Effects of a single trace mineral injection at beginning of fixed-time AI treatment regimen on reproductive function and antioxidant response of grazing Nellore cows

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ABSTRACT

There was evaluation of effects of injectable trace minerals (ITM) administered 11 d before artificial insemination (AI) on body weight (BW), body condition score (BCS), ovarian structures, pregnancy rate, and antioxidant response of Nellore cows. In Experiment 1, 20 multiparous cows were assigned to one of two treatments: subcutaneous injection (6 mL/cow; 11 d before AI) of saline solution or ITM (60, 10, 5, and 15 mg/mL of Zn, Mn, Se and Cu, respectively) and BW, BCS, ovarian structures and blood were evaluated. In Experiment 2, 1,144 multiparous cows were assigned to same treatments described in Experiment 1 and pregnancy rate on d 30 was evaluated. In Experiment 1, ITM did not affect ($P \geq 0.23$) BW, dominant follicle size, ovulation rate, and plasma concentrations of haptoglobin, ceruloplasmin and progesterone (P4). The ITM treatment tended to increase ($P = 0.06$) cow BCS and reduce ($P \leq 0.06$) corpus luteum (CL) diameter and volume. Furthermore, ITM treatment tended to increase ($P = 0.06$) plasma concentrations of SOD and increased ($P = 0.007$) GSH-Px compared with saline injection. In Experiment 2, ITM treatment tended ($P = 0.06$) to increase pregnancy rate of cows with BCS ≤ 5.0 but not cows with BCS > 5.0 ($P = 0.99$). The ITM treatment did not alter BW, plasma P4, and acute phase response, but enhanced plasma concentrations of antioxidant enzymes, and tended to enhance BCS and pregnancy rates to AI of cows with BCS ≤ 5.0, even though there was a smaller corpus luteum size.

Keywords: Ceruloplasmin; Corpus luteum; Haptoglobin; Oxidative stress; Pregnancy

1. Introduction

The use of injectable trace mineral (ITM) ensures the administration of a known amount of trace mineral (TM) to each animal, is not interfered by dietary antagonists (Arthington et al. 2014a; Hartman et al., 2018), and rapidly increases the TM status of animals (Hartman et al., 2018). Manufacturer recommendation is that ITM should be administered to beef cows

approximately 30 d before the beginning of the breeding season or AI (Multimin, Fort Collins, CO, USA). In studies where there was following of this recommendation there was either an increase (Mundell et al., 2012; Stokes et al., 2017; Vedovatto et al., 2019) or no effect on pregnancy rates compared to administration of a saline injection (Willmore et al., 2015; Maldonado et al., 2017; Stokes et al., 2017 and 2018).

Trace minerals are components of antioxidant enzymes. For example, Zn, Mn and Cu are components of the superoxide dismutase (SOD), whereas Se is a component of the glutathione peroxidase (GSH-Px), and both enzymes are essential for the control of oxidative stress in cells throughout the body (Sordillo and Aitken, 2009). Reactive oxygen species (ROS) may affect reproduction by affecting multiple physiological processes, such as oocyte maturation to the time of fertilization, embryonic survival and development, and pregnancy maintenance (Agarwal et al., 2005). The ITM treatment, however, effectively increased the plasma concentration of SOD (Tomasi et al., 2018) and GSH-Px (Pogge et al., 2012) for 10 and 15 d after application, respectively.

For cows submitted to fixed-time artificial insemination (AI) protocol (FTAI), the recommendation of using ITM 30 d before AI results in an additional handling in the corral, increasing the labor costs. With several FTAI protocols, there is initiation of treatment regimens 10 or 11 d before AI, and thus, ITM administration at the start of imposing the FTAI protocol will reduce operating costs. Thus, the hypothesis in the present study was that a single administration of ITM 11 d before AI will increase the reproductive performance of grazing beef cows through regulation of oxidative stress in the period after AI compared to the administration of saline.

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) for the protocol nº 754/2016.

2.1. Animals, treatments and samples collection

2.1.1. 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 [BCS = 4.7 ± 0.6, scale 1 to 9; BW = 396 ± 23.9 kg, and 5.4 ± 2.5 yr of age] were used in the experiment. The study started 11 d before AI and ended 30 d after AI (d -11 to 30). All cows were maintained in a single 12-ha paddock with marandu-grass as the primary pasture forage [*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 -11, cows were stratified by BCS and BW and randomly assigned into one of two treatments: subcutaneous injection (6 mL/cow; n = ten 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 treatment regimen from d -11 to 0. On d -11, cows were administered a 2-mg intramuscular injection of estradiol benzoate (Gonadiol; Zoetis, São Paulo, Brazil) and there was insertion of an intravaginal progesterone-releasing device containing 1.9 g of progesterone (P4; CIDR; Zoetis). On d -2, the CIDR device was removed, and each cow was administered intramuscular injections of

95 $PGF_{2\alpha}$ (12.5 mg/cow; Lutalyse; Zoetis), estradiol cypionate (1 mg/cow; ECP; Zoetis) and eCG
96 (300 IU/cow; Novormon; Zoetis). On d 0, there was AI of the cows by a single technician using
97 semen from a single Nellore bull. The dominant follicle diameter, corpus luteum (CL)
98 diameter, and pregnancy status were assessed using transrectal ultrasonography (7.5-MHz
99 transducer; Mindray DP 2200 VET, Shenzhen, China) on d 0, 14, and 30, respectively. The CL
100 volume (cm^3) was calculated using the formula for the volume of the sphere [$V = 4/3\pi(D/2)^3$
101 where D is the maximum diameter (mm) of the CL. The presence of CL on d 14 was used to
102 determine which cows had ovulations. Data for cow BW and BCS were collected on d -11, 0
103 and 30. Cow BCS was evaluated by a single technician, blinded with regard to which cows
104 were in what treatment group, using the procedures previously described by Herd and Sprott
105 (1986).

106 Blood samples were collected from the coccygeal vein on d -11, -9, -4, 0, 7, 14, 21 and
107 30 into 10-mL blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ,
108 USA) with sodium heparin. Immediately after collection, blood samples were stored on ice and
109 then centrifuged at 1200 × *g* for 30 min for plasma separation and collection. Plasma samples
110 were stored at -20 ºC for further analysis of the plasma concentrations of P4, superoxide
111 dismutase (SOD), glutathione peroxidase (GSH-Px), haptoglobin and ceruloplasmin. Plasma
112 concentrations of P4 were quantified on d 0, 7, 14, 21 and 30. Hand plucked samples of pastures
113 were collected on d -11, 0 and 30, and then dried at 60 °C for 5 d, ground to 1 mm, and analyzed
114 for chemical composition.

115

116 *2.1.2. Experiment 2*

117 In Experiment 2, 1,144 multiparous suckling Nellore cows (BCS = 4.8 ± 0.7; BW = 400
118 ± 35; approximately 4 yr of age) from three commercial cow-calf operations were used to
119 conduct the experiment. The commercial cow-calf operation 1 (Campo Verde) was located in

Jaraguari, MS, Brazil (20°24'29.8"S54°05'25.3"W), and in this operation, 192 cows were rotated between two paddocks (80 ha/paddock) containing marandu-grass pasture [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu]. Commercial cow-calf operation 2 (São José do Nabileque) was located in Corumbá, MS, Brazil (20°05'49.9"S 57°20'41.4"W), and in this operation, 425 cows were from three herds (129, 93, and 203 cows/herd) and were pastured in three pairs of paddocks (60 to 100 ha/paddock) containing humidicola-grass pasture [*Urochloa humidicola* (Rendle) Morrone & Zuloaga]. The commercial cow-calf operation 3 (Seriema) was located in Miranda, MS, Brazil (20°24'02.0"S 56°18'11.2"W), and in this operation, 527 cows were maintained in four herds (140, 137, 122 and 128 cows/herd) and each herd was rotated to maintain pasture quantity and quality between two paddocks (40 to 60 ha/paddock) containing decumbens-grass [*Urochloa decumbens* (Stapf) R.D. Webster]. Cows were rotated among all pastures approximately 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 -11, cows were randomly assigned to same treatments described for Experiment 1 (a single 6-mL injection of saline or ITM administered 11 d before AI). Cow BCS was evaluated on d -11 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 for Experiment 1. In each herd, cows were inseminated by the same technician using semen from a single Angus (cow-calf operations 1 and 3) or Nellore 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 -11, 0 and 30, and then dried at 60 °C for 5 d, ground to 1 mm, and analyzed for chemical composition.

143 *2.2. Laboratory analysis*

144 Forage samples (Exp. 1 and 2) were analyzed according to AOAC (2000): dry matter
145 (DM), method 930.15; crude protein (CP), method 976.05; ether extract (EE), method 920.39
146 and ashes, method 942.05. The concentrations of lignin, neutral detergent fiber (NDF) and acid
147 (ADF) were evaluated using the methodology of Van Soest et al. (1991). Analyses of mineral
148 concentrations were conducted using inductively coupled plasma mass spectrometry
149 procedures, and Se was analyzed as described by Oliveira et al. (2016) and the other minerals
150 as described by Braselton et al. (1997).

151 Plasma samples (Exp. 1) were analyzed as follows: GSH-Px and SOD concentrations
152 were determined using commercial kits for ELISA (Cayman Chemical, Ann Arbor, MI, catalog
153 number 703102 and 706002, respectively), whereas concentrations of haptoglobin were
154 analyzed as described by Cooke and Arthington (2013) and ceruloplasmin as described by
155 Demetriou et al. (1974). The inter- and intra-assay CV were 4.6% and 6.7% for SOD, 4.9%
156 and 9.1% for GSH-Px, 3.9% and 9.4% for haptoglobin, and 2.0% and 4.3% for ceruloplasmin,
157 respectively. Plasma P4 concentrations were determined using a solid-phase, competitive,
158 chemiluminescent enzyme immunoassay (IMMULITE 1000, Diagnostics Products Corp.)
159 previously validated for cattle samples (Martin et al., 2007). Detectable range and intra-assay
160 CV for plasma P4 concentrations were 0.2 to 9.9 ng/mL and 4.7%, respectively.

161

162 *2.3. Statistical analyses*

163 For all analyses, animal was considered the experimental unit. In Experiment 1, plasma
164 data, ovarian structures, BW, BW change, BCS and BCS change were analyzed using the
165 MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.4) with Satterthwaite
166 approximation to determine the denominator degrees of freedom for the test of fixed effects.
167 Data for ovarian structures, BW and BCS change were tested for fixed effect of treatment,

using cow(treatment) as random effect and BCS obtained on d -11 as covariate. 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 -11 as covariate. Plasma data on d -11 also were included as covariates in each respective analysis (except for P4) but removed from the model when $P > 0.10$. The toepliz covariance structure was selected for the analyses of haptoglobin, and first order autoregressive covariance structure was selected for BW SOD, GSH-Px, ceruloplasmin, P4 and BCS, as these generated the least Akaike information criterion. Ovulation and pregnancy rates were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, version 9.4) with the binomial distribution option and with Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Ovulation and pregnancy rates in Experiment 1 were tested for the fixed effect of treatment, using cow(treatment) as a random effect and BCS obtained on d -11 as a covariate. Pregnancy rate in Experiment 2 was tested for fixed effect of treatment, using cow(treatment × herd) and herd as random effects and BCS obtained on d -11 as a covariate. In Experiment 2, the *post hoc* analysis was also performed, where cows were stratified by BCS on d -11. Means were evaluated 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

3.1. Experiment 1

Effects of treatment × day and treatment were not detected ($P \geq 0.23$) for BW, BW change, dominant follicle size, ovulation rate, pregnancy rate and plasma concentrations of haptoglobin, ceruloplasmin and P4 (Table 3). Effects of treatment, but not treatment × day ($P = 0.22$) were detected ($P = 0.06$) for BCS, which was greater for ITM- compared with saline-

193 treated cows during the entire experiment (Table 3). There tended to be effects of treatment (*P*
194 = 0.06) for BCS change from d -11 to 30, but not ($P \geq 0.15$) from d -11 to 0 or 0 to 30 (Table
195 3). The ITM-treated cows tended (*P* = 0.06) to have an increase in BCS, whereas saline-treated
196 cows had a decrease in BCS during the experimental period (Table 3). There tended to be
197 effects of treatment (*P* = 0.06) for CL diameter, which was less for ITM- compared with saline-
198 treated cows (Table 3). There were effects of treatment (*P* = 0.03) for CL volume, which was
199 less for ITM- compared with saline-treated cows (Table 3). There tended to be effects of
200 treatment × day (*P* = 0.06) and there were effects of treatment (*P* = 0.03) on SOD plasma
201 concentrations (Table 3). The ITM-treated cows tended to have greater (*P* = 0.06) plasma SOD
202 concentrations from d -4 to 30 compared with saline-treated cows (Fig. 1). Effects of treatment
203 × day and treatment were detected ($P \leq 0.007$) for plasma concentrations of GSH-Px (Table 3).
204 The ITM-treated cows had greater (*P* = 0.007) plasma concentrations of GSH-Px on d -9 and -
205 4 compared with saline-treated cows (Fig. 1).
206
207 *3.2. Experiment 2*
208 When all cows were included in the statistical model, regardless of BCS group, there
209 tended to be effects of treatment (*P* = 0.09) for pregnancy rate, which was greater for ITM-
210 compared with saline-treated cows (Table 4). Results from further analyses of BCS indicated
211 there was a tendency (*P* = 0.06) for greater pregnancy rates for ITM- compared with saline-
212 treated cows with a BCS ≤ 5.0 and that there was no difference (*P* = 0.99) between treatments
213 for cows with BCS > 5.0 (Table 4).
214
215 **4. Discussion**
216 A single ITM injection did not affect cow BW. The ITM-treated cows, however, tended
217 to have a greater BCS and gained BCS from d -11 to 30, whereas saline-treated cows had a

decrease in BCS and had a lesser BCS during that same period. Results from studies with growing beef cattle indicated that ITM treatments can increase calf average daily gain (Arthington et al., 2014a; Genther and Hansen, 2014; Harsh et al., 2018). In the present study, because cows had already reached the maturity by the time the experiment was initiated, the effect of ITM may have occurred in the form of a greater deposition of adipose tissue. Although forage intake was not measured in the present study, one plausible explanation for the greater BCS of ITM-treated cows is that there was greater forage intake compared with saline-treated cows. In support of this rationale, heifers administered ITM tended to increase the voluntary dry matter intake (DMI; Harsh et al., 2018).

The supplementation of ITM resulted in a reduced CL size in the present study. In other studies, there were no effects of ITM supplementation on ovarian morphology, follicle population and follicular development in beef cows and heifers (Maldonado et al., 2017; Stokes et al., 2018; Vedovatto et al., 2019). The exact reasons for the reduced CL size following ITM-treatment in the current study are unknown. Although CL size is positively correlated with the P4 synthesis and secretion (Kastelic et al., 1990), the plasma concentration of P4 was not altered when there was a single administration of ITM. The correlation between corpus luteum size and P4 concentrations is not consistent among studies. Mann (2009) reported that CL size was strongly correlated with P4 concentration only on d 5, but not on d 8 and 16. In the present study, the CL was assessed only on d 14, and thus, at this time of the estrous cycle there may not have been a correlation between production of P4 and CL size at this stage of the estrous cycle. In addition, ITM-treated cows possibly have a greater P4 synthesis compared to saline-treated cows even though there is less CL tissue volume. In the present study, a single ITM administration resulted in an increase in the plasma concentrations of antioxidant enzymes and this outcome may have in turn resulted in a reduced amount of ROS, which functions as intracellular regulators of steroidogenesis in and progesterone release from the CL (Fujii et al.,

2005). In addition, Mn is a cofactor for the synthesis of cholesterol, a precursor of steroid hormones such as progesterone (Nocek et al., 2006). Further studies exploring the mechanisms by which ITM has actions of biological processes may be warranted.

A single ITM administration 11 d before AI resulted in an increase in the plasma concentration of SOD, which is an important metalloenzyme regulating oxidative stress in cells of the body (Sordillo and Aitken, 2009). This increase in plasma concentrations of SOD occurred because supplemental Zn, Mn and Cu are components of SOD, that is present in the body in the form of Cu/Zn-SOD and Mn-SOD (Markclund, 1980). This finding that there was an increase in the SOD concentration 41 d after the treatment with ITM are inconsistent with previously published results that treatment with of newborn calves with Zn and Cu, resulted in an increased concentration of SOD only until 10 d after treatment administration (Tomasi et al. 2018). The TM are temporarily stored in the liver following ITM administration. Liver concentrations of Zn and Cu may remain greater for as long as 79 d (Niedermayer et al., 2017) and 100 d (Arthington et al., 2014a) after the time of administration, respectively. In the present experiment, the relatively larger amount, as compared to the recommended amount to administer, of Zn and Cu supplied with the ITM treatment may have resulted in there being stores of these minerals in the liver for more than 41 d, thus, inducing a greater production of SOD during that period. The concentration of GSH-Px was greater by 4 to 7 d after ITM treatment administration, and this occurred due to the greater supply of Se (a component of GSH-Px; Rotruck et al., 1973).

In the present study, TM status at the start of the study was not determined. Before the start of the study, however, all cows were managed as a single group and were provided the same mineral/vitamin mix. 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 during the early lactation period (NASEM, 2016). Forage did not meet the requirements for Zn (except

for Exp. 2; operation 1) and Cu, and thus, TM deficiency of these elements may have prevailed in some cows even though there was TM supplementation (Arthington et al., 2014a).

A single administration of ITM 11 d before AI did not alter the plasma concentrations of haptoglobin or cause a local inflammation at the injection site. Although haptoglobin theoretically has no correlation with Zn, Mn, Se and Cu, Arthington et al. (2014a) observed increased plasma concentrations of haptoglobin by 6 to 10 d after ITM treatments of Brangus crossbred heifers, indicating a possible inflammatory reaction. In the present study, the application of ITM also did not result in alterations of the plasma concentrations of ceruloplasmin. This is a Cu-dependent acute phase protein, and cattle with greater ITM-induced liver Cu status may have greater plasma concentrations of ceruloplasmin following a stressful event (Arthington et al., 2014a). Although forage in the current experiment had a lesser than NRC recommended Cu concentration, the free-choice mineral supplementation may have provided sufficient supplemental Cu for maximal ceruloplasmin production in response to stress (caused by the application of ITM, blood collection and FTAI protocol), and thus, the additional Cu provided with the ITM treatment was likely not necessary. Also, in the study of Arthington et al. (2014a), Brangus crossbred heifers were treated with a larger ITM dosage than that administered in the current study (1 mL/45 kg compared with 1 mL/66 kg of BW, respectively) and were transported for 1,600 km, which likely explains the inconsistency in results between the two studies.

The ITM treatment in the present study tended to increase pregnancy rate only when cows had a BCS $\leq$ 5.0, which has also been previously reported (Arthington et al., 2014b; Vedovatto et al., 2019). This response might be attributed to the greater BCS in ITM- compared with saline-treated cows during the present study. The greater BCS likely had a greater effect in cows with a BCS $\leq$ 5.0 compared with cows with a BCS > 5.0. Body condition score is an indicator of energy reserves of the animal, and cows with a greater BCS have greater circulating

concentrations of some hormones such as insulin-like growth factor 1 (IGF-I) and leptin. The concentrations of these hormones have been associated with improved reproductive performance (Meikle et al., 2004). Another factor that may have contributed to the increased pregnancy rate of ITM-treated cows was the increased plasma concentrations of the antioxidant enzyme SOD from d -4 to 30, which likely contributed to a post-AI control of oxidative stress in the reproductive organs of the cows of this group. As described by Agarwal et al. (2005), ROS affects multiple physiological processes from oocyte maturation to fertilization, embryonic survival and development, and pregnancy maintenance. The greatest proportion of embryonic deaths occur during the first 21 d after AI (Inskeep and Dailey, 2005), and within this 21-d period, the most prevalent phase when mortality occurs is during the development of the morula to blastocyst stage (d 5 to 8 after AI; Maurer and Chenault, 1983). The greater regulation of oxidative stress at this stage that resulted from the ITM treatment may have contributed, therefore, to the enhanced pregnancy rate of ITM-treated cows.

5. Conclusion

A single ITM treatment administered 11 d before AI did not alter the body weight or elicit an acute phase response in grazing Nellore cows. The ITM treatment, however, tended to result in an enhanced BCS, increase the plasma concentrations of antioxidant enzymes, and tended to increase pregnancy rates to AI, compared with saline-treated cows with a BCS $\leq$ 5.0 even though there was a smaller corpus luteum size.

Conflict of interest

The authors declare that they have no conflict of interest.

316

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325

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Table 1

Chemical composition of forages grazed by cows in Experiments 1 and 2

Item	Pasture				Requirement[a] (NASEM, 2016)
	Exp. 1	Exp. 2 Operation 1	Exp. 2 Operation 2	Exp. 2 Operation 3	
Dry matter (DM), g/kg	281.1	310.5	360.6	515.5	-
g/kg of DM					
Crude protein	73.0	71.3	45.5	44.8	-
Neutral detergent fiber	713.7	720.3	700.6	781.6	-
Acid detergent fiber	425.7	347.0	383.9	488.7	-
Lignin	40.4	36.5	44.1	60.5	-
Ethereal extract	21.5	21.8	22.5	18.9	-
Ashes	100.4	86.5	75.2	77.6	-
Calcium	2.44	2.42	1.83	1.46	-
Phosphorus	1.19	1.45	0.76	0.92	-
Sodium	1.49	1.85	2.61	1.48	1.0
Potassium	9.76	10.59	7.50	10.01	7.0
Magnesium	2.51	1.88	2.08	1.12	2.0
mg/kg of DM					
Iron	115.01	177.3	113.44	155.02	50.0
Zinc	19.64	31.9	11.02	14.20	30.0
Manganese	132.16	138.8	88.98	71.55	40.0
Selenium	0.11	0.16	0.16	0.07	0.1
Copper	2.07	4.32	2.10	2.77	10.0

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

Table 2

Mineral composition of the complete trace mineral/vitamin mixtures offered to cows in Experiments 1 and 2

Item[a]	Mineral/vitamin mix			
	Exp. 1[b]	Exp. 2[c] Operation 1	Exp. 2[d] Operation 2	Exp. 2[e] Operation 3
g/kg of dry matter (DM)				
Calcium	196	150 – 220	139 – 155	111 – 135
Phosphorus	90	81	80	90
Sodium	99	114	130	141
Magnesium	20	-	10	-
Sulfur	20	14	40	18
mg/kg of DM				
Fluorine	900	810	800	900
Cobalt	200	60	80	60
Iodine	180	78	100	75
Iron	2400	-	-	1800
Zinc	3000	5250	5000	4500
Manganese	1670	1040	1040	1800
Selenium	40	22	26	17
Copper	1200	1500	1350	1500
UI/kg				
Vitamin A	150000	-	-	-
Vitamin D3	30000	-	-	-
Vitamin E	1500	-	-	-

[a]Source of zinc, manganese, selenium and copper used in Exp.1 and 2 were zinc oxide, manganese monoxide, sodium selenite and copper sulphate, 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)

452 [d]BellNutri (Trouw Nutrition, Mirassol, SP, Brazil; target consumption of 75 g/day)

453 [e]Fosbovi Reprodução (DSM Produtos Nutricionais, Campo Grande, MS, Brazil; target

454 consumption of 90 - 120 g/day)

455 **Table 3**

456 Data for body condition variables, ovarian structures, pregnancy rate, and plasma
457 measurements of Nellore cows administered a single subcutaneous injection (6 mL/cow) of
458 saline solution or injectable trace mineral (ITM) 11 d before AI (d -11; Exp. 1)

Item[a]	Treatment[b]		SEM	*P*-value	
	ITM	Saline		Trt. × day	Trt.
Body variables					
BW, kg	400.7	398.6	3.5	0.68	0.62
BW change, kg					
d -11 to 0	-12.4	-16.5	2.9		0.33
d 0 to 30	36.6	38.6	3.6		0.71
d -11 to 30	24.2	22.1	5.1		0.77
BCS	4.7	4.3	0.13	0.22	0.06
BCS change					
d -11 to 0	-0.2	-0.7	0.23		0.15
d 0 to 30	0.4	0.3	0.19		0.71
d -11 to 30	0.2	-0.4	0.21		0.06
Ovarian structures					
Dominant follicle size (d 0), mm	13.6	13.9	1.1		0.86
CL diameter (d 14), mm	30.1	38.0	2.6		0.06
CL volume (d 14), cm	15.6	34.1	5.1		0.03
Ovulation rate,[c] %	80 (8/10)	100 (10/10)	9.5		0.16
Pregnancy rate,[c] %	70 (7/10)	60 (6/10)	16.1		0.66
Plasma analyses					
Superoxide dismutase, U/mL	3.7	3.1	0.18	0.06	0.02
Glutathione peroxidase, nmol/min/mL	2.9	2.2	0.14	0.007	0.003
Haptoglobin, mg/mL	0.15	0.13	0.02	0.43	0.43
Ceruloplasmin, mg/mL	18.1	18.7	0.77	0.78	0.46
Progesterone,[d] ng/mL					
Cows pregnant	3.9	3.7	0.7	0.52	0.23

Overall	3.7	3.1	0.5	0.94	0.87

[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

[c]Values in parentheses represent the number of cows pregnant or that ovulated/number of cows evaluated

[d]Only cows that ovulated were included in the statistical analyses

Table 4

Pregnancy rate to AI of Nellore cows administered a single subcutaneous injection (6 mL/cow) of saline solution or injectable trace mineral (ITM) 11 d before AI (d -11; Exp. 2)

BCS[a]	Treatment[b]		SEM	*P*-value
	ITM	Saline		Treatment
3.5 to 5.0	53.7 (269/501)	48.1 (237/493)	3.9	0.06
5.5 to 6.5	57.9 (44/76)	58.1 (43/74)	6.5	0.99
All cows	54.2 (313/577)	49.4 (280/567)	8.1	0.09

[a]Body condition score (scale 1 - 9) evaluated on d -11 according to Herd and Sprott (1986) Number of cows per BCS: 3.5 (*n* = 28), 4.0 (*n* = 224), 4.5 (*n* = 63), 5.0 (*n* = 679), 5.5 (*n* = 32), 6.0 (*n* = 97), and 6.5 (*n* = 21)

[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); Values in parentheses represent the number of cows pregnant/number of cows evaluated

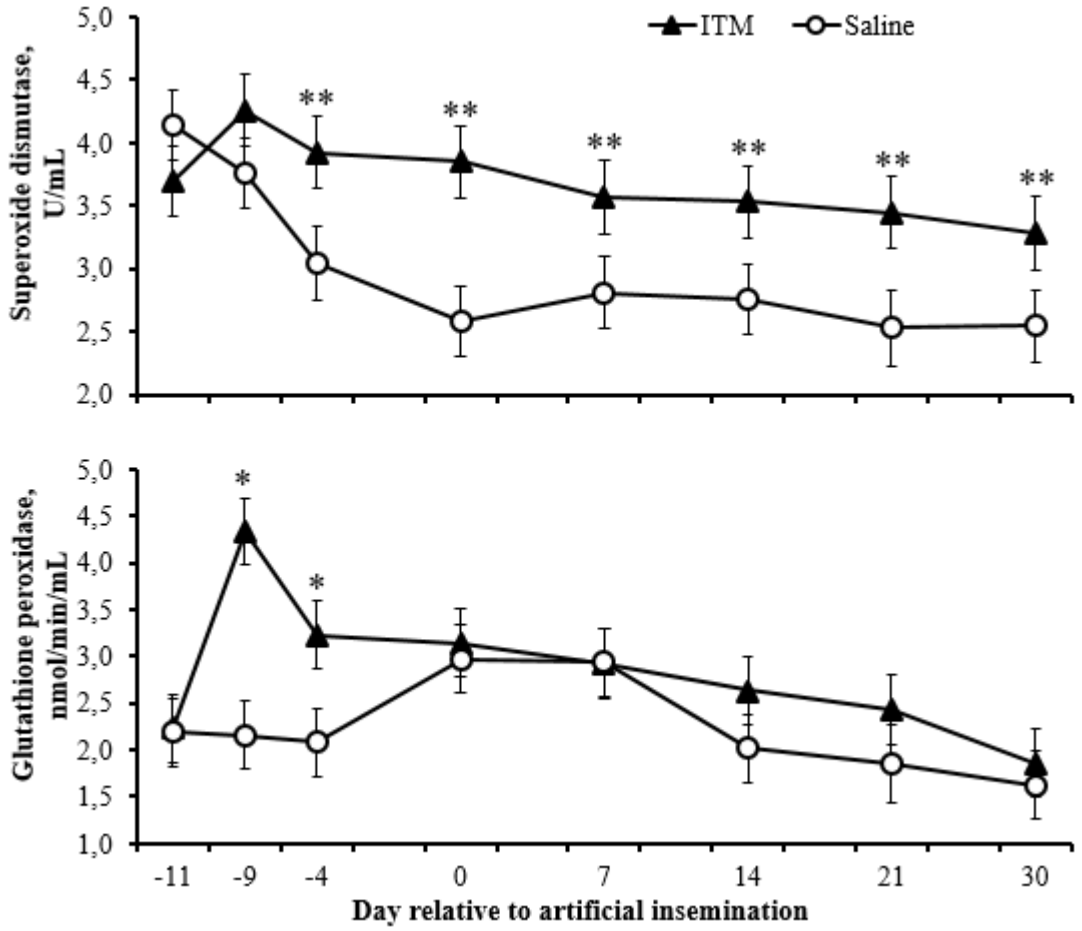
477 **Figure caption**

478

479 **Fig. 1.** Plasma concentrations of superoxide dismutase and glutathione peroxidase of Nellore
480 cows administered a single subcutaneous injection (6 mL/cow) of saline solution or injectable
481 trace mineral (ITM) 11 d before AI (d -11; Exp. 1); *On the same day indicate a difference (P
482 ≤ 0.05); **On the same day indicate a tendency to differ ($P \leq 0.10$)

483

484 **Fig. 1**


ITM
Saline
Superoxide dismutase, U/mL
5,0
4,5
4,0
3,5
3,0
2,5
2,0
**
**
**
**
**
**
Glutathione peroxidase, nmol/min/mL
5,0
4,5
4,0
3,5
3,0
2,5
2,0
1,5
1,0
*
*
-11
-9
-4
0
7
14
21
30
Day relative to artificial insemination

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