Effect of an injectable trace mineral at the initiation of a 14 day CIDR protocol on heifer performance and reproduction[1]

R. S. Stokes, A. R. Ralph, A. J. Mickna, W. P. Chapple, A. R. Schroeder, F. A. Ireland, and D. W. Shike[2]

Department of Animal Sciences, University of Illinois, Urbana 61801

ABSTRACT: Three experiments were conducted at separate locations to determine the effects of a trace mineral injection (TMI), Multimin 90, on heifer performance and reproduction. In Exp. 1, [spring-born, Angus, n = 93, body weight (BW) = 428 ± 45.2 kg], Exp. 2 (spring-born, Angus × Simmental, n = 120, BW = 426 ± 54.0 kg), and Exp. 3 (fall-born, commercial Angus, n = 199, BW = 345 ± 39.7 kg) heifers were stratified by BW within experiment and assigned to 1 of 2 treatments: a control, saline injection, or TMI at a dose of 1 mL/68 kg BW. Free choice mineral, containing Cu, Mn, Se, and Zn formulated to meet or exceed NRC recommendations, was supplemented to heifers. Injections were given 33 d prior to breeding at the initiation of a 14-d controlled internal drug release (CIDR)-prostaglandin protocol. There was no difference ($P \geq 0.37$) in BW during Exp. 1. Additionally, there was no difference ($P \geq 0.52$) in body condition score (BCS) at initiation or at artificial insemination (AI) and final pregnancy confirmation in Exp. 1; however, a greater ($P = 0.03$) BCS was noted for control heifers at breeding. Pregnancy rates to timed AI and overall pregnancy rates were also similar ($P \geq 0.74$) regardless of treatment. During Exp. 2, BCS and BW did not differ ($P \geq 0.44$) across treatments. There was a tendency ($P = 0.07$) for TMI heifers to have an increased AI pregnancy rate (62 vs. 45%) compared with control heifers despite no difference ($P = 0.51$) in overall pregnancy rate. In Exp. 3, BW was not different ($P \geq 0.39$) across all time points. Also, BCS did not differ ($P \geq 0.45$) at initiation, AI, or final pregnancy conformation. Interestingly, there was a tendency ($P = 0.10$) for TMI heifers to have an increased BCS at the time of breeding compared with control heifers. However, there were no differences ($P \geq 0.50$) in AI and overall pregnancy rates. In 1 of 3 experiments, an injectable trace mineral administered 33 d prior to the breeding season in conjunction with a 14-d CIDR protocol, tended to increased AI conception rates of heifers even when adequate trace mineral supplement was provided. The variable response observed across experiments may be caused by differences in breed, calving season, mineral sources, and management strategies.

Key words: artificial insemination, beef heifer, injectable trace mineral, pregnancy rate, reproduction

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INTRODUCTION

Trace minerals such as copper, manganese, selenium, and zinc play critical roles in biochemical processes and are key components of a ruminant animal's health and productivity (Suttle, 2010). Grazing cattle primarily receive trace minerals through forages; however, these sources often do not meet cattle requirements due to variation in soil composition (Smart et al., 1981). In these instances, producers commonly supplement through free-choice mineral, salt blocks fortified with trace minerals, or protein/energy supplements fortified with trace minerals (Arthington et al.,

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[2]Corresponding author: dshike@illinois.edu

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2014). Bioavailability of trace mineral sources can vary due to interactions with other minerals and feed components within the gastrointestinal tract (Spears, 2003). An injectable trace mineral provides the opportunity to supplement trace minerals that completely bypass the gastrointestinal tract and thus avoid the complex ruminal interactions. Also, a multi-element injectable trace mineral allows for targeted delivery of a specific amount to individual animals (Arthington et al., 2014) and eliminates the variability associated with voluntary intake of free choice mineral (Arthington and Swenson, 2004).

Pogge et al. (2012) recently demonstrated trace mineral injections are an effective way to increase the trace mineral status of calves, particularly Cu and Se. Increased mineral status may be of particular importance when biological needs are increased, such as breeding. In some research, an injectable trace mineral has improved reproductive performance. Sales et al. (2011) reported injectable trace mineral supplementation increased conception rates and chance of embryo survival after timed embryo transfer. Additionally, Kirchhoff (2015) reported an injectable trace mineral increased heifer artificial insemination (AI) conception rate. Multimin 90 is labeled for administration 30 d prior to breeding, which coincides with the initiation of a 14-d controlled internal drug release (CIDR) prostaglandin (PG) protocol. From a practical management standpoint, this allows for the administration of injectable trace mineral without any additional handling. Therefore, the objective of these experiments was to assess the effects of an injectable trace mineral administered at the initiation of a 14-d CIDR protocol on heifer performance and reproduction.

MATERIALS AND METHODS

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois (IACUC #16046) and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animal in Agricultural Research and Teaching (FASS, 2010).

Experiment 1

Animals and Experimental Design. Spring-born, Angus heifers [Year 1: initial body weight (BW) = 430 ± 489.9 kg, *n* = 40; Year 2: initial BW = 425 ± 40.5 kg, *n* = 53] housed at the University of Illinois Beef Cattle and Sheep Field Laboratory in Urbana, IL were utilized to assess the effects of an injectable trace mineral (Multimin 90, Multimin USA, Fort Collins, CO) on heifer reproduction and performance over a 2-yr period. Heifers were stratified by BW and assigned to 1 of 2 treatments: either an injection with sterilized saline (CON1) or an injection of Multimin 90 (MM1) administered subcutaneously at a dose of 1 mL/68 kg BW. The Multimin 90 contained 60 mg/mL of zinc as zinc oxide, 10 mg/mL of manganese as manganese carbonate, 5 mg/mL of selenium as sodium selenite, and 15 mg/mL of copper as copper carbonate. Prior to the start of the trial, heifers were weaned and developed on a diet consisting of roughage, corn co-products, and supplement. Heifers were then adapted to a total mixed ration (TMR, Table 1) that included both inorganic and organic trace mineral, and remained on the same diet through the initiation of the trial. In year 1 heifer initial BW was collected on d 0 (April 2015) and heifers were enrolled in a 14-d CIDR (Pfizer Animal Health, New York, NY) insert – PG and timed AI protocol (Mallory et al., 2012). On d 19 heifers were transported to pasture where they grazed 70% endophyte-infected fescue (*Festuca arundinacea*), and 30% red (*Trifolium pretense*) and white clover (*Trifolium repens*) pastures (60.91% NDF, 32.28% ADF, and 13.04% CP). While on pasture, heifers had access to free choice mineral [19.7%

Table 1. Ingredient composition of heifer diets (% DM basis) for Exp. 1

	Inclusion, % DM	
Item	Year 1	Year 2
Ingredient, %		
Corn silage	72	60
MDGS[1]	13	15
Treated corn stalks[2]	10	–
Grass hay	–	20
Supplement[3,4]	5	5
Analyzed nutrient content		
CP, %	10.1	9.9
NDF, %	51.1	50.7
ADF, %	28.9	27.7
Crude fat, %	2.9	3.5
S, %	0.18	0.20
Cu, mg/kg	4.8	13.6
Mn, mg/kg	29.3	29.1
Zn, mg/kg	21.9	36.6

[1]Experiment 1 determined the effects of a TMI on spring born Angus × Simmental heifer performance and reproduction.

[2]Modified distillers grains with solubles.

[3]Corn stalks were treated with Silage SAVOR Plus (Kemin Industries, Inc., Des Moines, IA) at 0.5 kg Mg^{-1} applied at bagging.

[4]Supplement contained 87.7% ground corn, 8.9% limestone, 1.8% trace mineral salt [8.5% Ca as calcium carbonate, 5% Mg as magnesium oxide and magnesium sulfate, 7.6% K as potassium chloride, 6.7% Cl as potassium chloride, 10% S as S8, prilled, 0.5% Cu as copper sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN), 2% Fe as iron sulfate, 3% Mn as manganese sulfate and Availa-4, 3% Zn as zinc sulfate and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as calcium iodate, 150 mg/kg Se as sodium selenite, 2,205 KIU/kg VitA as retinyl acetate, 662.5 KIU/kg VitD as cholecalciferol, 22,047.5 IU/kg VitE as DL-α-tocopheryl acetate, and less than 1% crude protein, fat, crude fiber, salt], 0.1% Rumensin 90 (198 g monensin/kg, Rumensin 90; Elanco Animal Health, Greenfield, IN), and 1.5% fat.

limestone, 19.7% trace mineral salt (8.5% Ca as calcium carbonate, 5% Mg as magnesium oxide and magnesium sulfate, 7.6% K as potassium chloride, 6.7% Cl as potassium chloride, 10% S as S8, prilled, 0.5% Cu as copper sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN), 2% Fe as iron sulfate, 3% Mn as manganese sulfate and Availa-4, 3% Zn as zinc sulfate and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as calcium iodate, 150 mg/kg Se as sodium selenite, 2,205 KIU/kg VitA as retinyl acetate, 662.5 KIU/kg VitD as cholecalciferol, 22,047.5 IU/kg VitE as DL-α-tocopheryl acetate, and less than 1% crude protein, fat, crude fiber, salt), 14.2% salt, 40.6% monocalcium phosphate, 4.2% dried molasses, 1.4% zinc sulfate, and 0.01% iodine]. Heifers were weighed, body condition scored (BCS), and AI on d 33. Ten d following AI, heifers were exposed to 1 bull that had previously passed a breeding soundness exam, for a 64 d breeding season. Artificial insemination conception rates, BCS, and BW were collected on d 70 and final BCS, weights, and overall pregnancy confirmation were collected on d 130. A trained technician determined artificial insemination conception and overall pregnancy rates via ultrasonography (Aloka 500, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

In year 2, heifers were managed similarly with the following exceptions. At the initiation of the trial heifer BCS was measured. Ten d following AI heifers were divided into 2 groups with equal treatment representation in each group and exposed to 1 bull/group that had previously passed a breeding soundness exam. On d 60 (17 d after heifers had been exposed to bulls) 1 bull had to be removed from the study, so heifers were again co-mingled and placed with 1 bull that had passed a breeding soundness exam. Final BCS, BW, and pregnancy confirmation were collected on d 145.

Sample Collection and Analytical Procedures. For nutrient composition analysis, individual feed ingredients were collected monthly and composited and dried at 55°C for a minimum of 3 d and ground through a 1-mm screen using a Wiley mill (Arthur, H. Thomas, Philadelphia, PA). Forage samples were collected from pastures on a monthly basis and composited and dried similarly. Ground feed samples were analyzed for CP (Leco TruMac, LECO Corporation, St. Joseph, MI), NDF and ADF using an Ankom 200 Fiber Analyzer (Ankom Technology, Macedon, NY), and crude fat using an Ankom XT10 fat extractor (Ankom Technology). Ground forage samples were also analyzed for NDF, ADF, and CP. Forage and feed samples were also sent to a commercial lab where they were subjected to nitric acid digestion and inductively coupled plasma spectroscopy analysis for complete minerals (method 975.03: AOAC, 1988; The Ohio State University, Service Testing and Research Lab, Wooster, OH).

Statistical Analysis. Body weight and BCS were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Artificial insemination rates and overall pregnancy rates were analyzed using the GLIMMIX procedure of SAS. Final pregnancy confirmation data included bull in the model; however, this was not significant (P = 0.57), so bull was removed from the model. Heifer was considered the experimental unit for all measures. The model included the fixed effect of treatment and the random effect of year. Significance was declared at $P \leq 0.05$, and tendencies were declared from $0.05 < P \leq 0.10$. Means reported in tables are least squares means ± SEM.

Experiment 2

Animals and Experimental Design. To determine the effects of an injectable trace mineral on heifer reproduction and performance over a 2-yr period, spring-born, Angus × Simmental heifers (Year 1: initial BW = 411 ± 48.1 kg, n = 65; Year 2: initial BW = 441 ± 59.8 kg, n = 55) were utilized. Heifers were either 0.5 Angus and 0.5 Simmental or 0.625 Angus and 0.375 Simmental. Cattle were stratified by BW into 2 groups and randomly assigned either a sterilized saline injection (CON2) or a Multimin 90 injection (MM2) at a rate of 1 mL/68 kg BW. In yr 1, prior to the start of the trial, heifers were housed at the University of Illinois Beef Cattle and Sheep field Laboratory in Urbana, IL and fed a diet consisting of alfalfa haylage, corn silage, corn co-products, and supplement. Heifers were then adapted to a TMR (Table 1) and remained on this diet until d –13, which included both an organic and inorganic trace mineral supplement. On d –13 heifers were transported to the Orr Agricultural Research and Demonstration Center in Baylis, IL. Heifers were housed in soil surface pens and offered a TMR and an inorganic trace mineral supplement (Table 2). Intake averaged 7.1 kg of DM/d per heifer across treatments. At the initiation of the study (April, 2015), individual heifer BW were measured, treatments applied, and heifers were enrolled in a 14-d CIDR-PG and timed AI protocol. On d 33 heifers were weighed, BCS, and timed AI. Immediately following timed AI heifers were transported to pasture for the duration of the breeding season. Heifers grazed pastures with an average coverage area of 70% endophyte-infected fescue (*Festuca arundinacea*) and 30% red clover (*Trifolium pretense*) and white clover (*Trifolium repens*, 9.05% NDF, 32.98% ADF, and 11.96% CP) for the remainder of the study and were given access to free choice mineral [12% Ca as calcium carbonate, 8% P, 18%

Table 2. Ingredient composition of heifer diets (% DM basis) for Exp. 2[1]

	Inclusion, % DM	
Item	Year 1	Year 2
Ingredient, %		
Corn silage	24.7	59.0
MDGS[2]	14.14	20.0
Grass hay	61.16	16.0
Supplement[3,4]	–	5.0
Analyzed nutrient content		
CP, %	12.1	10.5
NDF, %	53.9	41.1
ADF, %	33.2	21.0
Crude fat, %	2.3	4.2
S, %	0.27	0.22
Cu, mg/kg	6.4	7.0
Mn, mg/kg	40.2	67.2
Zn, mg/kg	30.0	37.8

[1]Experiment 2 determined the effects of a TMI on spring born Angus × Simmental heifer performance and reproduction.

[2]Modified distillers grains with solubles.

[3]In year 1 supplement was top dressed at a rate of 0.11 kg/heifer per d (23.4% Ca as calcium carbonate, 15.7% salt, 1.0% Mg as magnesium oxide, 3,500 mg/kg of Zn as zinc sulfate, 3,350 mg/kg of Cu as copper sulfate, 26.4 mg/kg of Se as sodium selenite, 181,437 IU/kg of vitamin A, and 181 IU/kg of vitamin E).

[4]Supplement contained 87.7% ground corn, 8.9% limestone, 1.8% trace mineral salt [8.5% Ca as calcium carbonate, 5% Mg as magnesium oxide and magnesium sulfate, 7.6% K as potassium chloride, 6.7% Cl as potassium chloride, 10% S as S8, prilled, 0.5% Cu as copper sulfate and Availa-4 (Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN), 2% Fe as iron sulfate, 3% Mn as manganese sulfate and Availa-4, 3% Zn as zinc sulfate and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as calcium iodate, 150 mg/kg Se as sodium selenite, 2,205 KIU/kg VitA as retinyl acetate, 662.5 KIU/kg VitD as cholecalciferol, 22,047.5 IU/kg VitE as DL-α-tocopheryl acetate, and less than 1% crude protein, fat, crude fiber, salt], 0.1% Rumensin 90 (198 g monensin/kg, Rumensin 90; Elanco Animal Health, Greenfield, IN), and 1.5% fat.

salt, 11% Mg as magnesium sulfate, 90 mg/kg I as calcium iodate, 108,862 IU/kg vitamin A, 18,144 IU/kg vitamin D_3, 454 IU/kg vitamin E, and 5,600 mg/kg of chlortetracycline (Aureomycin; Alpharma Inc. Animal Health, Bridgewater, NJ)]. Ten days following AI, a bull was placed with each pen of heifers for a 45 d breeding season. Artificial insemination conception rates, BW, and BCS were determined on d 70. Overall pregnancy rate, BW, and BCS were collected on d 144. A trained technician determined artificial insemination conception and overall pregnancy rates via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

In year 2, heifers were managed similarly with the following exceptions. The University of Illinois Beef Cattle and Sheep field Laboratory in Urbana, IL was used to house heifers from weaning until d 10 of the trial. On d 10, heifers were transported to the Orr Agricultural Research and Demonstration Center in Baylis, IL and were housed in soil surface pens and received a similar diet and trace mineral supplement (Table 2). Average heifer DMI was 8.5 kg/d per heifer across treatments. At the time of CIDR removal, all heifers' tail heads were painted and paint scores (1 = completely gone, 2 = partially gone, and 3 = untouched) were collected at the time of breeding. Heifers were managed in 2 groups and 10 d following AI were exposed for a 46 d breeding season to 1 bull per group that had previously passed a breeding soundness exam. Overall pregnancy rate, BW, and BCS were collected on d 145.

Sample Collection and Analytical Procedures. Feed ingredients and forage samples were collected and analyzed as described in Exp. 1.

Statistical Analysis. Data were analyzed as described in Exp. 1 with the following exception that group was included as a fixed effect. Treatment distributions of tail paint scores were determined using PROC GLIMMIX of SAS.

Experiment 3

Animals and Experimental Design. Fall-born, commercial Angus (*n* = 199, initial BW = 345 ± 39.7 kg) were utilized to determine the effects of an injectable trace mineral on heifer BW, BCS, and reproductive performance. Heifers were fed and managed at the Dixon Springs Agricultural Research Center, Simpson, IL. Body weight and sire were used to stratify cattle. Heifers were utilized previously in another study and thus heifers were also stratified by previous treatment (Kordas et al., 2017). On d 0 (October, 2015), initial BW and BCS were collected, heifers were synchronized with a 14-d CIDR-PG timed AI protocol as previously described by Mallory et al. (2012), and administered 1 of 2 treatments: a control sterile saline injection (CON3) or a Multimin 90 injection (MM3) both administered at a rate of 1 mL/68 kg of BW. Heifers were placed on pastures following AI, managed as a single group, and grazed 70% endophyte infected fescue (*Festuca arundinacea*) and 30% red clover (*Tri-folium pretense*) pastures (60.53% NDF, 34.41% ADF, and 9.81% CP). Heifers were offered a supplement consisting of 50% soybean hulls (62.53% NDF, 45.74% ADF, and 11.26% CP) and 50% corn gluten feed pellets (33.65% NDF, 9.19% ADF, and 23.77% CP) at a rate of 2.7 kg/heifer per d. Free choice loose mineral [Renaissance Nutrition, Roaring Springs, PA; 0.16% S, 17.88% Ca as calcium carbonate, 2.99% P as monocalcium phosphate, 24.5% salt, 9.35% Na, 5.74% Mg as magnesium oxide, 0.06% K, 2,214 mg/kg Fe as iron oxide, 2,013 mg/kg Mn as manganese hydroxychloride (Intellibond M, Micronutrients Inc., Indianapolis, IN), 2511 mg/kg Zn as hydroxyl zinc (Intellibond Z, Micronutrients Inc.), 1,001 mg/kg Cu as tribasic copper chloride (Intellibond C, Micronutrients Inc.), 27 mg/kg Co as cobalt carbonate, 36 mg/kg I as calcium iodate, 26 mg/kg Se as sodium selenite, 110,178 IU/kg vitamin A, 3,084 IU/kg vitamin D, 545 IU/kg vitamin E, and 1,179 mg/kg of chlortetracycline] was offered ad libitum to heifers.

On d 30, heat detection patches (Estrotect Heat Detectors, Rockway Inc., Spring Valley, WI) were placed on all heifers. On d 33 heifers were weighed, BCS, AI and heat patches were visually scored from 0 to 3 (0 = missing, 1 = fully activated, 2 = partially activated, and 3 = not activated). Nine days following AI, heifers were exposed to 5 yearling bulls that had all passed breeding soundness exams, for a 71 d breeding season. Due to limited pasture forage availability, on d 71 heifers were offered free choice grass hay (68.7% NDF, 39.9% ADF, and 6.01% CP) for the remainder of the trial. On d 76 individual BW, BCS, and AI pregnancy conception rates were collected. Overall conception rates and final BW and BCS were collected on d 153. A trained technician determined AI conception and overall pregnancy rates via ultrasonography (Aloka 500 instrument, Hitachi Aloka Medical America, Inc., Wallingford, CT; 7.5 MHz general purpose transducer array).

Sample Collection and Analytical Procedures. Feed ingredients and forage samples were collected and analyzed as described in Exp. 1.

Statistical Analysis. Heifer BW and BCS were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Heifer AI conception and overall pregnancy rates were analyzed using the GLIMMIX procedure of SAS. Treatment distributions of heat patch scores were determined using PROC GLIMMIX of SAS. The model included the fixed effects of treatment. Treatment effects were considered significant at $P \leq 0.05$ and tendencies were noted at $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

All heifers received the same diet (Table 1) or pasture regardless of treatment. There was no difference ($P \geq 0.37$, Table 3) for heifer BW at any time point. Also, there was no difference ($P \geq 0.52$) for heifer BCS at the initiation of the trial, AI pregnancy confirmation, or final pregnancy confirmation regardless of treatment. Interestingly, CON1 heifers had greater ($P = 0.03$) BCS at breeding than their MM1 treated counterparts. Pregnancy rates to timed AI and overall pregnancy rates were similar for CON1 and MM1 treated heifers ($P \geq 0.74$, Fig. 1).

Table 3. Influence of an injectable trace mineral on heifer BW and BCS over 2 consecutive years in Exp. 1

	Treatment[1]			
Item	CON1	MM1	SEM	*P*-value
n, heifer	47	46		
BW, kg				
Initial	429	426	4.6	0.72
Breeding	425	424	6.4	0.92
AI pregnancy confirmation	433	418	6.5	0.37
Final pregnancy confirmation	437	436	16.2	0.78
BCS				
Initial	5.4	5.4	0.07	0.52
Breeding	5.7	5.5	0.17	0.03
AI pregnancy confirmation	5.5	5.5	0.22	0.86
Final pregnancy confirmation	5.5	5.6	0.22	0.64

[1]Control (CON1) cattle received a sterilized saline solution at 1 mL/68 kg BW, and Multimin 90 (MM1) cattle received injectable trace mineral at 1 mL/68 kg BW.

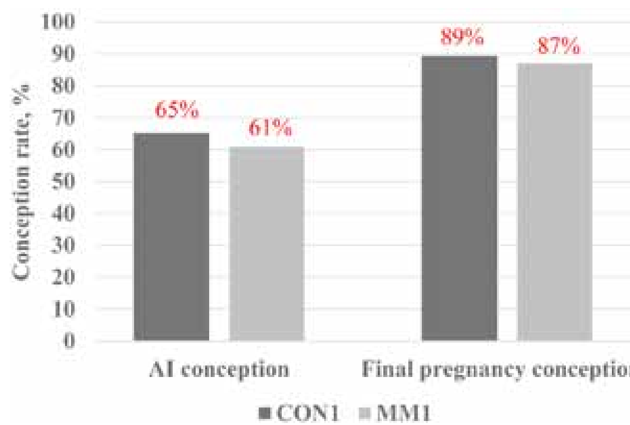

Figure 1. The effect of an injectable trace mineral (Multimin 90, MM1) or sterilized saline solution (CON1) at the initiation of a synchronization protocol on heifer AI and final pregnancy rates over 2 consecutive years in Exp. 1. Pregnancy rates to timed AI and overall pregnancy rates were similar for CON1 and MM1 treated heifers ($P \geq 0.74$).

Experiment 2

All heifers received the same diet (Table 2) or pasture regardless of treatment. Heifer BW and BCS did not differ across all time points ($P \geq 0.44$, Table 4). Additionally, the distribution of tail paint scores was similar ($P \geq 0.90$) across treatments. There was a tendency ($P = 0.07$, Fig. 2) for MM2 heifers to have greater AI pregnancy rates (62.1%) compared with their CON2 (45.2%) counterparts. However, by the time of overall pregnancy confirmation there was no difference ($P = 0.51$) for pregnancy rates regardless of treatment and overall pregnancy rate averaged 82.7%.

Experiment 3

Heifer BW did not differ across all time points ($P \geq 0.39$, Table 5). Additionally, heifer BCS did not dif-

Table 4. Influence of an injectable trace mineral on heifer BW, BCS, and tail paint score over 2 consecutive years in Exp. 2

	Treatment[1]			
Item	CON2	MM2	SEM	*P*-value
n, heifer	60	60		
BW, kg				
Initial	427	426	15.1	0.97
Breeding	413	413	10.3	0.96
AI pregnancy confirmation	432	427	9.3	0.44
Final pregnancy confirmation	434	435	27.5	0.81
BCS				
Initial	5.5	5.5	0.09	0.58
Breeding	5.8	5.8	0.09	0.78
AI pregnancy confirmation	5.6	5.6	0.14	0.80
Final pregnancy confirmation	5.5	5.5	0.17	0.58
Tail paint score[2,3], %				
1	25	23	–	0.90
2	75	74	–	0.95
3	–	–	–	–

[1]Control (CON2) cattle received a sterilized saline solution at 1 mL/68 kg BW, and Multimin 90 (MM2) cattle received injectable trace mineral at 1 mL/68 kg BW.

[2]Tail paint scores were visually assessed at the time of breeding (1 = completely gone, 2 = partially gone, 3 = untouched).

[3]Tail paint scores were not collected in year 1.

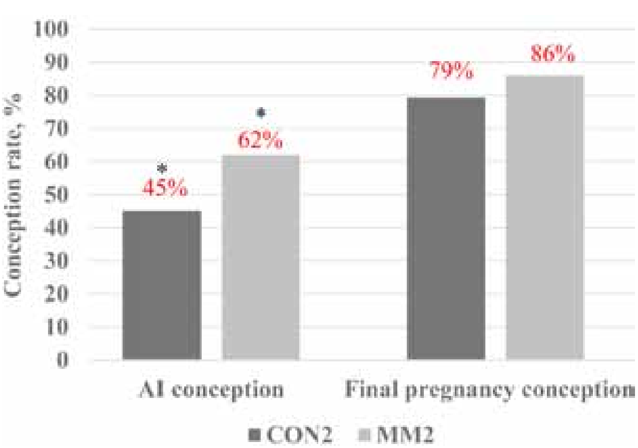

Figure 2. The effect of an injectable trace mineral (Multimin 90, MM2) or sterilized saline solution (CON2) at the initiation of a synchronization protocol on heifer AI and final pregnancy rates over 2 consecutive years in Exp. 2. * indicates a tendency ($P = 0.07$) for MM2 AI conception rates to be greater than CON2 heifers. Overall pregnancy rates were similar for CON2 and MM2 treated heifers ($P = 0.51$).

fer ($P \geq 0.45$) at the initiation of the trail or at AI and final pregnancy confirmation. However, there was a tendency ($P = 0.10$) for MM3 heifers to have a greater BCS at the time of breeding than their CON3 counterparts. There were also no differences ($P \geq 0.50$) across treatments, in the distribution of heat patch scores for Exp. 3. Pregnancy rates to timed AI and overall pregnancy rates were also similar for both CON3 and MM3 treated heifers ($P \geq 0.50$, Fig. 3).

Table 5. Influence of an injectable trace mineral on heifer BW, BCS, and heat patch scores in Exp. 3

	Treatment[1]			
Item	CON3	MM3	SEM	*P*-value
n, heifer	99	100		
BW, kg				
Initial	344	344	2.8	0.93
Breeding	347	348	2.9	0.79
AI pregnancy confirmation	346	348	3.1	0.76
Final pregnancy confirmation	374	370	3.3	0.39
BCS				
Initial	5.4	5.4	0.05	0.95
Breeding	5.6	5.7	0.05	0.10
AI pregnancy confirmation	5.0	5.1	0.05	0.45
Final pregnancy confirmation	4.9	4.9	0.05	1.00
Heat Patch Score[2], %				
1	58	59	–	0.89
2	17	20	–	0.59
3	25	21	–	0.50

[1]Control (CON3) cattle received a sterilized saline solution at 1 mL/68 kg BW, and Multimin 90 (MM3) cattle received injectable trace mineral at 1 mL/68 kg BW.

[2] Heat patches were visually scored at time of breeding from 0– 3 (0 = missing, 1 = fully activated, 2 = partially activated, 3 = not activated). No heifers were missing heat patches at the time of breeding.

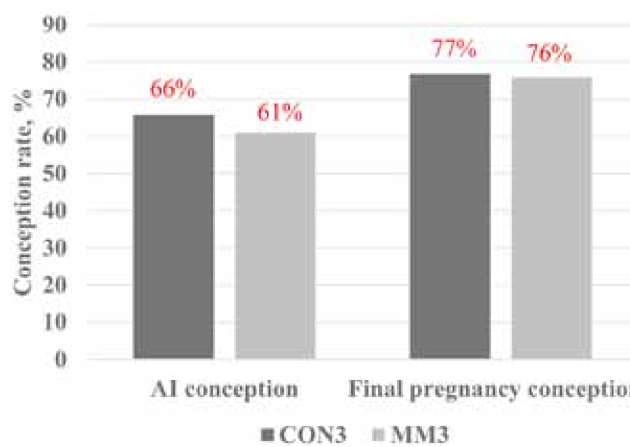

Figure 3. The effect of an injectable trace mineral (Multimin 90, MM3) or sterilized saline solution (CON3) at the initiation of a synchronization protocol on heifer AI and final pregnancy rates in Exp. 3. Pregnancy rates to timed AI and overall pregnancy rates were also similar for both CON3 and MM3 treated heifers ($P \geq 0.50$).

DISCUSSION

For all experiments, trace mineral injection did not affect heifer BW at any time point. Also, heifer BCS was similar at initiation, AI pregnancy confirmation, and final pregnancy confirmation regardless of treatment across these experiments. Since diet and pasture availability were the same for both treatments, regardless of experiment, it is likely this did not impact the results. These results are consistent with Gadberry and Baldridge

(2013), where BW and BCS did not differ for Angus cows receiving either an injectable trace mineral or no injectable trace mineral prior to breeding. However, the effects of injectable trace mineral supplementation on cow BW and BCS has been inconsistent across literature. Arthington et al. (2014) reported in beef heifers, those administered an injectable trace mineral tended to have greater ADG compared with heifers given saline. Still, it is important to note the heifers utilized by Arthington et al. (2014) were administered treatments at weaning and heifers remained on study through breeding, for a total of 177 d. In the current experiments, treatments were administered 33 d prior to breeding, at estrus synchronization, and heifers remained on study until the time of final pregnancy confirmation, making it challenging to draw comparisons across these experiments.

Interestingly, in Exp. 1, CON1 heifers tended to have a greater BCS at the time of breeding than their MM1 counterparts. Contrastingly in Exp. 3, CON3 heifers tended to have a lesser BCS than MM3 heifers at the time of breeding. It is important to note these differences in BCS were significant due to a small standard error (0.172 and 0.048 for Exp. 1 and Exp. 3, respectively) and it is likely this difference is physiologically insignificant. Still, other authors have noted decreased performance in heifers treated with an injectable trace mineral after stress due to shipping or transportation (Arthington et al., 2014). Arthington et al. (2014) hypothesized this performance decrease is due to an increase in Cu-dependent acute phase protein ceruloplasmin, which can affect nutrient metabolism and animal growth (Johnson, 1997), and could be greater in cattle with an increased Cu status. From a management standpoint, it is unlikely the heifers in the current experiments, had been subjected to any stress that would have increased these acute phase proteins. However, stress was not assessed in this trial so, it is unknown if this may have explained the BCS changes noted at breeding.

In both Exp. 1 and Exp. 3, pregnancy rates to timed AI and overall pregnancy rates were similar for both treatments. In an intensively managed dairy herd fed a TMR that met NRC requirements for trace mineral supplementation, no difference was reported in first-service conception rate when a single injection of trace mineral was administered prior to breeding (Vanegas et al., 2004). While it was physiologically unclear why this may have occurred, this data could suggest additional trace mineral supplementation in a herd provided with NRC recommended levels of trace minerals provides no beneficial effects. The heifers in the current study were provided trace mineral at or above NRC recommendations, suggesting they likely had an adequate mineral status.

It is important to note heifers in Exp. 3 weighed 348 kg at breeding, approximately 53% of the herd's 650 kg average mature BW. The NRC (2016) suggests Bos Taurus heifers should reach puberty at approximately 60% of mature weight. Additionally, Vera et al. (1993) noted heifers in a nutritionally restrictive environment will have an even greater percentage of mature BW at puberty than noted above. Mature cows also in a restrictive environment often weigh less than cows of a similar genotype not maintained in a restrictive environment (Pahnish et al., 1983). These heifers were more nutritionally challenged and thus below their expected percent of mature BW at breeding. The authors speculated the greatest response in trace mineral supplementation would be noted in Exp. 3. However, pregnancy rates to timed AI and overall pregnancy rates were similar across treatments, and there were no differences in the distribution of heat patch scores for Exp. 3. This data suggests all heifers responded similarly to estrus synchronization and there was no difference in the number of heifers exhibiting standing estrus at the time of AI. This is comparable to work by Brasche et al. (2015) who reported no effect of trace mineral injection on overall pregnancy rates or overall AI conception of commercial and purebred Angus heifers synchronized using a 14-d CIDR-PG protocol. Gadberry and Baldridge (2013) also noted no effect on pregnancy rate when Angus cows were treated with either an injectable trace mineral or no injection. Although the authors speculated the greatest response would be noted in this experiment, as heifers were less than the recommended 60% of mature BW, control heifer still had acceptable AI pregnancy rates (66%). Other researchers have reported favorable reproductive success when heifers were bred at 50 to 55% of mature BW (Funston et al., 2012; Gunn et al., 2015).

Exp. 2 heifers treated with a trace mineral injection (TMI) tended to have greater AI pregnancy rates compared with their control counterparts. However, overall pregnancy rates were similar regardless of treatment. Heifers that conceive to AI will not only have an increased probability of weaning more calves in their lifetime but also heavier calves (Burris and Priode, 1958). Additionally, when more heifers conceive to AI, this can result in a more uniform calf crop and a shortened breeding season (Dziuk and Bellows, 1983). Even though all heifers had access to free choice trace mineral during the grazing period and were provided with an organic trace mineral as part of a TMR from weaning until beginning the grazing period, a favorable response ($P = 0.07$) in AI pregnancy rates due to the trace mineral injection was still noted. Since offering free choice mineral can result in inconsistent consumption and erratic intake, it is possible the trace mineral status of MM2 heifers was more optimal than CON2 heifers. However, it is important to note the distribution of tail paint scores was similar across treatments, suggesting all heifers responded similarly to estrus synchronization, and thus conception differences were not due to differences in synchronization response. Interestingly, Kirchhoff (2015) reported a similar increase in pregnancy rate to AI when beef heifers were treated with an injectable trace mineral and noted no differences across treatment in estrous behavior as indicated by estrous detection patches. Mundell et al. (2012) also noted comparable results when heifers were treated with either a trace mineral injection or with sterile saline; heifers that received the trace mineral injection had greater fixed time AI conception rates and overall pregnancy rates were similar between treatments. These data suggest an injectable trace mineral may increase AI conception rates even if dietary trace minerals are meeting heifer's requirements.

However, as with performance parameters, the effects of an injectable trace mineral on reproductive performance has been inconsistent. Brasche et al. (2014a) treated Angus heifers with either a trace mineral or sterile saline injection 33 d prior to AI and reported greater AI and overall pregnancy rates for heifers receiving a trace mineral injection. These differences were noted despite the trace mineral injection having no effect on the liver concentration of Mn, Cu, or Zn. Though, Brasche et al. (2014a) did report an increase in liver Se concentrations of heifers treated with a trace mineral injection. In contrast, Daugherty et al. (2002) improved the Cu status of cows treated with trace mineral injection and vitamin E; however, saw no effect on the conception rate of beef cows compared to saline-treated cows. Brasche et al. (2014b) also noted no difference in AI or overall pregnancy rates when cows were given either a trace mineral or saline injection at breeding. While it is unknown what may be driving this variability across experiments it is possible other factors such as breed differences, management strategies, nutrient status, and potential mineral antagonists are playing a complex role in assessing reproductive performance in cattle supplemented with an injectable trace mineral.

The cattle utilized in Exp. 2 were predominantly Simmental influenced cattle, while those utilized in Exp. 1 were Angus, suggesting breed differences may account for some variability noted. Particularly with Cu, differences have been reported in how Simmental cattle absorb (Ward et al., 1995) and excrete (Gooneratne et al., 1994) this trace mineral, suggesting Simmental may have greater Cu requirements compared with Angus. Additionally, data has suggested Simmental cattle may have differences in liver Mn excretion compared with Angus cattle (Pogge et al., 2012). This data suggests the cattle utilized in Exp. 2, which the literature suggests were genetically already predisposed to a greater trace mineral requirement, could have had an altered trace mineral status at the initiation of the trial, allowing the TMI to improve heifer mineral status at breeding, when trace minerals are of utmost importance. This could help to explain why increased AI conception rates were noted for MM2 heifers over their CON2 counterparts. Unfortunately, since trace mineral status was not assessed in this trial, we are unable to conclude if these Simmental heifers did have a greater need for the trace minerals provided from the trace mineral injection.

Heifers utilized in Exp. 2 were developed in a drylot and then moved to an early grazing season pasture at breeding. The NRC (2016) suggests this management strategy can result in BW loss and greater instances of reproductive failure. The heifers in Exp. 2 lost 13.5 kg from the initiation of the trial to breeding (33 d), suggesting the negative energy balance immediately prior to breeding could have decreased reproductive performance. It is possible that the additional Cu, Mn, Se, and Zn provided to the MM2 treated heifers could have played an important role in maintaining reproductive success. Trace minerals play a crucial role in a ruminant animal's productivity (Suttle, 2010) and can play important roles in hormone synthesis (Paterson and Engle, 2005), which is vital for reproductive success. However, further research is needed to elucidate how trace minerals alter or improve embryo survival when cattle are in a negative energy balance at breeding.

In conclusion, under the conditions of these 3 experiments, an injectable trace mineral administered 33 d prior to the breeding season, in certain management settings, resulted in increased AI conception rates of heifers even when provided adequate trace mineral supplement. Due to the difficulty of assessing trace mineral status of an entire herd, supplementing trace minerals through an injection may be a viable way to ensure a consistent, adequate trace mineral supply to heifers for optimal reproductive performance. The reproductive response across these 3 experiments was variable. However, it is important to note that injectable trace minerals do not appear to incur any negative impacts on heifer performance or reproductive success. Further research is required to substantiate this hypothesis and to further understand the response differences elicited by injectable trace mineral supplementation across herds.

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