineral status or ensure adequate trace mineral status when prior status is unknown. Injectable trace mineral supplements increase liver Cu (Kurz, 2004; Pogge et al., 2012) and plasma and liver Se (Pogge et al., 2012) in feedlot steers while also increasing activity of Se- and Mn-dependent enzymes within 30 d of injection (Pogge et al., 2012; Genther and Hansen, 2014). Oral supplementation strategies such as a drench or paste may also be used in beef cattle; however, little information is available concerning their effects on plasma or liver trace mineral concentrations. Ruminoreticular boluses containing trace minerals, including Se and Cu, increase blood Se concentrations in calves and mature cows (Buckley et al., 1987; Hidiroglou et al., 1987), as well as liver Cu concentrations of mature cows (Sprinkle et al., 2006). However, trace minerals have low rates of intestinal absorption regardless of source (Spears, 1996), and a single pulse dose of trace minerals such as Cu or Zn provided by an oral drench or paste may not provide enough mineral to improve trace mineral concentrations of cattle (Greene, 1999). Trace minerals administered orally face additional challenges such as antagonists or binding to undigested feed particles, potentially decreasing absorption (Spears, 2003). The objective of this study was to deter-

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mine whether various trace mineral-containing products provided through oral or injectable routes increase trace mineral concentrations of beef cattle. It was hypothesized that injectable trace minerals would rapidly increase plasma and liver mineral concentrations, bolus products would increase liver mineral concentrations gradually, and drench or paste single-use, pulse-dose products would have little effect on trace mineral concentrations of steers.

## MATERIALS AND METHODS

### Pretrial Animal Background and Experimental Diet

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (8-17-8587-B).

Newly weaned Angus crossbred steers ( $n = 56$ ) were purchased from a single source and transported to the Iowa State University Beef Nutrition Research Center. Steers were weighed, vaccinated against viral infections (Bovi-Shield Gold One Shot, vaccine against bovine rhinotracheitis, virus diarrhea, parainfluenza<sub>3</sub>, and respiratory syncytial virus, Zoetis Inc., Kalamazoo, MI), and treated for parasites (Ivomec Eprinomex, Boehringer Ingelheim

Vetmedica Inc., St. Joseph, MO) on d  $-21$ . Steers were adapted to a corn silage-based diet for 21 d before start of the trial, and this diet was fed until d 36 at which time steers began transitioning to a finishing diet for the remainder of the trial (Table 1). Before initiation of the study, all feed ingredients were analyzed for trace minerals and a supplement was formulated such that the TMR met or slightly exceeded NASEM (2016) recommendations, using only inorganic supplemental trace mineral sources. Because the diet met requirements for Se and Zn, additional supplementation was not included. Feed was delivered daily at approximately 0800 h, and off-condition feed was discarded as necessary.

### Experimental Design

Steers were weighed (d  $-21$ ) and sorted by BW ( $248 \pm 14.3$  kg) to 1 of 7 treatments. Treatments were administered on d 0 of the trial and included **CON**: injection of sterilized saline at a dose of 1 mL/45 kg of BW; **ITM**: injectable Multimin90 (Multimin USA, Fort Collins, CO) at a dose of 1 mL/45 kg of BW; **MMD**: Mineral Max Drench (Aspen Veterinary Resources Ltd., Liberty, MO) at a dose of 6 mL/45 kg of BW; **MMP**: Mineral Max Paste (Aspen Veterinary Resources Ltd.) at a dose of 30 mL per steer;

**Table 1.** Common diet fed to all steers

Item	Growing diet <sup>1</sup>	Transition diet <sup>2</sup>	Finishing diet <sup>3</sup>
Ingredient, % DM			
Corn silage	40	24	16
Cracked corn	30	44	53
Corn dried distillers grains with solubles	25	27	26
Micronutrients and carrier <sup>4</sup>	5	5	5
Analyzed components			
CP, <sup>5</sup> %	15.4	—	15.9
NDF, <sup>5</sup> %	28.4	—	21.6
Ether extract, <sup>5</sup> %	4.0	—	4.6
NE <sub>m</sub> , <sup>6</sup> Mcal/kg	1.92	—	2.07
NE <sub>g</sub> , <sup>6</sup> Mcal/kg	1.28	—	1.41
Cu, mg/kg of DM	10.4	—	10.4
Se, mg/kg of DM	0.36	—	0.34
Mn, mg/kg of DM	19.0	—	18.8
Zn, mg/kg of DM	30.5	—	26.7

<sup>1</sup>Diet fed from d  $-21$  to 36 for group A and d  $-25$  to 41 for group B.

<sup>2</sup>Diet fed from d 37 to 43 for group A and d 42 to 48 for group B.

<sup>3</sup>Diet fed from d 44 to 120 for group A and d 49 to 120 for group B.

<sup>4</sup>Micronutrients and carrier includes dried distillers grains with solubles as a carrier and micronutrients provided as a percentage of diet DM: limestone (1.4%), Rumensin (0.015%; Elanco Animal Health, Greenfield, IN), and salt (0.31%). Trace minerals provided per kilogram of DM 0.15 mg of Co (cobalt carbonate), 6 mg of Cu (copper sulfate), 8 mg of Mn (manganese sulfate), and 0.5 mg of I (calcium iodate).

<sup>5</sup>Chemical analysis completed by Dairyland Laboratories (Arcadia, WI).

<sup>6</sup>Overall NE<sub>m</sub> and NE<sub>g</sub> values were calculated using NASEM (2016) values for individual feed ingredients.

**SFD:** Starting Fluid Drench (Kentucky Nutrition Service, Lawrenceburg, KY) at a dose of 11 mL per steer; **Se365:** Se365 bolus (Pacific Trace Minerals Inc., Ashland, OR) at a dose of 1 bolus per steer; and **Rel250:** Reloader250 bolus (Provimi North America Inc., Brookville, OH) at a dose of 1 bolus per steer, per manufacturer recommendations (Table 2). Steers that received an oral treatment (MMD, MMP, SFD, Se365, Rel250) also received an injection of sterilized saline (1 mL/45 kg of BW), and steers receiving injectable treatments (CON, ITM) had the bolus applicator inserted into their mouth such that any perceived stress from treatment administration was equalized across treatments.

To avoid pen effects, treatments were stratified across pens such that at least one steer per treatment was housed

in each pen ( $n = 8$  steers per pen; 7 pens in total). Pens were cement floored and under roof, and each pen was equipped with a bunk capable of measuring individual feed disappearance (Dahlke et al., 2008). Steer was the experimental unit ( $n = 8$  per treatment). Steers were divided into 2 groups (A and B) and stagger started by 5 d to accommodate the liver biopsy schedule. Both groups contained all treatments: group A (CON  $n = 5$ , ITM  $n = 5$ , MMD  $n = 5$ , MMP  $n = 4$ , SFD  $n = 3$ , Se365  $n = 3$ , Rel250  $n = 3$ ); group B (CON  $n = 3$ , ITM  $n = 3$ , MMD  $n = 3$ , MMP  $n = 4$ , SFD  $n = 5$ , Se365  $n = 5$ , Rel250  $n = 5$ ).

### Sample Collection and Analytical Procedures

Diet samples were collected weekly and dried at 70°C for 48 h in a forced-air oven for DM determination. Samples

**Table 2.** Concentration of trace mineral in products used and dosage<sup>1</sup> of trace mineral treatments

Treatment	Cu	Mn	Se	Zn
ITM <sup>2</sup>				
Concentration in product, mg/mL	15	10	5	60
Dosage, mg	97.5	65	32.5	390
MMD <sup>3</sup>				
Concentration in product, mg/mL	6.1	2.5	0.02	6.1
Dosage, mg	241.6	99	0.79	241.6
MMP <sup>4</sup>				
Concentration in product, mg/mL	6.1	2.5	0.06	6.1
Dosage, mg	183	75	1.8	183
SFD <sup>5</sup>				
Concentration in product, mg/mL	12.1	5	—	12.1
Dosage, mg	133.1	55	—	133.1
Se365, <sup>6</sup> mg	—	—	2,400	—
Rel250, <sup>7</sup> mg	541.5	1,425	256.5	24,700

<sup>1</sup>The amount of mineral provided per steer for each trace mineral treatment.

<sup>2</sup>Injectable trace mineral (Multimin90, Multimin USA, Fort Collins, CO). Amount of mineral provided per steer based off an average dose of 6.5 mL.

<sup>3</sup>Mineral Max Drench (Aspen Veterinary Resources Ltd., Liberty, MO). Values listed are per 29.6 mL and are the amount of mineral provided per steer based off an average dose of 39.6 mL. The product also contains Mg (0.95%), K (0.95%), Co (400 mg/kg), vitamin A (88,000 IU), vitamin D (44,000 IU), vitamin E (88 IU), vitamin B<sub>1</sub> (0.156 mg), vitamin B<sub>2</sub> (0.98 mg), vitamin B<sub>6</sub> (0.22 mg), vitamin B<sub>12</sub> (150 µg), and biotin (30 µg).

<sup>4</sup>Mineral Max Paste (Aspen Veterinary Resources Ltd.). Values listed are per 29.6 mL and are the amount of mineral provided per steer based off a dose of 30 mL. The product also contains Mg (0.95%), K (0.95%), Co (400 mg/kg), vitamin A (4,400,000 IU/kg), vitamin D (220,000 IU/kg), vitamin E (33,000 IU/kg), and vitamin B<sub>12</sub> (85,000 µg/kg).

<sup>5</sup>Starting Fluid Drench (Kentucky Nutrition Service, Lawrenceburg, KY). Values listed are per 11-mL dose and are the amount of mineral provided per steer based off a dose of 11 mL. The product also contains Mg (1.01%) and K (1.01%).

<sup>6</sup>Se365 bolus (Pacific Trace Minerals Inc., Ashland, OR). Values listed are per bolus. The bolus weighs 30 g.

<sup>7</sup>Reloader250 bolus (Provimi North America Inc., Brookville, OH). Values listed are per bolus. The bolus weighs 95 g and contains Ca (20–25 mg/g), Mg (88 mg/g), Na (0.6–2 mg/g), I (15.8 mg/g), Co (2.5 mg/g), vitamin A (440,000 IU/kg), vitamin D<sub>3</sub> (924,000 IU/kg), and vitamin E (22,000 IU/kg).

were composited by month within diet type (growing or finishing), acid digested, and analyzed by the Iowa State University Veterinary Diagnostic Laboratory for Cu, Mn, Se, and Zn using inductively coupled plasma mass spectroscopy (Analytik Jena Inc., Woburn, MA; Pogge et al., 2012). Dry matter intakes were calculated using intake data collected from the individual feed intake system (Dahlke et al., 2008) corrected for weekly DM to determine DMI.

Liver biopsies were performed on d -7 following the protocol of Engle and Spears (2000) to obtain initial liver mineral concentrations. Consecutive weights were taken on d -1 and 0 ( $303 \pm 15.2$  kg) to correctly calculate dosage of the treatments, and treatments were administered by a single person. Additional liver biopsies were performed on d 2, 15, 29, 49, 65, 91, and 120 after treatment. Samples were transported to the laboratory on ice and stored at  $-20^{\circ}\text{C}$  until analysis. Liver samples were dried and ashed according to Pogge et al. (2012). A total of 6 mL of blood was collected via the jugular vein using potassium EDTA vacuum tubes to analyze for trace minerals (Becton Dickenson, Rutherford, NJ) immediately before administration of the treatments (0 h) as well as 8, 24, and 48 h after treatment. Blood was stored on ice until return to the laboratory, where it was then centrifuged at  $1,000 \times g$  for 10 min at  $4^{\circ}\text{C}$ . Plasma was removed and stored at  $-20^{\circ}\text{C}$  until analysis. Liver and plasma samples were sent to the Iowa State University Veterinary Diagnostic Laboratory for analysis of Cu, Mn, Se, and Zn using inductively coupled plasma mass spectroscopy (Analytik Jena Inc.). Liver and plasma mineral analysis did not have technical replication. Quality controls (National Institute of Standards and Technology, Gaithersburg, MD) were included on each run to ensure accuracy of the instrument between runs. Plasma and liver intra-assay CV for all minerals was  $\leq 4.50\%$ , and inter-assay CV for all minerals was  $\leq 5.41\%$ .

### Statistical Analysis

Liver and plasma mineral data and DMI data were analyzed as repeated measures using PROC MIXED in SAS (SAS Institute Inc., Cary, NC). The model included the fixed effects of treatment, time, and treatment  $\times$  time, with time (day for liver, hour for plasma, and week for DMI) as the repeated effect. Steer was the experimental unit ( $n = 8$  per treatment). Initial liver concentrations (d -7) served as a covariate for all liver mineral analyses, and initial plasma concentrations (h 0) served as a covariate for all plasma mineral analyses. The covariance structure was compound symmetry and was selected based on the lowest corrected Akaike information criterion value (Littell et al., 1998). Group (A or B) was tested as a fixed effect initially in all models, found to be nonsignificant, and subsequently removed from the final model. Determination of outliers was conducted using the Cook's D statistic and removed if Cook's D was  $\geq 0.5$ . Liver Se and Zn and plasma Cu, Mn, Se, and Zn were natural log-transformed

to normalize the data, and the back transformed means and SEM values are presented. Significance was declared at  $P \leq 0.05$ , and tendencies of  $P \leq 0.10$  are reported. Pairwise differences were determined using the Pdiff option in SAS. Data shown are LSM and SEM.

## RESULTS AND DISCUSSION

### DMI

No treatment  $\times$  week ( $P \geq 0.65$ ) or treatment ( $P \geq 0.75$ ) effect was detected on DMI for steers during the growing or finishing period. Growing-period average DMI for each treatment (kg/d) were 7.59 for CON, 7.26 for ITM, 7.23 for MMD, 7.53 for MMP, 7.52 for SFD, 6.52 for Se365, and 6.67 for Rel250 with a SEM of 0.53. Finishing-period average DMI for each treatment (kg/d) were 9.57 for CON, 8.30 for ITM, 8.38 for MMD, 9.84 for MMP, 9.08 for SFD, 8.51 for Se365, and 8.92 for Rel250 with a SEM of 0.71.

### Copper

**Plasma.** There was no treatment  $\times$  hour ( $P = 0.52$ ), treatment ( $P = 0.43$ ), or hour ( $P = 0.11$ ) effect on plasma concentrations of Cu (data not shown), with concentrations averaging  $1.43 \pm 0.41$  mg/L across all treatments. Due to sensitive regulatory mechanisms, plasma Cu is known to be a poor indicator of Cu status in cattle, with concentrations remaining adequate until liver Cu concentration falls below 40 mg/kg of DM (Koh and Judson, 1987; Kincaid, 2000). In contrast to injectable routes of Cu delivery, oral supplementation of Cu through a drench or bolus likely encounters many antagonisms and interactions with Mo, Fe, and S, and apparent absorption of dietary Cu is generally less than 10% (Suttle, 2010). This may be why minimal changes in plasma Cu concentrations were observed across the initial 48-h period.

**Liver.** No treatment  $\times$  day or treatment effect ( $P \geq 0.26$ ; data not shown) was detected for liver Cu concentrations. Based on the reference range for adequacy (125 to 600 mg of Cu/kg of liver DM), all steers in the present study had adequate Cu status (Kincaid, 2000). There was an effect of day ( $P < 0.001$ ), where liver Cu concentrations were greater on d 15 and 29 than on all other days, which did not differ from one another (Figure 1A). The greater concentration of liver Cu on d 15 and 29 may be driven by a numerical increase in liver concentrations of Cu on d 15 in ITM compared with CON, which is consistent with past studies; however, the magnitude of increase (33 mg/kg of DM) is lesser than the approximately 40 to 50 mg of Cu/kg of DM increase observed by others (Pogge et al., 2012; Genther and Hansen, 2014; Hartman et al., 2018). Additionally, the ITM increase in liver Cu observed by others has been shown to last 28 to 40 d relative to controls (Genther and Hansen, 2014; Genther-Schroeder and Hansen, 2015), indicative of the quick but transient influence of ITM. Liver Cu concentrations measured in



the present study were variable compared with previous studies, perhaps due to the intensive study schedule early in the feeding period to accomplish blood draws and liver sampling, as the Cu-dependent enzyme ceruloplasmin fluctuates with stress (Ward and Spears, 1999).

## Selenium

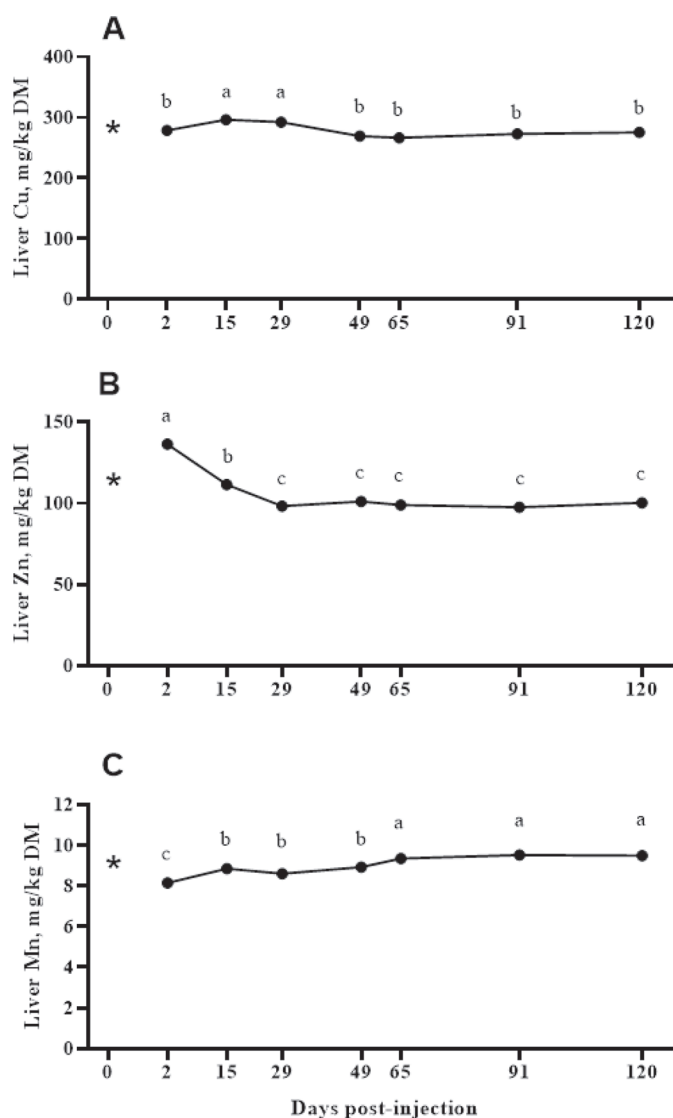
**Plasma.** There was a treatment  $\times$  hour effect ( $P < 0.001$ ) on plasma concentrations of Se, where ITM steers had greater plasma Se concentrations than all other treatments at h 8 and 24 and had greater plasma Se concentrations than SFD at h 48 (Figure 2). Consistent with previous studies (Pogge et al., 2012; Genther and Hansen, 2014), plasma Se peaked within the first day for ITM steers. Since the ITM treatment is delivered subcutaneously, there is an almost immediate response in plasma concentrations of Se. This is noted in previous research where plasma Se concentrations peaked 1 h after Multi-min90 injection in Holstein heifers (Hansen and Niedermayer, Iowa State University, Ames, IA, personal communication). The subsequent decrease in plasma Se is likely due to uptake by the liver (Figures 2 and 3).

Previous researchers have found that 30 to 40% of Se consumed by cattle is converted to selenite within the rumen and 10 to 15% is incorporated into microbial selenoproteins by replacing S within the cystine residue, leaving roughly half of the Se provided available for absorption by the animal (Gerloff, 1992; Serra et al., 1994). Wright and Bell (1966) observed absorption of Se in the gastrointestinal tract was approximately 34%. The low absorption of Se in the ruminant in combination with low Se content of the supplements may explain why no change was noted in the single, pulse-dose oral treatments used in this study. The bolus treatments both contained Se yet had no effect on plasma Se concentrations ( $P \geq 0.28$ ) when compared with CON. In contrast, Davy et al. (2016) used the same Se365 bolus in 2 separate trials and noted an increase in whole blood Se when compared with unsupplemented controls at various time points throughout the trials (trial 1: 30 and 90 d after treatment; trial 2: 21, 45, 64, 85 d after treatment). Both plasma and whole blood have a high correlation with changes in Se intake, but comparison of plasma and whole blood can be difficult because plasma responds quickly when Se intakes are altered, whereas whole blood responds more slowly (Stowe and Herdt, 1992).

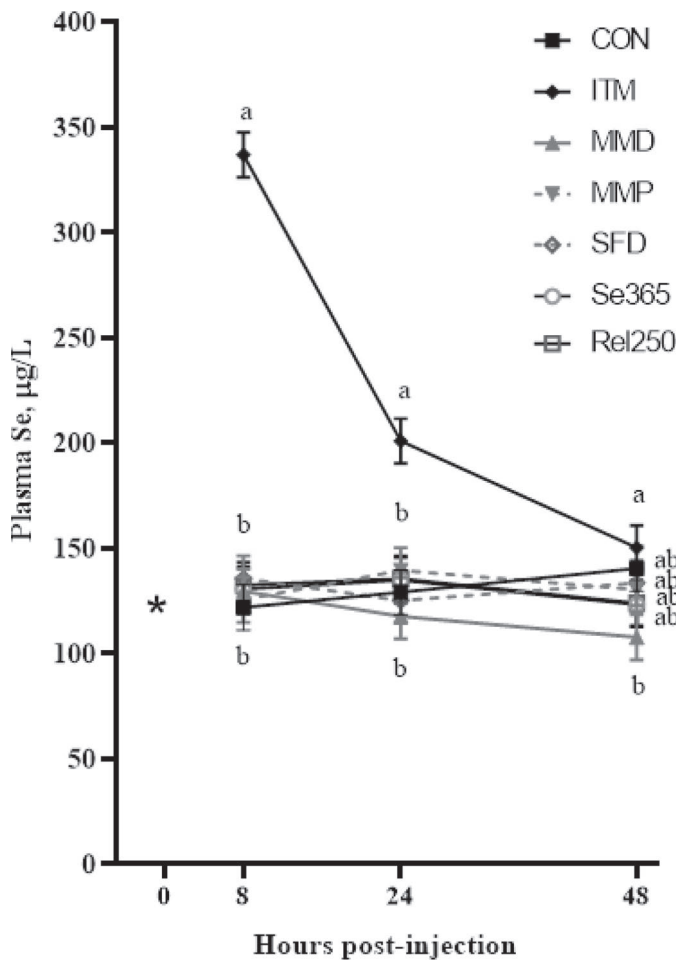
The cattle used by Davy et al. (2016) were Se deficient (Kincaid, 2000); thus, it is likely they had greater rates of absorption for Se in comparison with the Se-adequate steers used in the present study. No measures of bolus degradation were performed during the present study, but it appears that release and availability of Se from the bolus was not sufficient to affect plasma Se concentrations within the first 48 h in steers with adequate Se status. In contrast to Cu, Mn, and Zn, plasma Se concentrations provide more value as a status index (Kincaid, 2000), and the lack of discernable changes in plasma Se concentra-

tions during the 48 h after oral or bolus administration suggests Se provided in these products is not reaching the bloodstream in sufficient quantities to influence plasma Se concentrations of the animal.

**Liver.** A treatment  $\times$  day effect ( $P < 0.001$ ) for liver Se concentrations was detected (Figure 3). This is largely driven by greater liver Se concentrations in ITM steers on d 2 and 15 compared with all other treatments, whereas ITM led to greater liver Se concentrations than oral treatments and CON on d 29 and resulted in greater liver Se concentrations than MMD on d 49. The Rel250 treatment had greater liver Se concentrations than ITM and MMP on d 91 and greater liver Se concentrations than MMD,



**Figure 1.** Effects of day of sampling on liver concentrations of Cu (A), Zn (B), and Mn (C), over a 120-d postinjection period. Differing letters (a–c) within a mineral indicate a day difference ( $P \leq 0.05$ ). The d-0 values served as covariates in analysis. Asterisks denote the average starting liver concentrations within each mineral. The SEM for Cu, Zn, and Mn were 7.12, 1.03, and 0.15, respectively.



**Figure 2.** Effects of multiple trace mineral supplements on plasma concentrations of Se over a 48-h postinjection period. Treatments were saline (CON), Multimin90 (ITM; Multimin USA, Fort Collins, CO), Mineral Max Drench (MMD; Aspen Veterinary Resources Ltd., Liberty, MO), Mineral Max Paste (MMP; Aspen Veterinary Resources Ltd.), Starting Fluid Drench (SFD; Kentucky Nutrition Service, Lawrenceburg, KY), Se365 bolus (Se365; Pacific Trace Minerals Inc., Ashland, OR), and Reloader250 bolus (Rel250; Provimi North America Inc., Brookville, OH). Differing letters (a, b) within an hour indicate a difference ( $P \leq 0.05$ ). The h-0 values served as covariates in analysis. The asterisk denotes the average starting plasma Se concentration across treatments. Values are SEM  $\pm$  10.7.

MMP, SFD, and CON on d 120. The Se365 treatment had no effect on liver Se compared with CON throughout the trial ( $P \geq 0.36$ ).

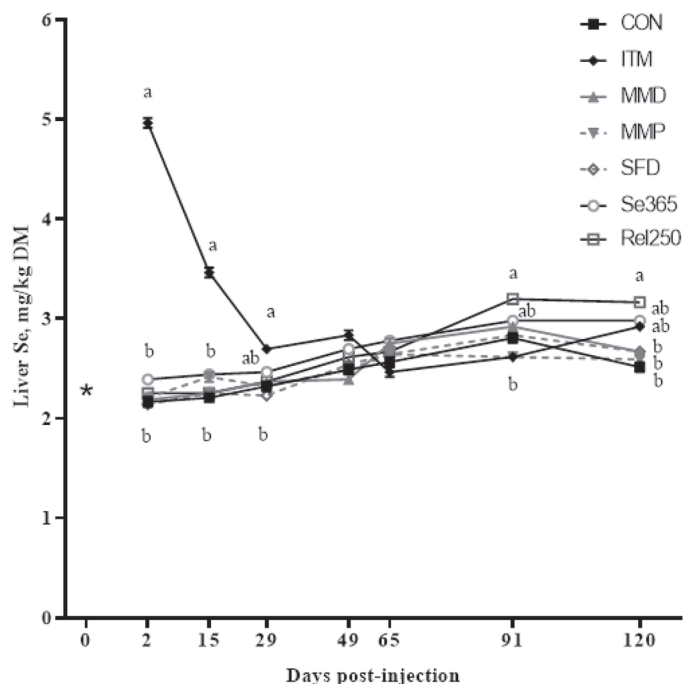
Consistent with previous work, liver Se concentrations in steers receiving injectable trace minerals were greater than those in CON steers through d 29 after injection (Pogge et al., 2012; Genther and Hansen, 2014). Administering Se in the oral drenches and pastes had no effect on liver Se concentrations, indicating the 3 single-use, pulse-dose products tested in this study had no effect on liver Se concentrations. However, the Rel250 steers displayed the greatest liver Se concentrations on d 91 and 120, indicating the bolus may be an effective way to eventually

increase liver Se. If the study were to be extended, it is predicted that there would be greater divergence between both bolus treatments and CON. In agreement with the present study, Hidiroglou et al. (1987) did not observe any difference in liver Se concentrations between cows given a ruminoreticular bolus containing Se and those not receiving a bolus over an 11-mo period. Previous research conducted by Koenig et al. (1997) found that sheep fed a concentrate diet had a greater absorption rate of Se than those receiving a forage diet, and it was suggested that the type of diet can influence Se availability for absorption. This could be a potential explanation as to why an increase in liver Se was not observed in the Rel250 bolus treatment until the cattle were transitioned over to the finishing ration, which contained a greater percentage of concentrates. Finishing diets, like the one used in the second half of this study, would lead to a lower ruminal pH compared with a growing diet, potentially affecting rate of degradation of the bolus. Globally, boluses are a common method of trace mineral supplementation for ruminants (Greene, 1999); however, there is a paucity of data regarding the effectiveness of this supplementation strategy and the effect of dietary changes on release of minerals from boluses.

## Zinc

**Plasma.** There was a treatment  $\times$  hour interaction ( $P < 0.001$ ) detected for plasma concentrations of Zn (Figure 4). At h 8, ITM steers had greater plasma Zn concentrations than all other treatments, plasma Zn concentrations were greater in MMD compared with CON, and all other treatments had intermediate plasma Zn concentrations between MMD and CON. By h 24 ITM steers displayed similar plasma Zn concentrations as other treatments and continued to follow this trend to h 48, similar to in the work by Pogge et al. (2012). Steers treated with MMD exhibited greater plasma Zn concentrations than CON at 8 h after treatment, possibly due to the greater dose of Zn provided in the MMD product (Table 2). The Rel250 bolus apparently did not release sufficient Zn to affect plasma Zn concentrations within 48 h of administration.

**Liver.** No treatment  $\times$  day or treatment effects on liver Zn were noted ( $P \geq 0.24$ ; data not shown). A day effect was detected ( $P < 0.0001$ ) where d-2 liver Zn concentrations were greater than all other days, and d-15 liver Zn was greater than all other sampling days excluding d 2 (Figure 1B). It is speculated that the liver Zn increase across all treatments, including those treatments not containing Zn, could be partially explained by inflammation arising from multiple blood draws and administration of the treatments. Though no measures of inflammation were assessed in the present study, others have established a connection between inflammation and an increase in liver Zn (Liuzzi et al., 2005; Tanaka et al., 2014). Although liver Zn is a valuable depot for body Zn, no strong biomarker for Zn status in cattle has yet been identified (Mills, 1987).



**Figure 3.** Effects of multiple trace mineral supplements on liver concentrations of Se over a 120-d postinjection period. Treatments were saline (CON), Multimin90 (ITM; Multimin USA, Fort Collins, CO), Mineral Max Drench (MMD; Aspen Veterinary Resources Ltd., Liberty, MO), Mineral Max Paste (MMP; Aspen Veterinary Resources Ltd.), Starting Fluid Drench (SFD; Kentucky Nutrition Service, Lawrenceburg, KY), Se365 bolus (Se365; Pacific Trace Minerals Inc., Ashland, OR), and Reloader250 bolus (Rel250; Provimi North America Inc., Brookville, OH). Differing letters (a, b) within an hour indicate a difference ( $P \leq 0.05$ ). The d-0 values served as covariates in analysis. The asterisk denotes the average starting liver Se concentration across treatments. Values are  $SEM \pm 0.13$ .

### Manganese

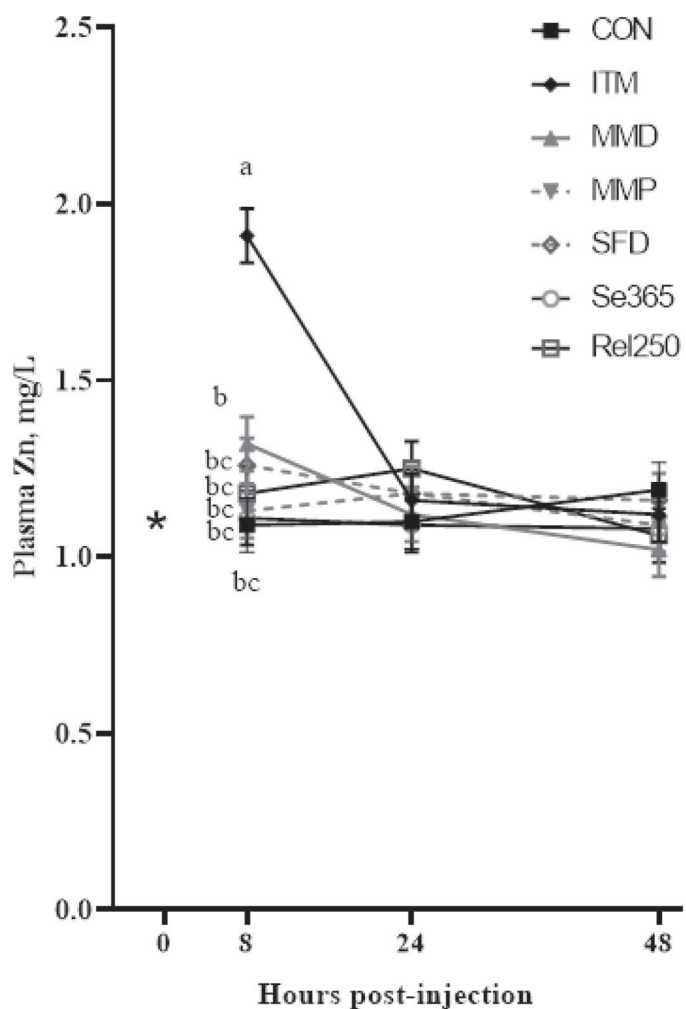
**Plasma.** There was a treatment  $\times$  hour interaction ( $P < 0.0001$ ) for plasma Mn concentrations (Figure 5). At 8 and 24 h, ITM had greater plasma Mn concentrations than all other treatments before returning to baseline at h 48. All other treatments showed no change in plasma Mn concentrations and were consistent with CON. The effects of ITM are similar to those found by Pogge et al. (2012), where plasma Mn concentrations in growing steers receiving the same injectable trace mineral product were greater than those in saline-injected steers through 10 h after injection. Even though the oral treatments all provided Mn, there was no effect on plasma Mn concentrations from the oral treatments compared with CON. The absorption rate of Mn by the gastrointestinal tract is very low, approximately 5% (Mena, 1981), which is likely why no differences were detected in plasma concentrations of Mn. Plasma Mn concentrations are tightly regulated by homeostatic mechanisms (Weiss and Socha, 2005), and it is unsurprising that only ITM altered these concentrations and only for a short period of time.

**Liver.** There was no treatment  $\times$  day or treatment effect ( $P \geq 0.32$ ; data not shown) on liver concentrations of Mn. A day effect ( $P < 0.0001$ ) was detected where d-2 liver Mn was lesser than all other days; d 65, 91, and 120 were greater in liver Mn than all other days; and concentrations of liver Mn on d 15, 29, and 49 were intermediate (Figure 1C). The day effect was partially due to the unexpected decrease in d-2 liver Mn concentrations in all treatments except for CON. There appears to be a decrease in liver Mn when steers receive a large amount of mineral, regardless of mineral supplementation route (injection, oral, or bolus), dosage, or which minerals were present in the product. Interestingly, Hansen and Jackson (unpublished data) previously noted a very similar response where a decrease in liver Mn on d 2 was observed in steers receiving injectable trace mineral. It is intriguing that a similar effect was noted not only in the ITM treatment but in all treatments containing trace minerals in the present study. Trace minerals can be pro-oxidant if not safely bound to a chaperone or storage protein in the body (Weisinger and Fridovich, 1973). The decrease in liver Mn may suggest an increase in antioxidant activity in response to a large amount of mineral delivered to the body as Mn is critical to the antioxidant enzyme Mn-superoxide dismutase (Paynter, 1980), though Mn superoxide dismutase activity was not assessed in the present study.

Similar to Zn, there are challenges when assessing Mn status of cattle. Underwood and Suttle (1999) reported that even at very high dietary concentrations of Mn, there are minimal differences in liver Mn concentrations. Though some suggest that liver Mn is a sufficient indicator of Mn status in cattle (Steger and Loeck, 1972), the liver does not possess a long-term storage mechanism, making it difficult to accurately assess Mn status (Hidiroglou, 1979). Up to 98% of Mn absorbed in the gastrointestinal tract is cleared from the liver and incorporated into bile (Papavasiliou et al., 1966), where Mn status is then controlled by re-uptake of Mn from bile into the liver by the membrane-bound Mn transporter ZIP8 (Lin et al., 2017). This may be why liver Mn is largely unaffected by the trace mineral treatments examined here. The increase in liver Mn across days on study may reflect decreasing need for Mn in the body for growth processes, as Mn is particularly important in proteoglycan formation in skeletal development (Suttle, 2010). However, Mn metabolism and requirements in feedlot cattle are poorly understood and require further study.

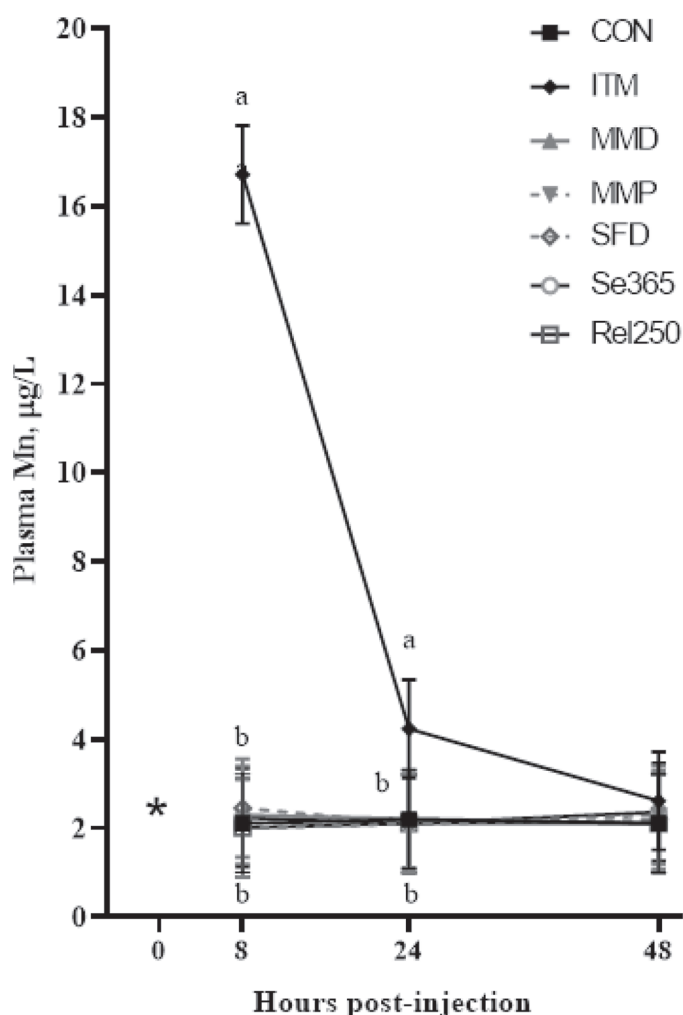
### APPLICATIONS

These results lend new insight into the use of single-use, pulse-dose trace mineral supplements. Combined with previous results, it appears the injectable trace mineral supplement studied here, in mineral-adequate cattle, is an effective method of increasing Se, Mn, and Zn concentrations in plasma (24 h) and liver Se (through d 29). If cattle coming into a feedlot are in a mineral deficient state, a



**Figure 4.** Effects of multiple trace mineral supplements on plasma concentrations of Zn over a 48-h postinjection period. Treatments were saline (CON), Multimin90 (ITM; Multimin USA, Fort Collins, CO), Mineral Max Drench (MMD; Aspen Veterinary Resources Ltd., Liberty, MO), Mineral Max Paste (MMP; Aspen Veterinary Resources Ltd.), Starting Fluid Drench (SFD; Kentucky Nutrition Service, Lawrenceburg, KY), Se365 bolus (Se365; Pacific Trace Minerals Inc., Ashland, OR), and Reloader250 bolus (Rel250; Provimi North America Inc., Brookville, OH). Differing letters (a–c) within an hour indicate a difference ( $P \leq 0.05$ ). The h-0 values served as covariates in analysis. The asterisk denotes the average starting plasma Zn concentration across treatments. Values are  $SEM \pm 0.08$ .

rapid increase in trace minerals may assist in restoring mineral reserves. A bolus may be of value if more gradual increases in liver trace mineral concentrations are desired; approximately 120 d were required before boluses began to increase liver concentrations of certain trace minerals. Dietary composition and initial trace mineral status may affect effectiveness of boluses in changing cattle mineral status. Additionally, while 8 steers per treatment is adequate to denote treatment effects with larger magnitude changes such as those caused by injectable mineral, it is possible this is insufficient to pick up more subtle increases in liver mineral over time such as those induced by the boluses.



**Figure 5.** Effects of multiple trace mineral supplements on plasma concentrations of Mn over a 48-h postinjection period. Treatments were saline (CON), Multimin90 (ITM; Multimin USA, Fort Collins, CO), Mineral Max Drench (MMD; Aspen Veterinary Resources Ltd., Liberty, MO), Mineral Max Paste (MMP; Aspen Veterinary Resources Ltd.), Starting Fluid Drench (SFD; Kentucky Nutrition Service, Lawrenceburg, KY), Se365 bolus (Se365; Pacific Trace Minerals Inc., Ashland, OR), and Reloader250 bolus (Rel250; Provimi North America Inc., Brookville, OH). Differing letters (a, b) within an hour indicate a difference ( $P \leq 0.05$ ). The h-0 values served as covariates in analysis. The asterisk denotes the average starting plasma Mn concentration across treatments. Values are  $SEM \pm 1.11$ .

Future work should expand cattle numbers assessing bolus treatments to determine the most effective place for these products in cattle production systems. Overall, the oral products assessed in the present study had no effect on plasma or liver trace mineral concentrations of trace mineral-adequate cattle, suggesting oral, single, pulse-dose products providing only daily recommendations for minerals are ineffective. Further work is necessary to determine whether a combination of these treatments, such as an injectable trace mineral dose and an oral bolus, could improve and maintain adequate trace mineral status of cattle over a longer period.



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