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Effects of a single trace mineral injection on body parameters, ovarian structures, pregnancy rate and components of the innate immune system of grazing Nellore cows synchronized to a fixed-time AI protocol

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ABSTRACT

Two experiments evaluated the effects of injectable trace minerals (ITM) administered 30 d before artificial insemination (AI) on body weight (BW), body condition score (BCS), ovarian structures, pregnancy rate, and components of the innate immune system of grazing Nellore cows. In Exp. 1, 20 multiparous cows (BCS = 4.3 ± 0.4, scale 1 to 9; BW = 388 ± 35 kg) were kept on marandu-grass pasture (*Urochloa* sp.) and offered free-choice access to a trace mineral supplement. Cows were stratified by BCS and BW and randomly assigned to 1 of 2 treatments: subcutaneous injection (6 mL/cow) of saline solution (0.9% NaCl) or ITM (Multimin 90; containing 60, 10, 5, and 15 mg/mL of Zn, Mn, Se and Cu, respectively) administered 30 d (d -30) before AI (d 0). Pregnancy diagnosis was evaluated on d 30, BW and BCS on d -30, 0 and 30, ovarian structures on d 0 and 14, and blood samples collected on d -30, -26, -22, -17, -11, 0, 7, 14, 21 and 30 for analysis of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), haptoglobin, ceruloplasmin and progesterone (P4). In Exp. 2, 573 multiparous cows (BCS = 4.8 ± 0.5; BW = 400 ± 35 kg) received free-choice access to a trace mineral supplement and were assigned randomly to 1 of 2 treatments (ITM or saline injection 30 d before AI). In Exp. 1, ITM did not affect ($P \geq 0.12$) BW, BCS, corpus luteum (CL) diameter and volume, pregnancy rate, and plasma concentrations of haptoglobin, ceruloplasmin and P4 compared to saline injection. Administration of ITM increased ($P \leq 0.008$) plasma concentrations of SOD on d -22 and -17 and GSH-Px on d -26 compared to saline. In Exp. 2, ITM tended to increase the pregnancy rate compared to saline injection for cows with BCS < 5 ($P = 0.09$; 58.4 vs. 46.8 ± 6.5% respectively), but not for cows with BCS > 5 ($P = 0.36$; 71.8 vs. 67.6 ± 3.5% respectively). Therefore, a single ITM injection 30 d before AI did not alter the body parameters, ovarian structures and acute phase response of grazing Nellore cows, but increased plasma concentrations of antioxidant enzymes, and tended to improve pregnancy rates to AI in cows with BCS < 5.

1. Introduction

Inadequate trace mineral (TM) status in beef cows may decrease conception rate and increase the anestrus period, fetal resorption, placental retention rates, abortions, premature calving, cystic ovaries, and metritis (Corah and Ives, 1991). Forage does not always meet the TM requirements of grazing beef cattle, so TM supplementation is often required. The conventional method of supplying TM is through free-choice mineral supplementation. However, this strategy leads to high variability on TM consumption between the animals of the same herd, and consequently, low TM status in some animals (Manzano et al., 2012). The use of injectable TM (ITM) ensures the supply of a known TM amount to each animal, is not interfered by dietary antagonists (Arthington et al., 2014a; Hartman et al., 2018), and rapidly increases

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the TM status of animals (Hartman et al., 2018).

Studies have reported that ITM increased the pregnancy rate following a fixed-time artificial insemination (AI) protocol (FTAI) in *Bos taurus* cows and heifers (Mundell et al., 2012; Stokes et al., 2017) and following embryo transfer in *Bos indicus* × *Bos taurus* heifers (Sales et al., 2011). Cattle breed may affect the amount of circulating antioxidant enzymes produced and the storage time of TM following ITM (Pogge et al., 2012), and thus, the use of ITM in purebred *Bos indicus* breeds may not elicit similar responses on reproduction compared to that observed in *Bos taurus* or *Bos taurus*-crossbred herds. We are unaware of studies evaluating the effects of ITM on reproductive performance, ovarian structures and metabolic alterations in *Bos indicus* cows. We hypothesized that the administration of ITM will enhance the plasma concentrations of antioxidant enzymes, development of ovarian structures, and pregnancy rate of cows undergoing FTAI. Thus, two experiments evaluated the effects of a single ITM injection administered 30 d before AI on body parameters, ovarian structures, pregnancy rate and components of the innate immune system of grazing Nellore cows.

2. Material and methods

All cows were managed in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and experimental protocols reviewed and approved by the ethics committee on animal use of the Universidade Federal de Mato Grosso do Sul (UFMS) under the protocol nº 754/2016.

2.1. Animals, treatments and samples collection

Experiment 1. The study was conducted at the Faculdade de Medicina Veterinária e Zootecnia of UFMS in Terenos, MS, Brazil (20°26′50.8″S 54°50′21.5″W). A total of 20 multiparous suckling Nellore cows [body condition score (BCS) = 4.3 ± 0.4, scale 1 to 9; body weight (BW) = 388 ± 35 kg, and 4.8 ± 1.4 yr of age] were used in the experiment. The study started 30 d before and ended 30 d after AI (d −30 to 30). All cows were kept in a single 12-ha paddock of marandu-grass [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu; Table 1] and had free-choice access to water and a complete trace mineral/vitamin mix throughout the study (Table 2).

On d −30, cows were stratified by BCS and BW (largest to smallest values) and randomly assigned into 1 of 2 treatments: subcutaneous injection (6 mL/cow; $n = 10$ cows/treatment) of saline solution (0.9% NaCl) and ITM. The ITM solution contained 60, 10, 5, and 15 mg/mL of Zn, Mn, Se and Cu, respectively (Multimin 90, Multimin, Fort Collins, CO, USA). All injections were administered on the right side of the neck of each cow. All cows were assigned to a FTAI protocol from d −11 to 0. On d −11, cows received a 2-mg intramuscular injection of estradiol benzoate (Gonadiol; Zoetis, São Paulo, Brazil) and inserted with an intravaginal progesterone-releasing device containing 1.9 g of progesterone (P4; CIDR; Zoetis, São Paulo, Brazil). On d −2, CIDR device was removed, and each cow received intramuscular injections of $PGF_{2\alpha}$ (12.5 mg/cow; Lutalyse; Zoetis, São Paulo, Brazil), estradiol cypionate (1 mg/cow; ECP; Zoetis, São Paulo, Brazil) and eCG (300 IU/cow; Novormon; Zoetis, São Paulo, Brazil). On d 0, cows were timed-AI by a single technician using semen from a single Nellore bull. The dominant follicle diameter, corpus luteum (CL) diameter, and pregnancy status were assessed by transrectal ultrasonography (7.5-MHz transducer; Mindray DP 2200 VET, Shenzhen, China) on d 0, 14, and 30, respectively. The CL volume (cm^3) was calculated using the formula for the volume of the sphere [$V = 4/3\pi(D/2)^3$ where D is the maximum diameter (mm) of the CL (Cooke et al., 2009). Cow BW and BCS were collected on d −30, 0 and 30. Cow BCS was evaluated by a single technician, blinded for treatments, according to Herd and Sprott (1986).

Blood samples were collected from the coccygeal vein on d −30, −26, −22, −17, −11, 0, 7, 14, 21 and 30 into 10-mL blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) with sodium heparin. Immediately after collection, blood samples were stored on ice and then centrifuged at 1200 × *g* for 30 min for plasma harvest. Plasma samples were stored at −20ºC for further analysis of the plasma concentrations of P4, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), haptoglobin and ceruloplasmin. Plasma

Table 1
Forage chemical composition in Exp. 1 and 2.

Items[a]	Pasture Exp. 1	Exp. 2 Operation 1	Exp. 2 Operation 2	Requirements[a] (NRC, 2016)
Dry matter (DM), g/kg	301.9	310.5	360.6	–
g/kg of DM				
Crude protein	64.6	71.3	45.5	–
Neutral detergent fiber	741.7	720.3	700.6	–
Acid detergent fiber	415.9	347.0	383.9	–
Lignin	47.0	36.5	44.1	–
Ethereal extract	20.4	21.8	22.5	–
Ashes	82.0	86.5	75.2	–
Non-fibrous carbohydrate	91.3	100.1	156.2	–
Calcium	1.46	2.42	1.83	–
Phosphorus	0.61	1.45	0.76	–
Sodium	2.24	1.85	2.61	1
Potassium	10.14	10.59	7.50	7
Magnesium	1.08	1.88	2.08	2
mg/kg of DM				
Iron	42.58	177.30	113.44	50
Zinc	19.68	31.90	11.02	30
Manganese	104.36	138.8	88.98	40
Selenium	0.08	0.16	0.16	0.10
Copper	3.20	4.32	2.10	10

[a] Requirements for cows at early lactation established by NRC (2016).

Table 2
Mineral composition of the complete loose meal trace mineral/vitamin mixtures offered in Exp. 1 and 2.

Items[a]	Trace mineral/vitamin mix Exp. 1[b]	Exp.2[c] Operation 1	Exp.2[d] Operation 2
g/kg of dry matter (DM)			
Calcium	196	150.0 – 220	139 – 155
Phosphorus	90	81	80
Sodium	99	114	130
Magnesium	20	–	10
Sulfur	20	14	40
mg/kg of DM			
Fluorine	900	810	800
Cobalt	200	60	80
Iodine	180	78	100
Iron	2400	–	–
Zinc	3000	5250	5000
Manganese	1670	1040	1040
Selenium	40	22	26
Copper	1200	1500	1350
UI/kg			
Vitamin A	150,000	–	–
Vitamin D3	30,000	–	–
Vitamin E	1500	–	–

[a] The source of Zn, Mn, Se and Cu used in Exp.1 and 2 were Zn oxide, Mn monoxide, Na selenite and Cu sulfate, respectively.
[b] Mega Fós 90 Milk (AgroMega Indústria de Alimentos Animal), Tamboara, PR, Brazil; target consumption of 100 g/day;).
[c] Fórmula Campo Verde (MCassab Comércio e Indústria, Campo Grande, MS, Brazil; target consumption of 90 g/day).
[d] BellNutri (Trouw Nutrition, Mirassol, SP, Brazil; target consumption of 75 g/day).

concentrations of P4 were analyzed on d 0, 7, 14, 21 and 30. Hand plucked samples of pastures were collected on d −30, 0 and 30, and then dried at 60 °C for 5 d, ground to 1 mm, and analyzed for chemical composition.

Experiment 2. In Exp. 2, 573 multiparous suckling Nellore cows (BW = 400 ± 35 kg; BCS = 4.8 ± 0.5; approximately 4 yr of age) were selected from two commercial cow-calf operations. Commercial cow-calf operation 1 (São José do Nabileque) was located in Corumbá, MS, Brazil (20°05′49.9″S 57°20′41.4″W). In operation 1, 191 cows were kept in a single 180-ha paddock of humidicola-grass [*Urochloa humidicola* (Rendle) Morrone & Zuloaga]. The commercial cow-calf operation 2 (Campo Verde) was located in Jaraguari, MS, Brazil (20°24′29.8″S54°05′25.3″W), and in this operation, 382 cows were divided into 3 herds (55, 180, and 147 cows/herd) and kept in 3 pairs of paddocks (30 to 60 ha/paddock) of marandu-grass pastures [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu]. Cows were rotated among all pastures every 14 d, and all cows had free-choice access to water and a complete trace mineral/vitamin mixture until d 30 (Table 2).

On d −30, cows were randomly assigned to same treatments described in Exp. 1 (a single 6-mL injection of saline or ITM administered 30 d before AI). Cow BCS was evaluated on d −30 by a single technician, according to Herd and Sprott (1986). All cows were submitted to a FTAI protocol from d −11 to 0, as described in Exp. 1. In each herd, cows were inseminated by the same technician using semen from a single Nellore (cow-calf operation 1) or Angus bull (cow-calf operation 2). Pregnancy status was assessed on d 30 by transrectal ultrasonography (7.5-MHz transducer; Mindray DP 2200 VET, Shenzhen, China). Hand plucked samples of pastures were collected on d −30, 0 and 30, and then dried at 60 °C for 5 d, ground to 1 mm, and analyzed for chemical composition.

2.2. Laboratory analysis

Forage samples (Exp. 1 and 2) were analyzed according to AOAC (2000): dry matter (DM), method 930.15; crude protein (CP), method 976.05; ethereal extract (EE), method 920.39 and ashes, method 942.05. The concentrations of lignin, neutral detergent fiber (NDF) and acid (ADF) were done according to the methodology of Van Soest et al. (1991), and non-fibrous carbohydrate (NFC) were calculated according to the NRC (2001): NFC (%) = 100 – (% NDF + % CP + % EE + % ashes). Analyzes of mineral concentrations were done by Laboratory of Animal Nutrition of the Embrapa Pantanal (Corumbá, MS, Brazil), with the exception of Se that was analyzed by Laboratory of Minerals of the Universidade de São Paulo (Pirassununga, SP, Brazil).

Plasma samples (Exp. 1) were analyzed as follows: GSH-Px and SOD concentrations were determined by commercial kits for ELISA (Cayman Chemical, Ann Arbor, MI, catalog number 703,102 and 706,002, respectively), whereas concentrations of haptoglobin were analyzed as described by Cooke and Arthington (2013) and ceruloplasmin as described by Demetriou et al. (1974). The inter- and intra-assay CV were 4.9 and 7.2% for SOD, 4.2 and 9.8% for GSH-Px, 4.23 and 2.90% for haptoglobin, and 4.43 and 5.06% for ceruloplasmin, respectively. Plasma P4 concentrations were determined using a solid-phase, competitive, chemiluminescent enzyme immunoassay (Immulite 1000, Diagnostics Products Corp.) previously validated for bovine samples (Martin et al., 2007). Detectable range and intra-assay CV for plasma P4 concentrations were 0.2 to 12.2 ng/mL and 4.69%, respectively.

2.3. Statistical analyzes

In Exp. 1, plasma data, ovarian structures, BW, BW change, BCS and BCS change were analyzed using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Ovarian structures, BW and BCS change were tested for fixed effect of treatment using cow(treatment) as random effect and BCS obtained on d −30 as covariate. However, plasma data, BW and BCS were analyzed as repeated measures and tested for effects fixed of treatment, day and resulting interaction, using cow(treatment) as random variable and subject, and BCS obtained on d −30 as covariate. Plasma data on d 0 also were included as covariates in each respective analysis (except for P4), but were removed from the model when $P > 0.10$. The toepliz covariance structure was selected for the analyses of haptoglobin and BW, and first order autoregressive covariance structure was selected for SOD, GSH-Px, ceruloplasmin, P4 and BCS, as they generated the lowest Akaike information criterion. Pregnancy rate was analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Pregnancy rate in Exp. 1 was tested for fixed effect of treatment, using cow(treatment) as a random effect and BCS obtained on d −30 as a covariate. Pregnancy rate in Exp. 2 was tested for fixed effect of treatment, using cow(treatment × herd) and herd as random effects and BCS obtained on d −30 as a covariate. In Exp. 2, post hoc analysis was also performed, where the cows were stratified by BCS on d −30 into two categories, BCS < 5 and ≥ 5. The value 5 represented the median of BCS all animals in Exp.2. Means were separated using PDIFF and all results were reported as LSMEANS followed by SEM. Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10.

3. Results

Experiment 1. Effects of treatment × day and treatment were not detected ($P \geq 0.21$) for BW and BCS. Effects of treatment were not detected ($P \geq 0.12$) for BW change and BCS change, dominant follicle diameter, CL diameter and volume, and pregnancy rate (Table 3). All cows had CL on d 14, indicating that all cows ovulated to the FTAI protocol. Effects of treatment × day ($P = 0.008$), but not treatment ($P = 0.16$), were detected for plasma concentrations of SOD (Table 3). The ITM cows had greater ($P = 0.008$) plasma concentrations of SOD on d −22 and −17 compared to saline cows (Fig. 1a). Effects of treatment × day were detected ($P = 0.004$) for plasma concentrations of GSH-Px, which were greater for ITM cows on d −26 compared to saline cows (Table 3; Fig. 1b). Effects of treatment × day and treatment were not detected ($P \geq 0.23$) for plasma concentrations of haptoglobin, ceruloplasmin, and P4 (Table 4).

Experiment 2. Effects of treatment were not detected ($P = 0.12$) for pregnancy rates when all cows were included in the model. However, after sorting cows into 2 groups based on BCS observed on d −30, treatment effects tended to be detected ($P = 0.09$) for pregnancy rate in cows with BCS < 5, but not ($P = 0.36$) for cows with BCS ≥ 5 (Table 4). The ITM injection tended to increase the pregnancy rate compared to saline injection in cows with BCS < 5 (Table 4).

4. Discussion

No effects of ITM were observed for BW and BCS of cows, suggesting that the access to free-choice trace mineral mixtures likely met the TM requirements for the maximum performance of these variables. According to Genther and Hansen (2014), when animals remained for a relatively long period consuming a diet with low concentrations of Zn, Mn, Se and Cu, and then met their TM requirements (through diet consumption and application of ITM), these animals experienced greater BW gain compared to animals administered saline injection. However, if animals were consuming a diet that always met their TM requirements, the use of ITM did not impact the BW gain. In addition, all cows in Exp. 1 had already reached maturity, reducing the chances of observing treatment effects on BW and BW change.

The ITM administration increased the plasma concentrations of antioxidant enzymes for up to 13 d (plasma SOD) and 4 d (plasma GSH-Px) after application. The increase in post-ITM plasma concentrations of

Table 3
Body parameters, ovarian structures, pregnancy rate, and plasma analyzes of Nellore cows administered a single subcutaneous injection (6 mL/cow) of saline solution or injectable trace mineral (ITM) on d −30 [30 d before artificial insemination (AI); Exp. 1].

Items[a]	Treatments[a]		SEM	*P*-value	
	ITM	Saline		Trt. × day	Trt.
Body parameters					
BW, kg	399	395	2.3	0.23	0.21
BW change, kg					
d −30 to 0	8.8	0.8	4.6		0.24
d 0 to 30	16.7	20.2	2.8		0.39
d −30 to 30	25.5	21.0	5.2		0.55
BCS	4.7	4.6	0.15	0.74	0.94
BCS change					
d −30 to 0	0.3	0.4	0.26		0.79
d 0 to 30	0.5	0.2	0.21		0.42
d −30 to 30	0.8	0.6	0.25		0.67
Ovarian structures					
Dominant follicle size (d 0), mm	13.7	13.7	1.1		0.99
CL diameter (d 14), mm	30.3	35.9	2.3		0.12
CL volume (d 14), cm^3	15.5	29.2	6.2		0.14
Pregnancy rate,[b]%	60 (6/10)	50 (5/10)	16.1		0.67
Plasma analyses					
Superoxide dismutase, U/mL	4.0	3.8	0.14	0.008	0.16
Glutathione peroxidase, nmol/min/mL	2.2	1.8	0.13	0.004	0.06
Haptoglobin, mg/mL	0.24	0.21	0.03	0.63	0.46
Ceruloplasmin, mg/mL	17.6	17.7	0.72	0.92	0.94
Progesterone, ng/mL					
Cows pregnant	4.8	5.9	0.6	0.52	0.23
Cows non-pregnant	4.4	4.7	0.9	0.80	0.87
Overall	4.7	5.3	0.5	0.85	0.40

[a] BW, body weight; BCS, body condition score, CL, corpus luteum.
[b] Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15 mg/mL of Zn, Mn, Se and Cu, respectively (Multimin 90, Multimin, Fort Collins, CO, USA), and both were administered on the right side of the neck of each cow. Values in parentheses represent the number of cows evaluated/number of pregnant cows.

Table 4
Pregnancy rate to artificial insemination (AI) of Nellore cows administered a single subcutaneous injection (6 mL/cow) of saline solution or injectable trace mineral (ITM) on d −30 (30 d before AI; Exp. 2).

BCS[a]	Treatments[b]		SEM	*P*-value
	ITM	Saline		Treatment
< 5,%	58.4 (51/90)	46.8 (34/74)	6.5	0.09
≥ 5,%	71.8 (133/194)	67.6 (137/212)	3.5	0.36
Overall,%	66.4 (184/284)	60.1 (171/289)	3.1	0.12

[a] Body condition score (BCS; scale 1 - 9) evaluated on d −30 according to Herd and Sprott (1986). Cow BCS < 5 = 4.26 ± 0.3, BCS ≥ 5 = 5.06 ± 0.3. Values in parentheses represent the number of cows evaluated/number of pregnant cows.
[b] Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15 mg/mL of Zn, Mn, Se, and Cu, respectively (Multimin 90, Multimin, Fort Collins, CO, USA), and both were administered on the right side of the neck of each cow.

SOD is likely due to the additional contribution of injected Zn, Mn, and Cu, which are components of Cu/Zn-SOD and Mn-SOD enzymes (Markclund, 1980). These metalloenzymes contribute to catalyze the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide (Sordillo and Aitken, 2009). However, Se is also a component of the GSH-Px enzyme, whose main function is to catalyze the reduction of hydroperoxides, such as hydrogen peroxides and lipid hydroperoxides (Rotruck et al., 1973). Therefore, the increased plasma concentrations of these antioxidant enzymes may lead to greater control of oxidative stress-induced cellular damage.

In the present study, plasma concentrations of ceruloplasmin and haptoglobin were relatively low (< 19.4 mg/dL and < 0.36 mg/mL respectively) for both treatments, suggesting that the inflammatory response was weak, which reduced the likelihood of detecting treatment effects. The lack of an ITM-induced acute phase response was unexpected because, in addition to a heightened production of neutralizing antibodies, ITM administration produced a pro-inflammatory acute phase reaction in beef cattle (Arthington et al., 2014a) similar to the acute phase reaction induced by vaccination (Arthington et al., 2013). Brangus-crossbred beef heifers administered a single dose of ITM

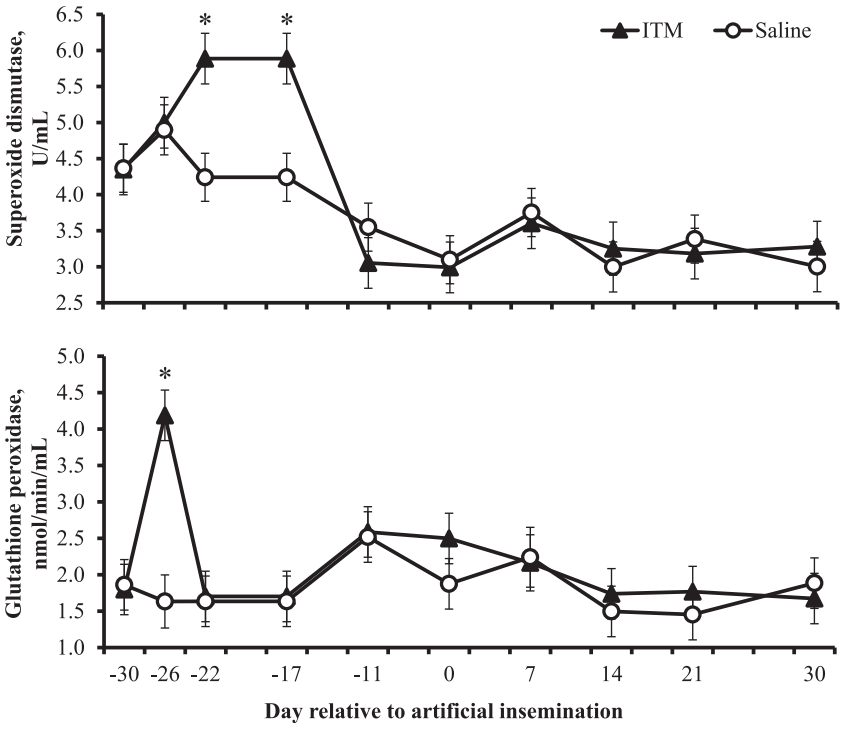

Fig. 1. Plasma concentrations of superoxide dismutase (Fig. 1a) and glutathione peroxidase (Fig. 1b) of Nellore cows administered a single subcutaneous injection (6 mL/cow) of saline solution or injectable trace mineral (ITM) on d −30 (30 d before artificial insemination; Exp. 1). Effects of treatment × day, but not for treatment ($P = 0.16$), were detected ($P = 0.008$) for plasma concentrations of superoxide dismutase. Effects of treatment × day were detected ($P = 0.004$) and effects of treatment tended to be detected ($P = 0.06$) for plasma concentrations of glutathione peroxidase. *$P \leq 0.05$.

at the time of weaning had greater post-weaning and post-transportation plasma concentrations of haptoglobin compared to saline heifers (Arthington et al., 2014a). At this point, it remains unknown whether this positive antigen × antibody response, with concurrent vaccine and ITM administration, is the result of a direct nutritional impact of supplemental trace minerals or an adjuvant-like response elicited from the ITM product. A likely explanation for the discrepancy among the studies described above and ours is the effects of breed on the magnitude of the acute phase response. Qiu et al. (2007) observed that plasma concentrations of fibrinogen at weaning and following transportation were 19 and 28%, respectively, less for Brahman calves compared to Brahman × Angus crossbred calves. Hence, it is possible that mature Nellore cows in our study did not exhibit an acute phase response following ITM as strong as expected due to their purebred *Bos indicus* genetics.

The administration of ITM also did not affect the ovarian structures and plasma concentrations of P4. In previous study, heifers administered multiple injections of ITM from weaning to AI (221, 319, 401, and 521 ± 22 d of age) had similar ovarian development (antral follicle count and ovarian size) compared to saline heifers (Stokes et al., 2018). In addition, the administration of ITM 30 d before AI in cows synchronized to FTAI did not affect follicle population, dominant follicle size, time of estrus after CIDR removal, and CL size (Gonzalez-Maldonado et al., 2017). Although antioxidant enzymes and reactive oxygen species may affect follicle development (Agarwal et al., 2012) and that ITM increased the production of antioxidant enzymes, this increase in plasma concentrations of antioxidant enzymes only occurred until d −17. Hence, these enzymes were not altered by the ITM during the development of the dominant follicle or CL. Progesterone synthesis is positively correlated with CL diameter (Kastelic et al., 1990). Therefore, the lack of treatment effects on plasma P4 concentrations can be explained by absence of ITM-induced effects on CL size. Manganese is a cofactor for the synthesis of cholesterol, a precursor of steroid hormones such as progesterone (Nocek et al., 2006). However, the Mn requirements of cows in the current study was met solely from forage, and thus, the additional Mn supplied by the ITM probably did not further stimulated the CL-production of P4.

The impact of ITM on reproduction of beef cows reported in the literature are inconsistent, with some studies reporting an increase (Sales et al., 2011; Mundell et al., 2012; Stokes et al., 2017) or no impact on pregnancy rates (Arthington et al., 2014b; Willmore et al., 2015; Gonzalez-Maldonado et al., 2017; Stokes et al., 2017, 2018). This inconsistency of results may be due to multiple factors such as breed, category, diet provided, reproductive management, environmental factors, BCS (as shown in the current study) and also TM status at the time of ITM administration. In the present study, TM status at the start of the study was not determined. However, it is unlikely that cows were deficient in Mn and Se because forage alone met the requirements for these trace elements (except for Se in Exp. 1) for cows at early lactation (NRC, 2016). Forage did not meet the requirements for Zn (except for Exp. 2; operation 1) and Cu, and thus, TM deficiency may have happened in some cows as free-choice mineral consumption is highly variable between the animals of the same herd (Manzano et al., 2012).

In the current study, pregnancy rates tended to increase for ITM cows with BCS < 5 compared to saline cows, but not for cows with BCS ≥ 5. To our knowledge, this is the first study to detect this response. In another study, a large number of cows (n = 3750) were administered ITM or saline injection within 30 d after the start of the breeding season, although pregnancy rate did not differ statistically (P = 0.62), the increment on pregnancy percentage attainment with ITM vs. saline numerically increased as cow BCS decreased (3.1, 1.8, and 0.1% for cows in low, medium, and high BCS, respectively; Arthington et al., 2014b). The exact reasons for such responses are currently unknown, but further studies exploring the mechanisms by which ITM may increase reproductive performance of cows in low BCS is warranted.

Our initial hypothesis was that the application of ITM would increase the plasma concentrations of antioxidant enzymes, and consequently enable greater oxidative control and pregnancy rate compared to saline injection. Reactive oxygen species affect multiple physiological processes from oocyte maturation to fertilization, survival and embryonic development and maintenance of pregnancy (Agarwal et al., 2012). In the current study, although ITM increased the plasma concentration of SOD (d −22 and −17) and GSH-Px (d −26), these enzymes returned to baseline levels during the development (d −17 to 30) of the physiological processes described by Agarwal et al. (2012). Thus, the ITM-induced increase on plasma concentrations of antioxidative enzymes assessed in the current study had no direct or carry-over effects on pregnancy rates.

5. Conclusion

A single ITM injection administered 30 d before AI did not alter the body weight and BCS change, ovarian structures, and did not elicit an acute phase response in grazing Nellore cows. However, ITM administration increased plasma concentrations of antioxidant enzymes in all cows, regardless of BCS category, and tended to improve pregnancy rates to AI in cows with BCS < 5, but not for those with BCS ≥ 5.

Conflict of interest

The authors declare that they have no conflict of interest.

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