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Injectable trace-mineral supplementation improves sperm motility and morphology of young beef bulls[1]

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ABSTRACT

This experiment evaluated effects of supplemental s.c. trace-mineral injections on growth and breeding soundness of bull calves. Weaned bulls (n = 488; initial BW = 308 ± 45 kg, initial age = 203 ± 17 d) of 2 breeds (Angus and Charolais) and originating from 13 ranches in the Great Plains were transported to a common confinement facility and assigned randomly to 2 treatments: (1) s.c. injections of trace mineral (TM) containing 15 mg/mL Cu, 5 mg/mL Se, 10 mg/mL Mn, and 60 mg/mL Zn or (2) s.c. injections of physiological saline (control). Treatments were administered at arrival (d −2 or −1; 1 mL per 45 kg of BW) and on d 90 ± 1 (1 mL per 68 kg of BW). On d 0, bulls were stratified by treatment, breed, and ranch of origin and assigned randomly to 8 pens in which they were fed a growing diet for 225 d. The diet was formulated to promote a 1.5-kg ADG at a DMI of 2.6% of BW and to meet or exceed NRC (2000) requirements for Ca, Co, Cu, I, Mg, Mn, Na, P, K, Se, and Zn. Initial BW were measured and pretreatment blood plasma samples were collected on d −2 or −1. Breeding soundness examinations (BSE) were conducted and BW were measured at 10 and 12 mo of age (d 90 ± 1 and d 150 ± 1, respectively). Scrotal circumference was measured and semen samples were collected via electro-ejaculation. Motility and morphology of sperm were evaluated via light microscopy. Scrotal circumference, BW, and ADG did not differ ($P \geq 0.16$) between treatments at any time. Proportions of control- and TM-treated bulls achieving minimal satisfactory BSE classifications did not differ at 10 mo of age ($P = 0.98$; 50 ± 3.8% for both TM and control) or at 12 mo of age ($P = 0.43$; 89 and 86 ± 2.2% for TM and control, respectively). Conversely, improved ($P = 0.05$) sperm motility was detected in TM-treated bulls compared with control-treated bulls at 12 mo of age; moreover, TM-treated bulls had greater ($P \leq 0.05$) improvements in sperm morphology and motility between 10 and 12 mo of age than control-treated bulls. Among bulls that failed BSE at 10 mo of age, more TM-treated bulls tended ($P = 0.10$) to pass BSE at 12 mo of age than control-treated bulls (98 and 94 ± 1.6% for TM and control, respectively). Under the conditions of this experiment, sperm motility and morphology at 12 mo of age were improved in bulls treated with injectable TM at 7 and 10 mo of age compared with bulls treated with saline.

Key words: breeding soundness, sperm motility, sperm morphology, trace minerals

INTRODUCTION

Costs associated with developing young beef bulls to sexual maturity motivate breeders to minimize the number of animals that are culled for inadequate reproductive fitness before sale. To minimize risk of reproductive failure, many breeders develop young bulls in confinement during the postweaning period to ensure adequate growth and timely puberty. Bull-development diets fed in confinement are generally formulated to meet or exceed NRC (2000) or NASEM (2016) recommendations for trace minerals; however, variation in DMI between bulls may limit mineral intake, and gut-level antagonisms may limit intestinal mineral absorption.

Injectable supplemental trace minerals may be used to bypass gut-level antagonisms and to overcome poor intestinal absorption and variation in trace-mineral intake. Minerals of particular significance to sexual development of bulls include Cu, Mn, Se, and Zn. They have integral roles in either spermatogenesis (Underwood and Somers, 1969), sperm motility and morphology (Brown and Burk, 1973; Swarup and Sekhon, 1976; Hunter, 1977; Parillo et al., 2014), testicular hypertrophy (Miller and Miller, 1962), tissue repair (Machado et al., 2014), or steroid hormone synthesis (Hurley and Doane, 1989; Kumar et al., 2006).

Because of the relatively high value of breeding bulls compared with nonbreeding bulls, small improvements in breeding soundness achieved through injectable trace-mineral supplementation may result in greater net revenue for seedstock producers. Injectable trace-mineral supplements may have particular value for seedstock producers because trace-mineral status can be elevated coincident with predicted onset of puberty or the timing of a breeding soundness examination (**BSE**). The process of complete sper-

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matogenesis (spermatogonia to mature sperm) in beef and dairy bulls averages 61 d (Amann, 1962; Johnson et al., 1994). Treatment with an injectable trace-mineral source approximately 61 d before a scheduled BSE may result in a greater number of bulls with satisfactory breeding soundness. Therefore, the objective of this experiment was to evaluate the performance and breeding soundness of weaned bull calves that were developed in confinement and subject to 2 s.c. injections of either trace minerals or physiological saline.

MATERIALS AND METHODS

Animal care practices used in our experiment were reviewed and approved by the Kansas State University Animal Care and Use Committee (protocol no. 3426).

Weaned, fall-born bull calves (n = 488; initial BW = 308 ± 45 kg; initial age = 203 ± 17 d) of 2 breeds (Angus and Charolais) originating from 13 ranches in Kansas, Oklahoma, and Colorado were used in this experiment. To initiate the experiment, bull calves from all ranches were shipped to a private confinement facility near Randolph, KS. Each participating ranch was required to deliver both Angus and Charolais bulls to the central confinement facility to be used in our experiment—307 Angus bulls and 181 Charolais bulls were included in our analyses.

Bulls from individual ranches of origin arrived at the confinement facility over a 2-d time period (i.e., d −2 or −1); deliveries were staggered such that all bulls originating from a single ranch could be processed as a group upon arrival. Each bull was identified with a unique visual ear tag and a radio-frequency transponder button (half-duplex RFID, Allflex USA Inc., Fort Worth, TX). Pretreatment blood samples (approximately 10 mL) were collected via caudal vessel puncture. Serum mineral concentrations were analyzed for Co, Cu, Fe, Mn, Mo, Se, and Zn concentrations via inductively coupled plasma spectrometry (Varian ICP, Santa Clara, CA) at the Diagnostic Center for Population and Animal Health, Michigan State University. Bulls were then weighed and assigned randomly to 2 treatments: (1) supplemental s.c. trace-mineral injections (**TM**; Multimin 90, Multimin North America Inc., Fort Collins, CO; Table 1) or (2) s.c. injections of physiological saline (control). Treatments were administered at initial processing (1 mL per 45 kg of BW) and on d 90 ± 1 (1 mL per 68 kg of BW).

Following initial processing, bulls were temporarily penned (i.e., d −2 and −1) with their respective ranch-of-origin group until all processing activities were complete. The following d (i.e., d 0), bulls were stratified by treatment, breed, and ranch of origin and assigned randomly to 8 pens. All pens contained bulls from each ranch of origin and breed; treatment allocation to each pen was approximately equal. Pens (minimum area ≥200 m^2 per bull; linear bunk space = 0.46 m per bull) afforded ad libitum access to water via concrete tanks. A growing diet (Table 2) formulated to promote a 1.5-kg ADG at a DMI of 2.6% of BW was fed for 225 d. Bunks were evaluated each morning at 0630 h, and feed was delivered once daily at 0700 h. Bunks were managed using a slick-bunk management method to minimize feed refusals. If all feed delivered to a pen was consumed, delivery at the next feeding was increased to approximately 102% of the previous delivery until intake was stable at approximately 2.6% of aggregate pen BW. Diet samples were collected from bunks weekly and frozen at −20°C. Samples were composited by weight at the conclusion of the experiment and submitted to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for estimation of TDN and analysis of DM, CP, Ca, Mg, P, S, Co, Cu, Fe, I, Mn, Se, and Zn (Table 2). Diet NE values were calculated from TDN using equations proposed by the NRC (2000). Bulls were again weighed individually on d 90 ± 1 and d 150 ± 1 beginning at 0600 h and before daily feed delivery.

Breeding soundness examinations were conducted on d 90 ± 1 and d 150 ± 1 at approximately 10 mo (301 ± 17 d) and 12 mo of age, respectively. One veterinarian measured scrotal circumference and collected semen samples using a programmable electro-ejaculator. Semen samples were assessed visually for motility and morphology via light microscopy by one experienced technician immediately following collection. Bulls with white blood cells (**WBC**) in ejaculate were presumed to have vesiculitis and were treated using a macrolide antibiotic (Draxxin; Zoetis Inc., Kalamazoo, MI) according to label directions under the supervision of a veterinarian. Breeding soundness classifications of satisfactory or unsatisfactory were assigned as specified by the Society for Theriogenology (Chenoweth et al., 1993). Briefly, bulls were considered to be unsatisfactory breeders when one or more of the following criteria was true: scrotal circumference ≤30 cm; sperm-cell motility ≤30% motile cells; sperm-cell morphology ≤70% normal cells; or presence of WBC in ejaculate. Bulls that passed the BSE on d 150 were marketed via live auction on d 225 at 14 to 15 mo of age.

Bulls were monitored daily during the experiment for symptoms of respiratory disease, infectious keratoconjunctivitis, and interdigital infection. Bulls with clinical signs

Table 1. Composition of injectable trace-mineral solution administered to weaned bull calves at 7 and 10 mo of age

Item	Multimin 90,[1] mg/mL
Cu[2]	15
Mn[2]	10
Se[3]	5
Zn[2]	60

[1]Multimin North America (Fort Collins, CO).

[2]Provided in complex with disodium EDTA.

[3]Provided as sodium selenite.

of illness, as judged by animal caretakers, were removed from pens and restrained. Bulls were assigned a clinical-illness score (scale: 1 to 4; 1 = normal, 4 = moribund) and assessed for febrile response. The treatment threshold for respiratory disease was determined before the experiment to be a clinical illness score >1, a rectal temperature >40°C, and no symptoms of conjunctivitis or interdigital infection; however, no bulls were diagnosed with respiratory disease. Bulls with signs of infectious keratoconjunctivitis (i.e., corneal ulcers or obvious eye irritation; 165 first treatments, 27 retreatments) were treated with Micotil 300 (Elanco Animal Health, Eli Lilly & Co., Greenfield, IN), whereas those with acute necrotic infection of the interdigital skin (37 first treatments, 13 retreatments) were treated with Nuflor (Merck Animal Health, Intervet Inc., Madison, NJ). All treatments for disease were administered according to label directions under the supervision of a veterinarian.

Pretreatment serum mineral concentrations were analyzed as a completely randomized design using a mixed model (PROC MIXED, SAS Institute Inc., Cary, NC). Ranch of origin, breed, and treatment were class factors. Bull was experimental unit. The model included terms for the fixed effects of breed, treatment, and breed × treatment; ranch of origin was treated as a random effect. Treatment × breed effects were not detected ($P \geq 0.33$); therefore, main effects of treatment and breed on pretreatment serum mineral concentrations were reported.

Data associated with growth performance, breeding soundness, and health were analyzed as a randomized block design with pen serving as the block. Class factors included ranch of origin, pen, breed, and treatment. Bull within treatment and pen was the experimental unit. The model statement included terms for the fixed effects of treatment, breed, and treatment × breed. Ranch of origin within pen was treated as a random variable. Breed and treatment × breed effects were not detected ($P \geq 0.67$); therefore, treatment main effects were reported. Continuous variables were analyzed using a mixed model (PROC MIXED, SAS Institute Inc.), whereas binomial variables associated with breeding soundness were analyzed using logistic regression (PROC GLIMMIX, SAS Institute Inc.).

For all analyses, least squares means were considered different when protected by a significant F-test ($P \leq 0.05$), whereas tendencies were discussed when $0.05 < P \leq 0.10$.

Table 2. Composition of the diet fed to weaned beef bulls

Item	Value
Ingredient, DM %	
Corn silage	52.7
Modified distillers grains	35.1
Ground prairie hay	10.0
Supplement[1]	2.2
Nutrient composition,[2] DM basis	
CP, %	15.1
Ca, %	0.58
Mg, %	0.29
P, %	0.41
S, %	0.32
Co, mg/kg	0.34
Cu, mg/kg	12.63
Fe, mg/kg	98.50
I, mg/kg	0.67
Mn, mg/kg	51.78
Se, mg/kg	0.26
Zn, mg/kg	60.65
NE_m,[3] Mcal/kg	1.60
NE_g,[3] Mcal/kg	1.06

[1]Supplement contained limestone, salt, Rumensin 90 (31 mg/kg of diet DM; Elanco Animal Health, Indianapolis, IN), and a trace-mineral premix.

[2]Analyses conducted by SDK Laboratories (Hutchinson, KS).

[3]Calculated from nutrient analyses according to the NRC (2000).

RESULTS AND DISCUSSION

Bulls used in our study were sourced from regions with differing forage mineral profiles (Mortimer et al., 1999). Authors anticipated that serum mineral concentrations likely varied with ranch of origin; therefore, bulls were stratified by ranch of origin before treatment assignment. Serum mineral concentrations have limited diagnostic value in most cases; however, pretreatment serum samples were analyzed *ex post* to verify that initial serum concentrations of Co, Cu, Fe, Mn, Mo, Se, and Zn were not different ($P \geq 0.42$) between bulls assigned to TM and control (Table 3). Mean pretreatment serum concentrations of Co, Fe, Mn, and Zn in bulls assigned to TM and control were within the range considered to be adequate, whereas mean serum concentrations of Cu and Zn were considered to be marginal (Puls, 1994; Kincaid, 2000; Herdt and Hoff, 2011). In addition, mean pretreatment serum Mo concentrations were deemed potentially toxic (i.e., >6 ng/mL; Puls, 1994). No clinical symptoms of Cu deficiency were observed in bulls during our study despite elevated pretreatment serum Mo and the fact that the growing diet contained significant S (Table 2). Thomas and Moss (1951) noted that young bulls fed large amounts of Mo lacked libido and exhibited degeneration of seminiferous tubules and testicular interstitial tissue. Conversely, Hurley and Doane (1989) reported that toxic effects of Mo were counteracted by increasing supplemental Cu.

Authors anticipated also that serum mineral concentrations varied between breeds; therefore, bulls were stratified by breed before treatment assignment. Differences in pretreatment serum mineral concentrations were detected between Angus and Charolais bulls (Table 4). Angus bulls had greater ($P < 0.01$) serum Cu, Mn, Se, and Zn than Charolais bulls. In contrast, Angus bulls had lesser ($P <$

Table 3. Pretreatment[1] serum mineral concentrations of weaned beef bulls

	Treatment			
Item[2]	Saline[3]	Trace mineral[4]	SEM	*P*-value
Co, ng/mL	1.02	1.11	0.111	0.42
Cu, µg/mL	0.60	0.60	0.041	0.95
Fe, µg/mL	1.66	1.58	0.121	0.51
Mn, ng/mL	2.07	1.82	0.385	0.51
Mo, ng/mL	13.23	12.68	1.389	0.69
Se, ng/mL	66.90	67.66	2.304	0.74
Zn, µg/mL	0.99	0.99	0.053	0.97

[1]Measured in serum samples collected at the time of experiment enrollment (n = 488 bulls; initial BW ± SD = 308 ± 45 kg, initial age ± SD = 203 ± 17 d).

[2]Serum mineral concentrations were analyzed via inductively coupled plasma spectrometry (Varian ICP, Santa Clara, CA) at the Diagnostic Center for Population and Animal Health, Michigan State University.

[3]Bulls were injected with physiological saline (1 mL per 45 kg of BW) subsequent to whole-blood collection via caudal vessel puncture.

[4]Bulls were treated with an injectable trace-mineral supplement (1 mL per 45 kg of BW; Multimin 90, Multimin North America, Fort Collins, CO) subsequent to whole-blood collection via caudal vessel puncture.

0.01) serum Co than Charolais bulls. Serum Fe and Mo concentrations did not differ ($P \geq 0.13$) between breeds. Ward et al. (1995) reported that Angus cows were less likely to display symptoms of Cu deficiency than Charolais cows, whereas Herd (1997) noted that Angus cattle may require less dietary Cu than Charolais cattle. Differences in serum Cu, Se, and Zn concentrations between British and Continental European beef breeds (Pogge et al., 2012) and *Bos taurus* and *Bos indicus* beef breeds (Sprinkle et al., 2006) were documented recently. Conversely, Littledike et al. (1995) found no differences in serum Cu and Zn between mature Angus and Charolais cows, and Pogge et al. (2012) reported no differences in serum Mn between Angus and Simmental steers.

Neither BW nor ADG differed ($P \geq 0.16$) between TM- and control-treated bulls during the experiment (Table 5). This was not unexpected because all bulls were pen fed a common diet (Table 2). In addition, overall ADG of TM- and control-treated bulls met the ADG goal set at the beginning of the experiment (i.e., 1.5 kg). Effects of injectable trace-mineral supplementation on performance of growing cattle have been inconsistent. Stressed calves treated with injectable trace minerals at feedlot arrival had improved DMI, G:F, and ADG during receiving when compared with calves that were not treated (Berry et al., 2000; Richeson and Kegley, 2011). In contrast, Clark et al. (2006) and Arthington et al. (2014) observed short-term decreases in receiving ADG (28 and 14 d, respectively) when stressed calves were treated with an injectable trace-mineral supplement compared with counterparts treated with placebo, whereas Roberts et al. (2016) reported that an injectable trace-mineral supplement did not influence ADG or DMI of mildly-stressed bull and steer calves during a 42-d receiving period. In each of the aforementioned studies, cattle were subject to weaning or transport stress and growth performance was monitored for <60 d. By design, stress on bulls in the current study was minimal during the150-d period of growth measurement. Dry matter intake and ADG did not differ during feeding periods of >160 d between environmentally acclimated steers in-

Table 4. Breed effects on pretreatment[1] serum mineral concentrations of weaned beef bulls

	Breed			
Item[2]	Angus	Charolais	SEM	*P*-value
Co, ng/mL	0.96	1.09	0.034	<0.01
Cu, µg/mL	0.63	0.57	0.011	<0.01
Fe, µg/mL	1.59	1.53	0.037	0.13
Mn, ng/mL	2.13	1.81	0.283	<0.01
Mo, ng/mL	13.23	12.68	1.389	0.78
Se, ng/mL	66.09	65.27	0.715	<0.01
Zn, µg/mL	1.00	0.87	0.081	<0.01

[1]Measured from whole-blood samples collected at the time of experiment enrollment (n = 488 bulls; initial BW ± SD = 308 ± 45 kg, initial age ± SD = 203 ± 17 d).

[2]Serum mineral concentrations were analyzed via inductively coupled plasma spectrometry (Varian ICP, Santa Clara, CA) at the Diagnostic Center for Population and Animal Health, Michigan State University.

jected with either a trace-mineral supplement or placebo (Clark et al., 2006; Niedermayer et al., 2017).

Breeding soundness exams were administered on d 90 ± 1 (**BSE-1**) and d 150 ± 1 (**BSE-2**) at 10 and 12 mo of bull age, respectively. Proportions of bulls receiving breeding soundness classifications of satisfactory for BSE-1, BSE-2, and the individual components thereof are presented in Table 6. Proportions of TM- and control-treated bulls receiving minimum satisfactory breeding classifications at BSE-1 did not differ ($P = 0.97$; 50 ± 3.8% for both TM and control). Likewise, the proportion of TM-treated bulls and control-treated bulls receiving minimum satisfactory breeding classifications at BSE-2 did not differ ($P = 0.43$; 89 and 86 ± 2.2% for TM and control, respectively). Of bulls that failed BSE-1, more TM-treated bulls tended ($P = 0.10$) to pass BSE-2 than control-treated bulls. The proportions of bulls that either passed or failed both BSE-1 and BSE-2 did not differ ($P \geq 0.66$) between TM and control. Likewise, proportions of TM- and control-treated bulls with minimum satisfactory scrotal circumference, sperm motility, and sperm morphology at both BSE-1 and BSE-2 did not differ ($P \geq 0.27$).

Authors did not anticipate major differences in binomial variables associated with breeding soundness. Kirchhoff et al. (2015) reported that yearling bulls fed diets formulated to meet or exceed NRC (2000) requirements for trace minerals that were injected once with either saline or a trace-mineral solution containing Cu, Mn, Se, and Zn passed a BSE 91 d after treatment at similar rates. In contrast, source of supplemental trace minerals may influence individual BSE traits. Bulls fed combinations of inorganic and organic Zn had greater percentages of normal sperm and fewer poor sperm-motility classifications than bulls fed inorganic sources of Zn (Arthington et al., 2002). Furthermore, Rowe et al. (2014) noted improvements in sperm motility among bulls fed organic Cu, Co, Mn, and Zn compared with bulls fed inorganic sources of those minerals. Such findings are not universal. Peripubertal bulls fed organic Cu, Co, Mn, and Zn tended to reach puberty earlier than contemporaries fed inorganic Cu, Co, Mn, and Zn; however, semen characteristics and scrotal circumference did not differ between treatments (Geary et al., 2016). Mature bulls fed either no supplemental trace minerals, inorganic trace minerals, or hydroxychloride-complexed trace minerals (i.e., Cu, Mn, and Zn) had similar scrotal circumference, sperm motility, and sperm morphology after 70 d of treatment (Van Emon et al., 2016).

Continuous variables associated with BSE-1 and BSE-2 are presented in Table 7. Scrotal circumference did not differ ($P \geq 0.16$) between TM- and control-treated bulls for BSE-1 or BSE-2. Change in scrotal circumference from BSE-1 to BSE-2 also was not different ($P = 0.26$) between treatments. In general, scrotal circumference among the bulls enrolled in the experiment was adequate: less than 10% of bulls had unsatisfactory scrotal circumference at BSE 1 and 1% of bulls had unsatisfactory scrotal circumference at BSE 2. The proportions of morphologically normal sperm cells at BSE-1 and BSE-2 did not differ ($P \geq 0.13$) between TM and control, whereas the percentage change in morphologically normal sperm cells from BSE-1 to BSE-2 was 6% greater ($P = 0.05$) for TM than control. Sperm motility at BSE-1 (% motile cells) was not different ($P = 0.74$) between TM- and control-treated bulls. In contrast, sperm motility at BSE-2 and percentage change in sperm motility between BSE-1 and BSE-2 were greater ($P \leq 0.05$) in TM-treated bulls than in control-treated bulls.

Treatment-associated changes in sperm morphology and sperm motility were consistent with the literature regarding trace-mineral roles in male fertility. Hunter (1977) noted that an injectable Cu-glycinate supplement (400 mg) was related to a general increase in fertility among dairy cattle. Copper content in seminal plasma of bulls was posi-

Table 5. Effects of bolus injections[1] of either a trace-mineral solution or physiological saline administered at 7 and 10 mo of age on growth performance of weaned beef bulls

	Treatment			
Item	Saline[2]	Trace mineral[3]	SEM	*P*-value
Initial BW, kg	308	308	2.9	0.86
BW on d 90, kg	437	437	3.4	0.98
BW on d 150, kg	525	522	3.5	0.32
ADG d 1 to 90, kg	1.4	1.4	0.02	0.85
ADG d 91 to 150, kg	1.6	1.5	0.03	0.16
ADG d 1 to 150, kg	1.5	1.5	0.02	0.22

[1]Bolus injections were administered at the time of experiment enrollment (d 0) and d 90.

[2]Bulls were injected with physiological saline at 7 mo of age (1 mL per 45 kg of BW) and at 10 mo of age (1 mL per 68 kg of BW).

[3]Bulls were treated with an injectable trace-mineral supplement at 7 mo of age (1 mL per 45 kg of BW) and at 10 mo of age (1 mL per 68 kg of BW; Multimin 90, Multimin North America, Fort Collins, CO).

Table 6. Effects of bolus injections[1] of either a trace-mineral solution or physiological saline administered at 7 and 10 mo of age on breeding soundness of weaned beef bulls

	Treatment			
Item	Saline[2]	Trace mineral[3]	SEM	*P*-value
Satisfactory BSE-1,[4] %	50	50	3.9	0.97
Satisfactory BSE-2,[5] %	86	89	2.2	0.43
Failed BSE-1 but passed BSE-2, %	94	98	2.0	0.10
Passed both BSE-1 and BSE-2, %	48	49	3.8	0.66
Failed both BSE-1 and BSE-2, %	11	10	2.1	0.80
Satisfactory scrotal circumference at BSE-1, %	93	91	2.0	0.27
Satisfactory scrotal circumference at BSE-2, %	99	99	0.5	0.58
Satisfactory sperm motility at BSE-1, %	79	76	2.9	0.47
Satisfactory sperm motility at BSE-2, %	91	93	2.0	0.55
Satisfactory sperm morphology at BSE-1, %	53	54	3.9	0.91
Satisfactory sperm morphology at BSE-2, %	88	90	2.1	0.33

[1]Bolus injections were administered at the time of experiment enrollment (d 0) and on d 90.

[2]Bulls were injected with physiological saline at 7 mo of age (1 mL per 45 kg of BW) and at 10 mo of age (1 mL per 68 kg of BW).

[3]Bulls were treated with an injectable trace-mineral supplement at 7 mo of age (1 mL per 45 kg of BW) and at 10 mo of age (1 mL per 68 kg of BW; Multimin 90, Multimin North America, Fort Collins, CO).

[4]BSE-1 = initial breeding soundness examination was conducted on d 90 at approximately 10 mo of age.

[5]BSE-2 = final breeding soundness examination was conducted on d 150 at approximately 12 mo of age.

tively related to progressive motility, total antioxidant status, and sperm-cell viability following ejaculation (Tvrda et al., 2012).

Gut-level absorption of Mn is known to be extremely poor (i.e., <1%); moreover, factors influencing dietary Mn absorption have not been widely investigated (Van Bruwaene et al., 1984; NASEM, 2016). Little is known about specific roles of Mn in male fertility. Hurley and Doane (1989) speculated that Mn deficiency may be related to a generalized inhibition of steroid hormone production in

Table 7. Effects of bolus injections[1] of either a trace-mineral solution or physiological saline administered at 7 and 10 mo of age on scrotal circumference, sperm morphology, and sperm motility of weaned beef bulls

	Treatment			
Item	Saline[2]	Trace mineral[3]	SEM	*P*-value
Scrotal circumference at BSE-1,[4] cm	34.1	33.7	0.29	0.16
Scrotal circumference at BSE-2,[5] cm	36.6	36.5	0.25	0.45
Change in scrotal circumference from BSE-1 to BSE-2, cm	2.6	2.8	0.14	0.26
Normal sperm cells at BSE-1, % of total	56.9	54.9	2.46	0.46
Normal sperm cells at BSE-2, % of total	70.3	73.2	1.42	0.13
Change in normal sperm cells from BSE-1 to BSE-2, %	13.6	18.7	2.25	0.05
Sperm motility at BSE-1, % motile cells	33.8	33.3	1.02	0.74
Sperm motility at BSE-2, % motile cells	40.2	42.2	0.86	0.05
Change in sperm motility from BSE-1 to BSE-2, %	6.2	8.7	1.12	0.04

[1]Bolus injections were administered at the time of experiment enrollment (d 0) and on d 90.

[2]Bulls were injected with physiological saline at 7 mo of age (1 mL per 45 kg of BW) and at 10 mo of age (1 mL per 68 kg of BW).

[3]Bulls were treated with an injectable trace-mineral supplement at 7 mo of age (1 mL per 45 kg of BW) and at 10 mo of age (1 mL per 68 kg of BW; Multimin 90, Multimin North America, Fort Collins, CO).

[4]BSE-1 = initial breeding soundness examination was conducted on d 90 at approximately 10 mo of age.

[5]BSE-2 = final breeding soundness examination was conducted on d 150 at approximately 12 mo of age.

monogastrics via disruptions in cholesterol biosynthesis. This was corroborated by Hurley and Keen (1987) in a review on the effects of severe Mn deficiency in rats and rabbits—sterility and poor libido were coincident with low sperm count and degeneration of the epididymis and seminiferous tubules. In addition, Mn effects on reproduction may be related to antioxidant capabilities of Mn superoxide dismutase (Miriyala et al., 2012), which is exclusive to mitochondria (Karnati et al., 2013). Paynter (1980) reported that Mn superoxide dismutase activity was depressed when dietary Mn was restricted. Garner et al. (1997), in a subsequent publication, noted that oxidative stress in mitochondria was associated with decreased mitochondrial membrane potential and that motility was greater in cryopreserved bovine spermatozoa having more favorable mitochondrial membrane potential.

Selenium may be particularly critical for normal spermatogenesis, sperm morphology, and sperm motility. Selenium deficiency inhibited spermatogenesis, independent of vitamin E status in rats (Wu et al., 1973). Increased motility of bull sperm in response to exogenous Se in vitro was documented by Siegel et al. (1980). The selenoprotein phospholipid hydroperoxide glutathione peroxidase (**PHGPx**) is a mid-piece structural protein that is required for normal development and function of bull spermatozoa. Selenium deficiency resulted in poor expression of PHGPx in the mid-piece mitochondria and was associated with low sperm count and poor motility (Parillo et al., 2014). In addition, Se deficiency and limited expression of PHGPx caused mechanical instability resulting in morphological mid-piece abnormalities and flagellum loss (Ursini et al., 1999).

Smith and Senger (1978) reported Se concentrations in bull semen were 16× greater than that in blood. Sequestration of Se in semen may be related to relatively large mid-piece mitochondrial volume of bovine sperm cells. Bull spermatozoa had greater mid-piece mitochondrial volume than that of human, stallion, boar, or ram (Saaranen et al., 1989). As a result, bull spermatozoa also had greater concentrations of structural Se, seminal plasma Se, and greater PHGPx activity than other species.

Zinc is known to play multiple roles in male fertility. Swarup and Sekhon (1976) reported that Zn was critical for sperm-cell plasma membrane integrity, tail morphology, and, thus, motility. Testicular growth in bulls was stunted during Zn deficiency (Miller and Miller, 1962; Pitts et al., 1966) but reverted to normal when Zn status was restored (Pitts et al., 1966). Supplemental Zn improved morphological (Arthington et al., 2002) and motility (Kumar et al., 2006) characteristics of bull spermatozoa. Hurley and Doane (1989) noted also that Zn was necessary for testosterone biosynthesis and normal libido. Kumar et al. (2006) documented a 45% increase in serum testosterone in bulls fed 70 mg/kg supplemental Zn sulfate for 6 mo compared with bulls fed no supplemental Zn.

Overall health of bulls during our experiment was within acceptable limits. The proportion of bulls that failed BSE-1 because of the presence of WBC in ejaculate was small and not different ($P = 0.35$) between treatments (3.2 and 4.9% for TM and control, respectively; data not shown). Approximately half as many bulls subsequently failed BSE-2 because of WBC in ejaculate (1.6 and 2.1% for TM and control, respectively), and failure rates did not differ ($P = 0.71$) between treatments. The percentages of bulls treated once (43.4 and 41.1% for TM and control, respectively) or twice (7.6 and 8.2% for TM and control, respectively) for any combination of interdigital infection or keratoconjunctivitis were not affected ($P \geq 0.60$) by treatment (data not shown).

Treatment differences in animal health were not anticipated during our experiment. Health responses to supplemental injectable trace-mineral supplements seem to be common during periods of physiological stress (Richeson and Kegley, 2011; Machado et al., 2013; Arthington et al., 2014; Teixeira et al., 2014) but less likely when stress is minimal (Clark et al., 2006; Roberts et al., 2016), as was the case in the present experiment.

The bulls enrolled in the present experiment were developed with the intention of marketing them as breeding stock during October of 2014. Sale averages and treatment costs were averaged across both breeds. Bulls that passed BSE-2 were marketed via live auction and averaged $7,500 per bull. Bulls that failed BSE-2 but passed a third BSE approximately 60 d later were marketed private treaty for an average of $6,000. Bulls that failed the third BSE were sold as nonbreeding culls at a local auction market for an average price of $2,040.

Treatment of weaned bulls with 2 supplemental s.c. trace-mineral injections at 7 and 10 mo of age cost $5.67 per bull (1 mL per 45 kg of BW and 1 mL per 68 kg of BW at 7 and 10 mo of age, respectively; approximately 13.5 mL per bull at $0.42/mL). The addition of a $3 per bull chute charge (2×) brought the total cost of treatment to $11.67 per bull or $1,167 per 100 bulls. Per 100 TM-treated bulls, 89 were eligible for live auction, 6 for private treaty, and 5 for nonbreeding culls, resulting in gross revenue of $713,700. Per 100 control-treated bulls, 86 were live auction eligible, 8 private-treaty eligible, and 6 nonbreeding culls, resulting in gross revenue of $705,240. The gross difference between the 2 treatments, minus TM treatment cost, resulted in a net benefit of $7,293 per 100 bulls or $72.93 per bull enrolled in this experiment.

IMPLICATIONS

Under the conditions of this experiment, the proportions of bulls meeting minimum satisfactory requirements for breeding soundness at 12 mo of age were not different between bulls treated with injectable trace minerals (89%) or treated with saline (86%). In contrast, sperm motility and morphology were improved in bulls treated with trace minerals compared with those treated with saline. Of bulls that failed breeding soundness evaluations at 10 mo of age (50% of all bulls), more trace mineral–treated bulls (98%)

tended to pass breeding soundness evaluations at 12 mo of age than saline-treated bulls (94%). For seedstock producers, the value of breeding bulls is significantly greater than that of nonbreeding bulls. Small improvements in sperm motility and morphology achieved through injectable trace-mineral supplementation may allow sales of more breeding bulls with greater net revenue. In addition, injectable trace-mineral supplements may have particular value for seedstock producers because trace-mineral status can be elevated to coincide with the 61-d process of sperm-cell development and maturation.

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