Tropical Animal Health and Production
https://doi.org/10.1007/s11250-019-02056-0

SHORT COMMUNICATIONS

# Effect of a trace mineral injection at weaning on growth, antioxidant enzymes activity, and immune system in Nellore calves

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Received: 28 May 2019 / Accepted: 19 August 2019


## Abstract

The objective of this study was to evaluate the effects of injectable trace minerals (ITM) at the time of weaning on growth, antioxidant, and immune response of Nellore (*Bos indicus*) calves. Weaned calves ($n = 159$; 213 ± 32 kg) were stratified by body weight (BW) and randomly assigned to 1 of 2 treatments: injection (1 mL/45 kg of BW) of saline or ITM. Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15 mg/mL of Zn, Mn, Se, and Cu, respectively. The application of ITM increased ($P \leq 0.04$) the plasma concentrations of superoxide dismutase on day 7 and plasma glutathione peroxidase on day 7 and day 21. The ITM calves had greater leukocyte concentration on day 64 ($P = 0.04$), whereas neutrophil and mast cell concentrations did not differ ($P \geq 0.67$) between treatments. The ITM calves tended ($P \leq 0.08$) to have greater concentrations of lymphocytes on day 64 and eosinophils on day 21, but not ($P \geq 0.15$) monocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin compared to saline calves. The ITM calves had less mean corpuscular hemoglobin concentration (MCHC; $P = 0.02$) and tended to have increased platelet concentrations on day 21 ($P = 0.08$). Growth performance did not differ between treatments ($P \geq 0.78$). Thus, ITM at weaning did not impact growth performance, but increased plasma concentrations of antioxidant enzymes and blood platelets, improved components of the immune system, and reduced MCHC of Nellore calves.

**Keywords** Cattle · Glutathione peroxidase · Hemogram · Superoxide dismutase · Weight gain

## Introduction

Weaning is a stressful event in the calf's life that impacts behavior, stress-related hormones, immune function (Lynch et al. 2012), antioxidant enzyme activity, and consequently the oxidative stress (Burke et al. 2009). Some trace minerals (TM) as Zn, Mn, Se, and Cu are essentials for the proliferation of immune system cells (Maggini et al. 2007) and are components of antioxidant enzymes (Rotruck et al. 1973; Markclund 1980). Zinc, Mn, Se, and Cu are essential for beef cattle (NRC 2016) and for grazing animals are normally provided by free-choice mineral supplementation (Arthington et al. 2014). However, weaning may affect post-weaning voluntary intake of trace mineral (TM) supplement and lead to highly variable TM status within the herd (Manzano et al. 2012). Injectable trace minerals (ITM) eliminates the individual variability on TM status through the application of a known quantity in each animal, and increase the control of oxidative stress and immunological status (Teixeira et al. 2014; Soldá et al. 2017; Vedovatto et al. 2019). Thus, ITM may be an alternative to enhance the TM status of grazing recently weaned beef calves.

Arthington et al. (2014) evaluated the application of ITM in newly weaned calves and observed a tendency to increase weight gain. These animals were Brangus-crossbred and weaned at a time of year with optimum forage quantity and quality. In tropical regions, however, it is common to use *Bos indicus* breeds and wean calves during the dry season with low availability of low-quality forages. Furthermore, calf breed may affect the amount of antioxidant enzymes produced and the storage time of TM following ITM (Pogge et al. 2012), and thus, the use of ITM in *Bos indicus* breeds may

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Published online: 30 August 2019

not elicit similar responses on calf performance compared to that observed in Brangus-crossbred calves (Arthington et al. 2014). We are unaware of a study evaluating the effect of ITM on growth and components of the immune system of *Bos indicus* calves. We hypothesized that the application of ITM in calves at weaning will increase the plasma concentrations of antioxidant enzymes and growth performance, and improve the immune system, compared to saline-injected calves. Thus, the objective of this study was to evaluate the impact of ITM at weaning on post-weaning plasma concentrations of antioxidant enzymes, components of the immune system, and growth performance of Nellore calves grazing low-quality, warm-season grasses.

## Material and methods

### Animals, treatments, and samples collection

The experiment was conducted from 29 May to 21 September 2016, at Bela Vista Farm, located in Camapuã, MS, Brazil (19° 0′ 41.15″ latitude south and 53° 45′ 13.28″ longitude west). A total of 159 Nellore calves (8 ± 1 months of age; 213 ± 32 kg) were numerically identified with incandescent iron on the right leg and received a subcutaneous injection of ivermectin 1% (Ivomec, Merial, Brazil) at the time of weaning (day 0). On day 0, calves were also stratified by body weight (BW) and randomly assigned into 1 of the 2 treatments: saline injection (Saline; 0.9% NaCl; 1 mL/45 kg of BW) and ITM (1 mL/45 kg of BW). Calves were processed twice at weaning, first time for BW collection and second for treatment administration. The trace mineral injection solution had 60 mg of Zn/mL, 10 mg of Mn/mL, 5 mg of Se/mL, and 15 mg of Cu/mL (Multimin 90, Multimin, Fort Collins, CO, USA). Saline and TM injections were administered subcutaneously on the right side of the neck of each calf.

Immediately after treatment application (day 0), all calves were transferred to a single pasture (pasture A; 20 ha) for 21 days. Thereafter, calves were transferred to another pasture on day 22 (pasture B; 40 ha), where they remained until the end of the experiment (day 115). Both pastures consisted of xaraés-grass (*Urochloa brizantha* cv Xaraés) and had feed bunks for free-choice mineral supplementation and water drinkers with automatic replenishment. Trace mineral supplementation (8.8% Ca, 6.5% P, 14.5% Na, and 4690 mg/kg S, 44.5 mg/kg Co, 1500 mg/kg Fe, 60 mg/kg I, 650 mg/kg F, 2880 mg/kg Zn, 1050 mg/kg Mn, 10 mg/kg Se, and 1200 mg/kg Cu; Fosbovi 15, DSM Nutritional Products, Brazil) was offered free choice to all calves for a target daily intake of 60 g/calf. According to the manufacturer, the mineral sources used in the mixture were sodium chloride, flowers of sulfur, dicalcium phosphate, sulfur transchelate, calcium iodate, manganese monoxide, cobalt transchelate, copper transchelate, manganese transchelate, selenium transchelate, zinc transchelate, cobalt sulfate, copper sulfate monohydrate, iron sulfate, zinc sulfate, and kaolin. Nutritional composition of pastures was evaluated by manual harvesting and simulating grazing, and performed on days 0, 21, 64, and 115. Forage samples were then dried at 60 °C for 5 days, ground to 1 mm, and analyzed for chemical composition (Table 1).

Live BW (LBW) of calves were collected individually at 0800 h on days 0, 21, 64, and 115. Fasted BW was not obtained to avoid shrink-induced stress effects on blood parameters evaluated in the study. Blood samples were collected from jugular vein (10 calves/treatment) on days 0, 7, 21, and 64 into blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin (10 mL) or $K_2$EDTA (4 mL). After collection, blood samples were immediately stored on ice. Blood samples containing sodium heparin were centrifuged at 1200×*g* for 30 min for plasma harvest. Plasma samples were stored at − 20 °C for further analysis of the concentration of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Blood samples containing $K_2$EDTA were stored at 4 °C overnight, and hematological analysis were performed in these samples 24 h after collection.

### Laboratory analysis

The concentration of dry matter (DM), crude protein (CP), ethereal extract (EE), and ashes in forage samples were analyzed as described by AOAC (2000; methods 930.15, 976.05, 920.39, and 942.05, respectively). The concentration of neutral detergent fiber (NDF) and acid (ADF) was performed as described by Van Soest et al. (1991). The TM concentration was analyzed via inductively coupled plasma mass spectrometry, and Se was analyzed as described by Oliveira et al. (2016) and the other minerals as described by Braselton et al. (1997). The enzymes GSH-Px and SOD were analyzed using commercial kits for ELISA (Cayman Chemical, Ann Arbor, MI, USA, catalog number 703102 and 706002, respectively). The inter and intra-assay coefficients of variation for SOD were 4.9 and 7.2% and for GSH-Px were 4.2 and 9.8%, respectively. The hemogram was analyzed in complete blood samples in automated equipment (pocH-100iV DIFF Sysmex) as described by Riond et al. (2011).

### Statistical analysis

All data were analyzed as a completely randomized design using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. The Satterthwaite approximation was used rather than pooled variance because the variances of the two treatments were not equal. The average daily gain (ADG), BW, and blood data were considered dependent variables, while treatment,

**Table 1** Chemical composition of xaraés-grass during the experiment

| Items | Pasture A | | Pasture B | | Requirements (NRC 2016) |
|---|---|---|---|---|---|
| | Day 0 | Day 21 | Day 64 | Day 115 | |
| DM (%) | 27.3 | 29.9 | 35.2 | 34.4 | – |
| CP (% of DM) | 9.06 | 8.09 | 4.00 | 4.96 | – |
| NDF (% of DM) | 73.1 | 70.2 | 69.8 | 67.8 | – |
| ADF (% of DM) | 37.6 | 35.2 | 37.4 | 37.1 | – |
| Lignin (% of DM) | 4.06 | 3.76 | 3.11 | 3.21 | – |
| EE (% of DM) | 2.18 | 2.27 | 2.28 | 2.45 | – |
| Ashes (% of DM) | 6.03 | 5.63 | 8.02 | 8.91 | – |
| Ca (% of DM) | 0.13 | 0.24 | 0.15 | 0.24 | – |
| P (% of DM) | 0.18 | 0.15 | 0.09 | 0.12 | – |
| Na (% of DM) | 0.18 | 0.19 | 0.15 | 0.19 | 0.06–0.08 |
| K (% of DM) | 1.15 | 1.23 | 1.00 | 1.08 | 0.60 |
| Mg (% of DM) | 0.18 | 0.29 | 0.11 | 0.17 | 0.10 |
| Fe (mg/kg of DM) | 128 | 135 | 155 | 111 | 50.0 |
| Zn (mg/kg of DM) | 28.7 | 22.1 | 14.2 | 11.3 | 30.0 |
| Mn (mg/kg of DM) | 139 | 157 | 71.5 | 127 | 20.0 |
| Se (mg/kg of DM) | 0.07 | 0.08 | 0.08 | 0.06 | 0.10 |
| Cu (mg/kg of DM) | 5.99 | 5.36 | 2.77 | 1.43 | 10.0 |

*DM*, dry matter; *CP*, crude protein; *NDF*, neutral detergent fiber; *ADF*, acid detergent fiber; *EE*, ethereal extract

day, and calf as independent variables. The statistical model for ADG had treatment as a fixed effect and calf (treatment) as random effect. However, BW and all blood data were analyzed as repeated measures and the statistical model had treatment, day, and resulting interaction as fixed effects, and calf (treatment) as a random effect. Also, calf (treatment) was used as subject. The BW and blood data obtained on day 0 were included as covariates in each respective analysis, but were removed from the model when $P > 0.10$. The compound symmetry covariance structure was selected for all statistical analyses as it generated the lowest Akaike information criterion. Means were separated using PDIFF, and all results were reported as LSMEANS followed by standard error of the means (SEM). Significance was defined when $P \leq 0.05$, and tendency when $P > 0.05$ and $\leq 0.10$.

## Results

Effects of treatment × day, but not treatment ($P \geq 0.39$), were detected ($P \leq 0.04$) for plasma concentrations of SOD and GSH-Px (Table 2). The application of ITM increased the plasma concentration of SOD on day 7 ($P = 0.01$) and plasma concentrations of GSH-Px on days 7 and 21 ($P \leq 0.02$), compared with saline injection (Table 2).

Effects of treatment × day, but not treatment ($P = 0.32$), were detected ($P = 0.04$) for blood concentrations of leukocytes (Table 2). The application of ITM increased the leukocyte concentration only on day 64 ($P = 0.01$) compared with saline injection (Table 2). Effects of treatment × day and treatment were not detected ($P \geq 0.67$) for blood concentrations of neutrophils and mast cells (Table 2). Effects of treatment × day, but not treatment ($P = 0.27$), tended ($P = 0.08$) to be detected for blood concentrations of lymphocyte (Table 2). The ITM application tended to increase lymphocyte concentration only on day 64 ($P = 0.06$; Table 2). Effects of treatment × day and treatment, were not detected ($P \geq 0.22$) for blood concentration of monocytes (Table 2). Effects of treatment × day, but not treatment ($P = 0.51$), tended ($P = 0.06$) to be detected for blood concentration of eosinophils (Table 2). The application of ITM tended to increase the concentration of eosinophils only on day 21 ($P = 0.06$), compared to saline injection (Table 2).

Effects of treatment × day and treatment, were not detected ($P \geq 0.15$) for erythrocytes, hemoglobin, hematocrit (Table 2). Effects of treatment × day and treatment were not detected ($P \geq 0.33$) for mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH; Table 2). Effects of treatment, but not treatment × day ($P \geq 0.12$), were detected ($P = 0.03$) for MCHC (Table 2). The application of ITM reduced the mean corpuscular hemoglobin concentration ($P = 0.03$; MCHC), compared with saline injection (Table 2). Effects of treatment × day, but not treatment ($P \geq 0.38$) tended to be detected ($P = 0.08$) for blood concentrations of platelets (Table 2). The application of ITM tended to increase the blood concentrations of platelets on day 21 ($P = 0.07$) compared with saline calves (Table 2).

**Table 2** Blood concentrations of antioxidants enzymes, leukogram, erytogram, and platelets of Nellore calves grazing low-quality warm-season grasses and administered a single subcutaneous injection of saline solution or injectable trace mineral (ITM) at weaning

| Items[a] | Treatments[b] | | SEM | *P* value | |
|---|---|---|---|---|---|
| | Saline | ITM | | Treatment × day | Treatment |
| Antioxidants enzymes | | | | | |
| Superoxide dismutase (U/mL) | | | | 0.04 | 0.43 |
| day 0 | 3.83 | 3.82 | 0.33 | | |
| day 7 | 3.45[b] | 4.65[a] | 0.33 | | |
| day 21 | 3.39 | 3.51 | 0.33 | | |
| day 64 | 3.37 | 2.82 | 0.33 | | |
| Glutathione peroxidase (nmol/min/mL) | | | | 0.01 | 0.39 |
| day 0 | 1.50 | 1.57 | 0.27 | | |
| day 7 | 1.73[b] | 2.45[a] | 0.27 | | |
| day 21 | 2.01[b] | 2.97[a] | 0.27 | | |
| day 64 | 2.18 | 2.25 | 0.27 | | |
| *Leukogram* | | | | | |
| Leukocytes ($\times 10^3$ cells/μL) | | | | 0.04 | 0.32 |
| day 0 | 20.3 | 18.9 | 1.34 | | |
| day 7 | 17.2 | 18.6 | 1.34 | | |
| day 21 | 18.1 | 19.1 | 1.34 | | |
| day 64 | 17.6[b] | 22.6[a] | 1.34 | | |
| Neutrophils ($\times 10^3$ cells/μL) | 4.51 | 4.78 | 0.43 | 0.76 | 0.67 |
| Lymphocytes ($\times 10^3$ cells/μL) | | | | 0.08 | 0.27 |
| day 0 | 14.1 | 13.6 | 1.09 | | |
| day 7 | 11.1 | 12.2 | 1.09 | | |
| day 21 | 12.3 | 12.3 | 1.09 | | |
| day 64 | 11.5[b] | 15.7[a] | 1.09 | | |
| Monocytes ($\times 10^3$ cells/μL) | 0.63 | 0.64 | 0.11 | 0.22 | 0.97 |
| Eosinophils ($\times 10^3$ cells/μL) | | | | 0.06 | 0.51 |
| day 0 | 1.34 | 1.08 | 0.21 | | |
| day 7 | 0.38 | 0.72 | 0.21 | | |
| day 21 | 0.23[b] | 0.85[a] | 0.21 | | |
| day 64 | 0.64 | 0.48 | 0.21 | | |
| Mast cells ($\times 10^3$ cells/μL) | 0.04 | 0.03 | 0.03 | 0.99 | 0.89 |
| Erytogram | | | | | |
| Erythrocytes ($\times 10^6$ cells/μL) | 9.69 | 9.93 | 0.18 | 0.15 | 0.39 |
| Hemoglobin (g/dL) | 11.9 | 11.9 | 0.16 | 0.36 | 0.81 |
| Hematocrit (%) | 35.8 | 36.7 | 0.49 | 0.29 | 0.25 |
| MCV (fL) | 37.1 | 37.1 | 0.21 | 0.99 | 0.97 |
| MCH (pg) | 12.3 | 12.1 | 0.16 | 0.53 | 0.33 |
| MCHC (pg) | 33.3 | 32.5 | 0.22 | 0.12 | 0.03 |
| Platelets ($\times 10^3$ cells/μL) | | | | 0.08 | 0.38 |
| day 0 | 287 | 292 | 42.4 | | |
| day 7 | 365 | 371 | 88.1 | | |
| day 21 | 215[b] | 431[a] | 73.5 | | |
| day 64 | 240 | 232 | 51.1 | | |

[a] *MCV*, mean corpuscular volume, *MCH*, mean corpuscular hemoglobin, *MCHC*, mean corpuscular hemoglobin concentration

[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 (1 mL/45 kg of body weight) on the right side of the neck of each calf

Effects of treatment × day and treatment were not detected ($P \geq 0.85$) for calf BW, and the final LBW were 233 and 234 ± 0.85 kg for Saline and ITM calves, respectively. Calf ADG did not differ ($P \geq 0.78$) between treatments. The ADG from day 0 to day 64 were 0.10 and 0.10 ± 0.03 and from day 64 to day 115 were 0.26 and 0.27 ± 0.02 for Saline and ITM calves, respectively.

## Discussion

The use of ITM increased the production of the antioxidants enzymes SOD on day 7 and GSH-Px on day 7 and day 21 relative to application. Others studies also showed that the application of ITM increases the antioxidant enzyme activity in calves (Teixeira et al. 2014) and cows (Machado et al. 2014; Vedovatto et al. 2019). Zinc, Mn, Se, and Cu are components of a series of enzymes controlling the oxidative stress of cells and components of the SOD enzymes found in the form of Cu/Zn-SOD and Mn-SOD (Markclund 1980), whereas Se is a component of the GSH-Px (Rotruck et al. 1973).

Injectable TM increased the leukocyte concentration in the blood and also showed a tendency to increase the blood concentration of lymphocytes, eosinophils, and platelets. This result is in agreement with the findings of other studies that show that the application of ITM stimulates the immune system (Teixeira et al. 2014; Soldá et al. 2017). In our study, the increased production of SOD and GSH-Px following ITM possibly reflected the lower lipid peroxidation and cellular damage to leukocytes and platelets, and this possibly caused an increase in the concentration of these, compared to saline-injected calves. Furthermore, Zn is essential for highly proliferating cells, especially in the immune system, and Cu stimulates neutrophil and monocyte responses (Maggini et al. 2007). In addition, Se is component of the enzyme thioredoxin reductase, that affects the redox regulation of several key enzymes, transcription factors, and receptors that affect the expression of genes-encoding proteins involved in immune response (Maggini et al. 2007).

Weaning is a stressful event in the calf's life and impairs the immune function (Lynch et al. 2012) and the DM intake (Eckert et al. 2015). Although the mineral supplement intake was not assessed in the current study, this was possibly reduced by weaning stress. Thus, the application of ITM may have helped to maintain greater TM status after weaning and, thus, better immune response. Furthermore, in previous study, the intake of mineral supplement provided by free-choice for grazing steers, ranged from 0 to 400 g/animal (Manzano et al. 2012), indicating highly variable on mineral intake and consequently TM status within the same herd. Therefore, the application of ITM may have elevated the TM status of animals which has low voluntary mineral supplement intake and thus improved the immune system in these calves.

Injectable TM reduced blood MCHC. Arthington et al. (2014) observed that the ITM application in preweaned calves reduced the liver concentrations of Fe and increased the plasma concentrations of ceruloplasmin. According Roeser et al. (1970), ceruloplasmin is the primary Cu transport protein in the blood and is also an important mediator for Fe mobilization in the body. Thus, it is possible that the greater Cu supply following ITM negatively impacted Fe supply to hemoglobin, and this response reduced MCHC. Nonetheless, MCHC values reported in the present study were within the accepted normal range for cattle (30 to 36 g/dL; Jackson and Cockcroft 2002).

The application of ITM did not affect the growth performance, and this may have happened because the animals might not be TM deficient. When animals remain for a period consuming a diet deficient only in Zn, Mn, Se, and Cu, and subsequently consume a diet that meets the nutritional requirements associated to the application of ITM, they present greater ADG compared to those that did not receive ITM (Genther and Hansen 2014). However, if they are not TM deficient, the TM injection did not improve the growth performance (Genther and Hansen 2014). In our study, it is not possible to confirm if calves were deficient in Zn, Se,s and Cu during the weaning period, because liver samples were not collected during the study. However, calves probably were not deficient in Mn, since only pasture met the requirement of this TM, and the free-choice mineral provided may have been sufficient to complement the Zn, Se, and Cu provided by pasture and meets the requirements. In addition, in the current study, the greatest deficiencies were protein and energy, and thus, despite the higher supply of TM through ITM, the high deficiency of protein and energy may have limited growth and prevented ITM-induced increase on calf growth performance.

Thus, injectable trace minerals at the time of weaning increased the plasma concentration of antioxidant enzymes, blood concentrations of platelets, and components of immune system, but reduced the blood concentration of MCHC, and did not affect the body weight gain of Nellore calves grazing low-quality tropical grasses.

**Acknowledgments** We would like to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by the scholarship provided to the first author, the company Multimin (Fort Collins, CO, USA,) by the donation of the ITM, and to Bela Vista Farm (Camapuã, MS, Brazil) by the possibility of performing the experiment.

**Funding information** This study is funded by the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Técnologia do Estado de Mato Grosso do Sul (FUNDECT; 116/2016).

## Compliance with ethical standards

This study was conducted according to the ethical standards applied to animal research 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.

**Conflict of interest** The authors declare that they have no conflicts of interest.

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