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Effect of the injection of trace minerals on growth performance, health, antioxidant enzymes activity, and immune system of newborn Boer kids

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ABSTRACT - The objective of this study was to evaluate the effects of injectable trace minerals (ITM) on growth, health, antioxidant enzyme activity, and immune system of newborn Boer kids. Newborn kids (n = 125) were assigned to one of two treatments: injection (0.1 mL 4.5 kg^{-1}) of saline or ITM. Injectable trace minerals had 60, 10, 5, and 15 mg mL^{-1} of Zn, Mn, Se, and Cu, respectively. Kids were evaluated daily for the presence of diarrhea and weighted on d 0, 28, and 56. Blood samples were obtained on d 0, 3, 7, 14, 28, and 56. The ITM injection increased the plasma concentration of superoxide dismutase (d 14), glutathione peroxidase (d 3 and 7), and blood platelets (d 7) compared with saline injection. Kids receiving ITM showed greater amount of blood eosinophils and less mean corpuscular hemoglobin (MCH; d 3) and mean corpuscular hemoglobin concentration (MCHC) compared with kids receiving saline injection. The ITM injection did not affect other components of leukogram and erytogram. The ITM-injected kids tended to have less diarrhea incidence compared with saline-injected kids (20.7 vs. 34.8±7.10% respectively). The ITM injection did not affect mortality rate and growth. Therefore, a single ITM injection administered to newborn Boer kids increases the plasma concentration of antioxidant enzymes, platelets, and eosinophils, reduces MCH, MCHC, and tends to reduce the incidence of diarrhea, but does not affect mortality and growth.

Keywords: ADG, diarrhea, glutathione peroxidase, goats, hemogram, superoxide dismutase

Introduction

Low status of trace minerals (TM) in animals is usually associated with increased damage from oxidative stress to cells, poor immune system, greater incidence of disease, and reduced growth and reproductive performance (NRC, 2005). According to the NRC (2005), TM requirements are affected by several factors, including the interaction between TM and dietary antagonists. For example, Cu absorption coefficient is negatively affected by the dietary concentration of Mo, S, and Fe, whereas Zn absorption coefficient is affected by the dietary amount of phytate, Fe, Ca, and Cu (NRC, 2005). An alternative method for providing TM and avoid these problems is through the injectable TM (ITM; Arthington et al., 2014). In addition, for newborn kids, solid feed intake is relatively small and highly variable. Hence, injection may be an alternative to provide a known amount of TM for all animals from the same cohort.

In calves, ITM containing Zn, Mn, Se, and Cu increases the liver concentration of these TM (Arthington et al., 2014), and in cattle and sheep, it improves several components of the immune system and increases the plasma concentration of antioxidant enzymes (Cazarotto et al., 2018;

Teixeira et al., 2014; Tomasi et al., 2018; Vedovatto et al., 2019a,b; Vedovatto et al., 2020), resulting in improved health (Teixeira et al., 2014) and growth performance (Cazarotto et al., 2018). Although several studies have evaluated the use of ITM containing Zn, Mn, Se, and Cu in cattle (Arthington et al., 2014; Teixeira et al., 2014; Tomasi et al., 2018; Vedovatto et al., 2019a,b; Vedovatto et al., 2020) and lambs (Cazarotto et al., 2018), we are unaware of studies evaluating the effects of ITM in goats. In addition, the most susceptible stage of mortality of goats is from birth to weaning, because their immune system is not fully developed and is, thus, more susceptible to diseases.

We hypothesized that the application of ITM in newborn kids will improve the immunological system, reduce the incidence of diarrhea, and increase growth compared with saline injection. Thus, the objective of this study was to evaluate the effects of ITM on growth, health, antioxidant enzyme activity, and immune system of newborn Boer kids.

Material and Methods

This study was conducted according to the ethical standards applied to animal research and approved by the institutional ethics committee on animal use (case no. 754/2016). The experiment lasted 56 d and was conducted in Bandeirantes, MS, Brazil (19°53'15.9" South latitude and 54°24'43.0" West longitude).

Before the beginning of the experiment, 71 goats were selected from a fixed-time artificial insemination season and diagnosed as pregnant. These remained during the first four months of gestation on pasture of Marandu-grass [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu] and, in the last month of gestation, were transferred to a feedlot, and allocated in six pens (±12 goats pen^{-1}). Each pen (±50 m^2) had feed bunks, water drinkers with automatic replenishment, and creep-feeding structure (±6 m^2; for concentrate supply only for kids). A total of 125 Boer kids [62 males and 63 females; body weight (BW) = 6.6±1.82 kg; 0 to 10 (4.4±3.6) d of age] were identified with a numbered earring at birth.

In the last month of gestation, the goats received concentrate at 1.5% of BW [dry matter (DM) basis], and free-choice access to mineral mixture and corn silage (Table 1). The silage and concentrate were mixed immediately before offering to animals and provided into two daily equal meal amounts (08.00 and 16.00 h). The amount of silage was adjusted daily to provide orts at 50 g kg^{-1}. The mineral mixture was supplied in separate feed bunks and replenished as needed. After the goats gave birth, the amount of concentrate was changed to 1% of BW (DM basis). Diets were formulated to meet or exceed the requirements for crude protein (CP) and minerals for goats weighing 50 kg (double-kids) at late gestation and early lactation (NRC, 2007). Kids received their concentrate (Table 1) *ad libitum* in goat exclusion areas (creep-feeding). The concentrate was divided and offered in two equal daily meal amounts (at 08.00 and 16.00 h). Concentrate amount was adjusted daily to provide orts at 50 g kg^{-1}. The concentrate offered to kids met or exceeded the requirements for CP and minerals for Boer kids weighing 10 kg and gaining 0.1 kg of BW daily (NRC, 2007). In addition to the concentrate provided in the creep-feeding, all kids had access to the diet offered to their respective goats.

Of the 71 selected goats, six had triplets, 42 twins, and 23 single kids. All kids with 4.4±3.6 d of age were stratified by type of birth, sex, and BW and then randomly assigned into one of two treatments: subcutaneous injection (0.1 mL 4.5 kg^{-1}) of saline solution (saline; 31 and 32 males and females, respectively and 9, 42, and 12 kids born from triplet, twin, and single births, respectively) or ITM (31 and 31 males and females, respectively and 9, 42, and 11 kids born from triplet, twin, and single births, respectively). Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15 mg mL^{-1} 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 kid. At 30 d of age, all kids were vaccinated against clostridiosis (Sintoxan 9TH, Merial Saúde Animal, Brazil), pasteurellosis, and paratyphoid (Tifopasteurina, Hertape Calier Saúde Animal, Brazil).

Table 1 - Chemical composition of corn silage, concentrate, and mineral mixture provided for goats and kids

Item	Corn silage[1]	Concentrate for goats[2,3]	Concentrate for kids[2,4]	Mineral mixture[2,5]
g kg^{-1} of dry matter (DM)				
Crude protein	63.0	160	215	-
Neutral detergent fiber	467	-	-	-
Ether extract	24.3	30.0	30.0	-
Ash	52.2	-	-	-
Non-fibrous carbohydrates	393	-	-	-
Calcium	0.03	12.0	10.0	160
Phosphorus	0.79	4.80	4.00	47.3
Sodium	1.07	2.10	1.50	133
Potassium	6.00	6.50	5.50	18.8
Magnesium	0.95	2.00	2.00	13.3
mg kg^{-1} of DM				
Iron	0.49	51.9	85.0	166
Zinc	0.01	56.2	35.0	1133
Manganese	7.83	35.0	35.0	900
Selenium	0.07	0.42	1.20	10.0
Copper	0.01	14.0	25.0	267

[1] Chemical composition analyzed.
[2] Guaranteed levels described by the manufacturers.
[3] Caprinos Manutenção Caprivales, Só Sal Nutrição e Saúde Animal, Campo Grande, MS, Brazil.
[4] Caprinos Creep Caprivales, Só Sal Nutrição e Saúde Animal, Campo Grande, MS, Brazil.
[5] Caprinofós, DSM Produtos Nutricionais, Brasil. This product was mixed with NaCl in the ratio of 2:1 as recommended by the manufacturer, and the values presented were adjusted for this final mixture. Target intake for lactating goats of 20 g day^{-1}.

All kids were weighed on d 0 (before the ITM or saline injection), 28, and 56. The occurrence of diarrhea was observed daily following the methodology described by Larson et al. (1977), which is based on fecal score of fluidity: 1 - normal; 2 - soft; 3 - runny; and 4 - watery. The animal was considered with diarrhea if presented scores 3 or 4 for two or more consecutive days. Blood samples were collected from jugular vein from 12 kids per treatment (six males and six females randomly selected on d 0) on d 0, 3, 7, 14, 28, and 56 into blood collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) containing sodium heparin (5 mL) or K_2EDTA (4 mL). During the collection, blood samples were immediately stored on ice. Then, 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_2EDTA were stored at 4 °C overnight, and hematological analyzes were performed in these samples 24 h after collection. The farm where the study was conducted was not close to the laboratory; therefore, it was not possible to analyze the samples on the same day that they were collected. Silage samples were collected at different locations in the silo, dried at 60 °C for 5 d, ground to 1 mm, and analyzed for chemical composition.

Silage samples were analyzed according to AOAC (2000): DM, method 930.15; CP, method 976.05; ether extract (EE), method 920.39; and ashes, method 942.05. The concentration of neutral detergent fiber (NDF) was determined according to the methodology of Van Soest et al. (1991), and non-fibrous carbohydrates (NFC) were calculated according to NRC (2001): NFC (%) = 100 – (% NDF + % CP + % EE + % ash). The TM concentration was analyzed via inductively coupled plasma mass spectrometry, 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 determined by commercial ELISA kits (Cayman Chemical, Ann Arbor, MI, USA, catalog number 703102 and 706002, respectively). The inter and intra-assay coefficients of variation for SOD were 5.4 and 8.1%, and for GSH-Px, they were 5.2 and 9.6%, respectively.

Erythrocyte and total leukocyte count and hemoglobin concentration were analyzed in an automated equipment (pocH-100iV DIFF Sysmex). Hematocrit was obtained after capillary centrifugation (1000 × *g* for 5 min). Leucocyte differential counts were performed in blood smears stained with commercial dye (*Panótico Rapido*) using a light microscope at 1000X magnification (Feldman et al., 2000).

The TM status of the animals was not accessed in this study because liver biopsy is a very invasive procedure for newborn kids, and TM analysis in blood is not a reliable indicator of TM status (Herdt et al., 2000; Ranches et al., 2018).

Plasma concentrations of all enzymes, blood concentrations of immune cells, BW, and average daily gain (ADG) were analyzed using the MIXED procedure, and mortality and diarrhea rate were analyzed using the GLIMMIX procedure of SAS (Statistical Analysis System, version 9.4) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects.

The statistical model for ADG, mortality, and diarrhea rate was:

$$Y_{ijkl} = \mu + T_i + P_j + A_k + S_l + \varepsilon_{ijkl},$$

in which Y_{ijkl} = observation of the effect of treatment i in pen j, of animal k and sex l, in which μ is the overall mean; T_i = effect of treatment i [i = 1 (Saline) and 2 (ITM)]; P_j = effect of pen j (j = six pens); A_k = effect of animal k (k = 125 animals); S_l = effect of sex (l = 2); and ε_{ijkl} = random error associated with each observation.

Kid BW, plasma concentrations of all enzymes, and blood concentrations of immune cells were analyzed as repeated measures, and the statistical model was:

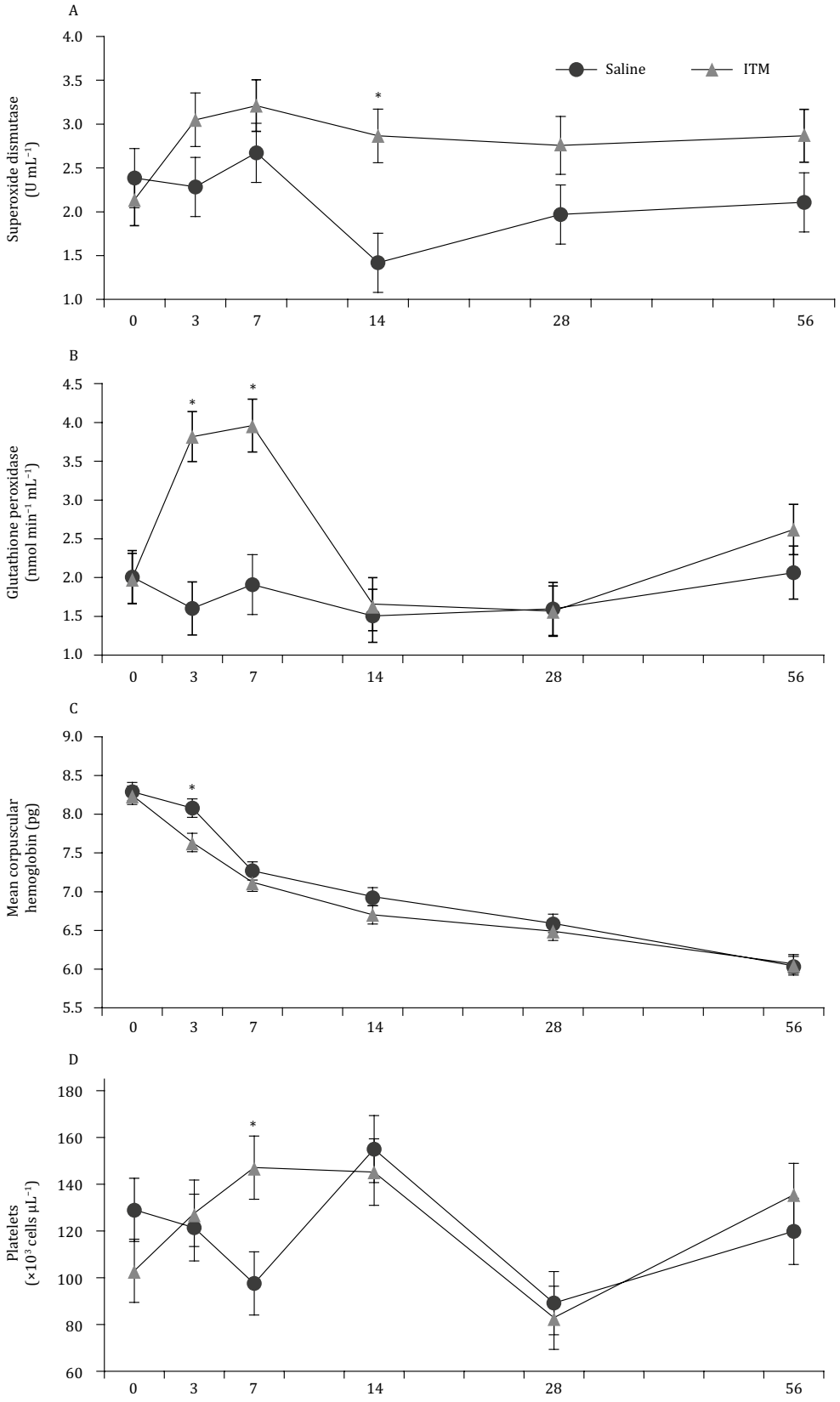
$$Y_{ijkl} = \mu + T_i + D_j + A_k + P_j + S_l + (TD)_{ij} + \varepsilon_{ijkl},$$

in which Y_{ijkl} = observation of the effect of treatment i per days of collection j in animal k; and pen j and sex l; μ = overall mean; T_i = effect of treatment [i = 1 (Saline), 2 (ITM)]; D_j = effect of days of collection (j = 0, 3, 7, 14, 28, and 56); A_k = animal effect (k = 125 animals), P_j = effect of pen (j = six pens); S_l = effect of sex (l = 2); TD_{ij} = interaction between treatment i and day j; and ε_{ijkl} = random error associated with each observation.

All results obtained on d 0 for each variable were included as covariates in each respective analysis, but were removed from the model when P>0.10. The toepliz covariance structure was selected for the analyses of BW, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), monocytes, and eosinophils; compound symmetric covariance structure was selected for SOD and lymphocytes; and first-order autoregressive covariance structure was selected for GSH-Px, erythrocytes, hemoglobin, mean corpuscular hemoglobin concentration (MCHC), platelets, leukocytes, and neutrophils, as they generated the lowest Akaike information criterion. Means were separated using PDIFF, and all results were reported as LSMEANS followed by SEM. Significance was defined when P≤0.05, and tendency when P>0.05 and P≤0.10.

Results

Effects of treatment × day were detected (P = 0.05), and treatment effects tended to be detected (P = 0.06) for plasma concentrations of SOD (Table 2). The ITM-injected kids had greater (P = 0.05) plasma concentrations of SOD on d 14 compared with saline-injected kids (Figure 1A). Effects of treatment × day and treatment were detected (P≤0.01) for plasma concentrations of GSH-Px, which was greater for ITM- vs. saline-injected kids on d 3 and 7 (Table 2; Figure 1B). Effects of treatment × day and treatment were not detected (P≥0.14) for blood concentrations of leukocytes, neutrophils, lymphocytes, and monocytes (Table 2). Effects of treatment, but not treatment × day (P = 0.63), were detected (P = 0.04) for blood concentrations of eosinophils, which was greater for ITM- vs. saline-injected kids (Table 2). Effects of treatment × day and treatment were not detected (P≥0.28) for blood concentrations of erythrocytes, hemoglobin, hematocrit, and MCV (Table 2). Effects of treatment × day, but not treatment (P = 0.18), were detected (P = 0.02) for MCH, which was lower for ITM- vs.



Effects of treatment × day were detected for superoxide dismutase (P = 0.05), glutathione peroxidase (P = 0.01), mean corpuscular hemoglobin (P = 0.02), and platelets (P = 0.01).
* P≤0.05.

Figure 1 - Blood concentrations of superoxide dismutase (A), glutathione peroxidase (B), mean corpuscular hemoglobin (C), and platelets (D) of Boer kids administered a single subcutaneous injection (0.1 mL 4.5 kg^{-1} of body weight) of saline solution or injectable trace minerals (ITM) at 4.4±3.6 d of age; d 0).

saline-injected kids on d 3 (Table 2; Figure 1C). Effects of treatment, but not treatment × day (P = 0.18), were detected (P = 0.02) for MCHC, which was greater for ITM- vs. saline-injected kids (Table 2). Effects of treatment × day, but not treatment (P = 0.87), were detected (P = 0.01) for platelets. The ITM-injected kids had greater blood concentrations of platelets on d 7 compared with saline-injected kids (Table 2; Figure 1D).

Effects of treatment were not detected (P≥0.15) for mortality rate during the study (Table 3). Effects of treatment tended to be detected (P≤0.09) for diarrhea incidence, which was lower for ITM- vs. saline-injected kids from d 0 to 28, 0 to 56, or when evaluating the animals that showed twice the incidence during the study (Table 3). Effects of treatment were not detected (P = 0.69) for diarrhea incidence from d 28 to 56 (Table 3). Effects of treatment × day and treatment were not detected (P≥0.72) for BW, whereas effects of treatment were not detected (P≥0.67) for ADG during the study (Table 3).

Discussion

It has been reported that the ITM application increases the plasma concentrations of SOD (Tomasi et al., 2018; Vedovatto et al., 2019a,b; Vedovatto et al., 2020) and GSH-Px (Teixeira et al., 2014; Vedovatto et al., 2019a,b; Vedovatto et al., 2020) in calves and cows, and SOD (Cazarotto et al., 2018) in newborn lambs. In the current study, the use of ITM in kids also increased the production of plasma SOD on d 14 and GSH-Px on d 3 and 7 compared with the saline solution. 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 (Marklund, 1980), whereas Se is a component of GSH-Px (Rotruck et al., 1973). Therefore, the increase in plasma concentration of SOD and GSH-Px following ITM may indicate a greater control of the oxidative stress of cells for at least 14 d after the injection.

Table 2 - Blood concentrations of antioxidant enzymes, leukogram, erytogram, and platelets of Boer kids administered a single subcutaneous injection (0.1 mL 4.5 kg^{-1} of body weight) of saline solution or injectable trace minerals (ITM) at 4.4±3.6 d of age, d 0

Item	Treatment[1]		SEM	P-value	
	Saline	ITM		Treatment × day	Treatment
Antioxidant enzymes					
Superoxide dismutase (U mL^{-1})	2.14	2.81	0.21	0.05	0.06
Glutathione peroxidase (nmol min^{-1} mL^{-1})	1.78	2.60	0.13	0.01	0.01
Leukogram					
Leukocytes (×10^3 cells μL^{-1})	14.7	15.6	0.38	0.15	0.14
Neutrophils (×10^3 cells μL^{-1})	8.12	7.95	0.60	0.86	0.86
Lymphocytes (×10^3 cells μL^{-1})	7.56	7.65	0.35	0.33	0.88
Monocytes (×10^3 cells μL^{-1})	0.29	0.17	0.20	0.53	0.67
Eosinophils (×10^3 cells μL^{-1})	0.10	0.25	0.05	0.63	0.04
Erytogram					
Erythrocytes (×10^6 cells μL^{-1})	16.2	16.0	0.60	0.70	0.81
Hemoglobin (g dL^{-1})	10.5	10.8	0.25	0.28	0.37
Hematocrit (%)	31.5	33.2	2.73	0.96	0.66
MCV (fL)	20.0	20.1	0.21	0.40	0.58
MCH (pg)	7.20	7.04	0.08	0.02	0.18
MCHC (pg)	36.0	35.0	0.28	0.18	0.02
Platelets (×10^3 cells μL^{-1})	125	124	7.54	0.01	0.87

MCV - mean corpuscular volume; MCH - mean corpuscular hemoglobin; MCHC - mean corpuscular hemoglobin concentration; SEM - standard error of the mean.
[1] Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15 mg mL^{-1} 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 kid.

Table 3 - Mortality, diarrhea, and growth performance of Boer kids administered a single subcutaneous injection (0.1 mL 4.5^{-1} kg of body weight) of saline solution or injectable trace minerals (ITM) at 4.4 ± 3.6 d of age, d 0

Item	Treatment[1]		SEM	P-value	
	Saline	ITM		Treatment × day	Treatment
Mortality (%)					
Day 0 to 28	6.46	1.44	2.52		0.15
Day 28 to 56	1.69	3.25	2.17		0.57
Day 0 to 56	8.07	4.71	3.25		0.45
Diarrhea (%)					
Once					
Day 0 to 28	27.0	14.6	5.47		0.09
Day 28 to 56	7.92	6.12	3.77		0.69
Day 0 to 56	34.8	20.7	7.10		0.07
Twice					
Day 0 to 56	10.5	2.99	5.33		0.07
Body weight (kg)				0.92	0.72
Day 0	6.61	6.62	0.24		
Day 28	9.11	9.21	0.24		
Day 56	12.1	12.3	0.24		
Average daily gain (kg d^{-1})					
Day 0 to 28	0.09	0.09	0.01		0.72
Day 28 to 56	0.13	0.13	0.01		0.98
Day 0 to 56	0.10	0.10	0.01		0.67

[1] Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15 mg mL^{-1} 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 kid.

The ITM application increased the concentration of eosinophils by 2.5-fold compared with the saline application. These results may be a consequence of better control of the damage caused by oxidative stress to these leukocytes due to the greater production of SOD and GSH-Px in ITM-injected kids. According to Spears and Weiss (2008), immune cells are sensitive to oxidative stress because they have membranes with high concentrations of polyunsaturated fatty acids, which are highly susceptible to lipid peroxidation. The ITM application increased the blood concentration of platelets only on d 7 compared with saline injection, coinciding with the peak of plasma GSH-Px. These results may indicate that platelets are also highly susceptible to oxidative stress or have their production in the bone marrow affected by this.

The concentrations of MCH and MCHC were reduced by ITM application. In agreement, Solaiman et al. (2007) observed that a high level of Cu inclusion in the diet of goats (100 mg day^{-1}) also reduced MCH compared with no supplemental Cu. Arthington et al. (2014) observed that the ITM application in pre-weaned calves reduced the liver concentrations of Fe and increased the plasma concentrations of ceruloplasmin. According to 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 MCH and MCHC. Nonetheless, MCH and MCHC values reported in the present study were within the accepted normal range for goats (5.2 to 8.0 and 30 to 36 g dL^{-1}, respectively; Jackson and Cockcroft, 2002). The reasons why ITM decreased MCH and MCHC, but not the concentration of hemoglobin, in the current study are unknown, and further studies exploring this may be warranted.

The ITM-injected kids tended to have less diarrhea compared with saline-injected kids. Similarly, the application of ITM in calves on d 0 and 30 of age reduced the incidence of diarrhea (41.7 and 49.7%

for ITM and saline, respectively) and pneumonia, otitis, or both (41.6 and 49.1% for ITM and saline respectively; Teixeira et al., 2014). In the present study, ITM injection increased plasma concentrations of GSH-Px (d 3 and d 7) and SOD (d 14), which may have reduced the damage caused by the oxidative stress to the immune cells leading to reduced incidence of diarrhea, primarily from d 0 to 28. In addition, ITM-injected kids showed greater blood concentrations of eosinophils, which may have enhanced the control of endoparasites known to cause diarrhea (Gleich and Adolphson, 1986; Chartier and Paraud, 2012). In support of this rationale, ITM injection in newborn lambs reduced the fecal number of *Eimeria* spp oocysts (Cazarotto et al., 2018).

The use of ITM enhanced the concentrations of multiple components of the immune system, increased antioxidant activity, and tended to reduce the incidence of diarrhea. However, these outcomes did not lead to enhanced growth performance compared with saline injection. Other studies observed that ITM injections increased the growth performance of newborn lambs (Cazarotto et al., 2018), but not of newborn calves (Arthington et al., 2014; Teixeira et al., 2014), or when applied at weaning of calves (Vedovatto et al., 2020), compared with saline injection. However, in the current study, we believe that the experimental period (56 d) was not sufficient to observe differences on performance, and, thus, future experiments can be performed for a longer time, involving the post-weaning period.

Conclusions

A single administration of injectable trace minerals to newborn kids increases the blood concentrations of antioxidant enzymes, platelets, and eosinophils, reduces mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and tends to reduce the incidence of diarrhea, but does not affect mortality rate and growth performance.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: M. Vedovatto and G.L. Franco. Data curation: M. Vedovatto, I.M. Cortada Neto, C.S. Pereira, A.L.L. Bento, R.F.A.T. Rocha and G.L. Franco. Formal analysis: M. Vedovatto, C.S. Pereira, A.L.L. Bento, R.F.A.T. Rocha, P. Moriel and G.L. Franco. Funding acquisition: M. Vedovatto and G.L. Franco. Investigation: M. Vedovatto, C.S. Pereira, A.L.L. Bento, R.F.A.T. Rocha, P. Moriel and G.L. Franco. Methodology: M. Vedovatto, I.M. Cortada Neto, C.S. Pereira, A.L.L. Bento, R.F.A.T. Rocha, P. Moriel and G.L. Franco. Project administration: G.L. Franco. Resources: G.L. Franco. Supervision: I.M. Cortada Neto and G.L. Franco. Writing-original draft: M. Vedovatto, P. Moriel and G.L. Franco. Writing-review & editing: M. Vedovatto and G.L. Franco.

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