Effects of a single trace minerals injection on antioxidant and immune response, resistance to endoparasites, health and growth of newborn Boer kids

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ABSTRACT: Vedovatto M., Cortada Neto I.M., Borges D.G.L., Coelho R.N., Ranches J. & Franco G.L. 2021. Effects of a single trace minerals injection on an antioxidant and immune response, resistance to endoparasites, health and growth of newborn Boer kids. Pesquisa Veterinária Brasileira 41:e06722, 2021. Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Av. Senador Felinto Muller 2443, Campo Grande, MS 79070-900, Brazil. E-mail: gumercindo.franco@ufms.br

This study evaluated the effects of injectable trace minerals (ITM) on antioxidant and immune response, resistance to endoparasites, health and growth of newborn Boer kids. Forty-six Boer kids [24 males and 22 females; 3.94±1.03kg of body weight (BW); 6.2±2.4 d of age] were enrolled in the study. Kids were stratified by type of birth (twins or singlet), sex, and BW and assigned to 1 of 2 treatments: one subcutaneous injection (0.1mL/4.5kg of BW) of (1) saline solution or (2) ITM (60, 10, 5, and 15mg/mL of Zn, Mn, Se and Cu, respectively). Blood samples were collected on d 0, 7, 14, 28 and 56. Feces samples were collected on d 56 and BW on d 0, 28 and 56. Kids were checked daily for signs of diarrhea. ITM kids had greater (P<0.01) plasma concentration of superoxide dismutase and tended (P=0.06) to have greater plasma concentration of glutathione peroxidase. ITM kids had greater (P=0.05) concentration of eosinophils, but no differences (P≥0.11) were observed for other hemogram variables. The ITM application did not affect (P≥0.11) the EPG count. However, ITM kids had less (P=0.02) cumulative incidence of diarrhea until d 42 (3.85 vs. 25.93±6.8% for ITM vs. Saline kids, respectively) but no differences (P>0.10) were observed after d 42. The ITM application did not affect (P=0.40) the growth of kids (0.071 vs. 0.065±0.005kg/day for ITM vs. Saline kids, respectively). Thus, the ITM application, increased the plasma concentration of antioxidant enzymes and eosinophils, decreased the incidence of diarrhea only in the middle of the experiment, but did not affected the EPG count and growth of Boer kids.

INDEX TERMS: Mineral injection, antioxidant, immune response, resistance, endoparasites, newborn Boer kids, diarrhea, Eimeria, glutathione peroxidase, hemogram, superoxide dismutase.
maior concentração de glutathiona peroxidase no plasma. Os animais ITM apresentaram maior ($P<0.05$) concentração de eosinófilos, mas não foram observadas diferenças ($P>0.11$) para outras variáveis do hemograma. A aplicação de ITM não afetou ($P>0.11$) a contagem de EPG. No entanto, os cabritos ITM apresentaram menor incidência cumulativa de diarreia ($P=0.02$) até 42 (3,85 vs. 25,93±6,8% para animais ITM vs. animais salina, respectivamente), mas nenhuma diferença ($P>0.10$) foi observada após 42. A aplicação do ITM não afetou ($P>0.40$) o crescimento dos animais (0.071 vs. 0.065±0.005kg/dia para ITM vs. Salina, respectivamente). Assim, a aplicação do ITM aumentou a concentração plasmática de enzimas antioxidantes e eosinófilos, diminuiu a incidência de diarreia somente na metade do experimento, mas não afetou a contagem de OPG e crescimento de cabritos Boer recém-nascidos.

**MATERIALS AND METHODS**

**Ethics statement.** The 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.

**Animals, treatments and samples collection.** The study was conducted in a commercial farm located in Bandeirantes/MS, Brazil (19°53’15.9” latitude south and 54°24’43.0” longitude west) during the Spring season (temperature-humidity index ranged from 73.6 to 77.6) and lasted 56 days. The goats (mothers) remained during the first four months of gestation on pasture of marandu-grass (*Urochloa brizantha* [Hochst. ex A. Rich] R. D. Webster, cv. Marandu). Further, on the last month of gestation, were transferred to a feedlot, were they received corn silage and concentrate to meet or exceed the requirements for crude protein and minerals for goats weighing 50kg (double-kids) at late gestation and early lactation (NRC 2007). After the delivery, the kids remained with the mothers until at least 3 days post-partum to allow Colostrum consumption. Subsequently, the kids were transferred on the same day to a single experimental pen, where they were kept as a single group until the end of the study. Forty-six Boer kids (24 males and 22 females; 3.94±1.03kg of BW; 62±2.4 d of age) were enrolled in the study. Kids were stratified by type of birth (twins or singlet), sex and body weight (BW), and then randomly assigned into 1 of 2 treatments: one single subcutaneous injection (0.1mL/4.5kg of BW) of saline solution (Saline) or ITM. Saline solution consisted of 0.9% NaCl, whereas ITM composition was 60, 10, 5, and 15mg/mL of Zn, Mn, Se and Cu, respectively (Multimin 90, Multimin, Fort Collins/CO, USA). Injections were administered on the right side of the neck of each kid.

The experimental pen (60m²) was located in a covered shed with open lateralis, providing good natural ventilation. The pen was equipped with one automatic drink fountain and two feed bunks with 10cm linear/animal. One feed bunk was used to provide fresh chopped forage [*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs; cv. Colonia] and the other feed bunk was used to provide concentrates (Table 1). The feed was offered twice daily (0800h and 1600h) ad libitum. Milk replacer (Sprayfo Lamb Primo®, Trouw Nutrition, Mirassol/SP, Brazil) was diluted in water (1:6 respectively) at 37°C and provided 250mL/kid, twice daily (0800h and 1600h), using an individual graduated bottle.

On day 0, all kids were weighed (before the ITM or Saline injection) with following body weight collections on days 28 and 56. The kids were vaccinated against clostridiosis (*Sintoxan®* 9TH, Merial Saúde Animal, Brazil), pasteurellosis and paratyphoid (*Tifopasteurina®*, Hertape Calier Saúde Animal, Brazil) at 30 days of age. No deworming practice was conducted during the study. Additionally, the occurrence of diarrhea was observed daily by the same trained veterinarian, 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. Diarrhea was confirmed if the animals presented scores 3 or 4 for 2 or more consecutive days. For this, the veterinarian remained in the farm throughout the study, and evaluate the kids individually, many times during the day, to detected the fluidity of the feces, that was noted in spreadsheets.

**INTRODUCTION**

Trace minerals injection (ITM) is an alternative method to the conventional mineral supplementation strategies. Among benefits within the use of ITM is that it provides a known amount of trace minerals for all animals of the same group and avoids the interference of antagonists that might be present in the diet (Arthington et al. 2014). Additionally, ITM is an interesting method for trace minerals supplementation to new-borns mammals that have low solid feed intake, and consequently low minerals intake, if they are provided by the conventional forms.

It has been reported that the application of ITM containing Zn, Mn, Se and Cu in beef cows (Vedovatto et al. 2019a, 2019b), weaned calves (Vedovatto et al. 2019c) and lambs (Cazarotto et al. 2018, 2019) increased the concentration of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Increased concentration of antioxidants enzymes by ITM, results in improved immune system (Cazarotto et al. 2018, 2019) and avoids the interference of antagonists that might be present in the diet (Arthington et al. 2014). Additionally, ITM is an interesting method for trace minerals supplementation to new-borns mammals that have low solid feed intake, and consequently low minerals intake, if they are provided by the conventional forms.

**RESULTS**

**Hematology:** The hemogram of kids ITM vs. Saline showed no differences ($P>0.10$) for all measured parameters. There was no significant difference ($P>0.10$) in the concentration of RBC (12.81±2.40 vs. 12.70±2.40×10⁶ cells/μL) or Hb (9.82±1.24 vs. 9.57±1.24g/dL) between groups. No differences were found in the concentration of WBC, lymphocytes, neutrophils, eosinophils, basophils, monocytes, and large unstained cells ($P>0.10$). There were no differences ($P>0.10$) in the concentration of Reticularocytes (0.71±0.18 vs. 0.69±0.19%). Animals ITM presented higher ($P<0.05$) concentration of monocytes (2.67±0.54 vs. 1.55±0.54×10⁶ cells/μL) and eosinophils (0.05±0.01 vs. 0.03±0.01×10⁶ cells/μL) compared to the animals Saline. Animals ITM presented higher ($P<0.05$) RBC and Hb concentration (11.31±1.28 vs. 10.64±1.28×10⁶ cells/μL and 9.17±1.24 vs. 8.86±1.24g/dL respectively, compared to the animals Saline).

**Parasitology:** The eggs were collected from the rectum of the kids via FEC (Fecal Egg Count) on days 14, 28 and 56. The eggs were identified using a light microscope (×400) and the results were registered with a computer. The parasite burden of kids ITM vs. Saline was not affected ($P>0.10$) by ITM for any of the measured species, including *Trichostrongylus colubriformis*, *Fasciola hepatica*, *Necator americanus* and *Strongyloides vulgaris*. There was no difference ($P>0.10$) in the number of eggs per gram of feces (EPG) between groups (823±349 vs. 1333±349). The incidence of naked eggs was not affected ($P>0.10$) by ITM for any of the measured species, including *Trichostrongylus colubriformis*, *Fasciola hepatica*, *Necator americanus* and *Strongyloides vulgaris*.

**DISCUSSION**

The main results of this study were that ITM supplementation at birth, 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. Diarrhea was confirmed if the animals presented scores 3 or 4 for 2 or more consecutive days. For this, the veterinarian remained in the farm throughout the study, and evaluate the kids individually, many times during the day, to detected the fluidity of the feces, that was noted in spreadsheets.

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Arthington, W.L., Nascimento, D.C. e D.P. Trinca, 2014. Nutrition, Mirassol/SP, Brazil) was diluted in water (1:6 respectively) at 37°C and provided 250mL/kid, twice daily (0800h and 1600h), using an individual graduated bottle.

On day 0, all kids were weighed (before the ITM or Saline injection) with following body weight collections on days 28 and 56. The kids were vaccinated against clostridiosis (*Sintoxan®* 9TH, Merial Saúde Animal, Brazil), pasteurellosis and paratyphoid (*Tifopasteurina®*, Hertape Calier Saúde Animal, Brazil) at 30 days of age. No deworming practice was conducted during the study. Additionally, the occurrence of diarrhea was observed daily by the same trained veterinarian, 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. Diarrhea was confirmed if the animals presented scores 3 or 4 for 2 or more consecutive days. For this, the veterinarian remained in the farm throughout the study, and evaluate the kids individually, many times during the day, to detected the fluidity of the feces, that was noted in spreadsheets.

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Blood samples were collected from jugular vein on d 0, 7, 14, 28 and 56 into blood collection tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes/NJ, USA) containing sodium heparin (5 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 SOD and GSH-Px. Blood samples containing K$_2$EDTA were stored at 4°C overnight, and haematological analyses performed in these samples until 24 hours after collection.

Fecal samples were collected on day 56, directly from the rectal ampulla, immediately stored on ice, and analysed within 24 hours after collection. Chopped forage samples were collected, dried at 60°C for 5 days, ground to 1mm and analysed for chemical composition.

**Laboratory analysis.** Forage was analysed according to AOAC (2000): crude protein, method 976.05; ether extract, method 920.39 and ash, method 942.05. The concentration of neutral detergent fiber was done according to the methodology of Van Soest et al. (1991). The trace minerals concentration of forages was analysed via inductively coupled plasma mass spectrometry, and Se was analysed as described by Oliveira et al. (2016) and the other minerals as described by Braselton et al. (1997). The trace minerals status of the kids was not accessed in this study as a liver biopsy procedure (best indicator for trace minerals status) would be too invasive for newborn kids. Further, the TM analysis in blood is not the most reliable indicator of trace minerals status (Herdt et al. 2000, Ranches et al. 2018). Thus, the lack of knowledge of the TM status of the kids is limitation of this study.

| Table 1. Chemical composition of forage ([*Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs *Panicum maximum*; cv. Colonião]), concentrate and milk replacer provided to kids during the study |
|-----------------------------------------------|
| **Items** | **Forage** | **Concentrate** | **Milk replacer** |
| g/kg of dry matter (DM) | | | |
| Crude protein | 71.51 | 215.00 | 220.00 |
| Neutral detergent fiber | 781.46 | 214.00 | 1.00 |
| Ether extract | 23.74 | 30.00 | 220.00 |
| Ash | 76.05 | 96 | 89.00 |
| Calcium | 1.85 | 10.00 | 9.00 |
| Phosphorus | 1.77 | 4.00 | 6.00 |
| Sodium | 2.59 | 1.50 | 7.00 |
| Potassium | 17.09 | 5.50 | - |
| Magnesium | 0.98 | 2.00 | - |
| mg/kg of DM | | | |
| Iron | 144.48 | 85.00 | 30.00 |
| Zinc | 25.95 | 35.00 | 84.00 |
| Manganese | 32.47 | 35.00 | 45.00 |
| Selenium | 0.06 | 1.20 | 0.3 |
| Copper | 5.09 | 25.00 | 10 |

| a Chemical composition analyzed; b guarantee levels described by the manufacturers; c Caprinos Creep Caprivesal, “Sô Sal Nutrição e Saúde Animal”, Campo Grande/MS, Brazil; d Sprayfo Lamb Primo®, Trouw Nutrition, Mirassol/SP, Brazil; Diluted in water (1:6 respectively) at 37°C |

The enzymes GSH-Px and SOD were determined by commercial kits (Cayman Chemical, Ann Arbor/MI, USA, catalogue number 703102 and 706002, respectively). The inter and intra-assay coefficients of variation for SOD were 4.5 and 7.2% and for GSH-Px were 6.1 and 8.1%, respectively.

The hemogram analysis was performed in whole blood samples in automated equipment (poCh-100IV DIFF Sysmex) as described by Riond et al. (2011). The parasite load determined by the EPG was performed as described by Gordon & Whitlock (1939).

**Statistics.** Kid was considered the experimental unit for all analyses. All dependent variables were tested for normality and using Univariate procedure of SAS (SAS Inst. Inc., Cary/NC, USA; version 9.4). The variable EPG did not have normal distribution and were transformed to log$_e$(X+1). Plasma concentrations of SOD and GSH-Px, blood concentration of immune cells, BW, average daily gain (ADG) and EPG (transformed) were analysed using MIXED procedure of SAS. Satterthwaite approximation was used to determine the denominator degrees of freedom for the test of fixed effects. Kid ADG and EPG were tested for fixed effect of treatment using sex and kid (treatment) as random effects. Kid BW, plasma concentrations of SOD and GSH-Px, and blood concentrations of immune cells were analysed as repeated measures and tested for fixed effects of treatment, day, and treatment × day, using sex and kid (treatment) as random variables and kid (treatment) as subject. All results obtained on day 0 for each variable were included as covariates in each respective analysis but were removed from the model when $P > 0.10$. The first order autoregressive covariance structure was selected for BW, eosinophils, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelets. The compound symmetric covariance structure was selected for SOD, leukocytes, lymphocytes, monocytes, haemoglobin and haematocrit. The Toeplitz covariance structure was selected for neutrophils, erythrocytes and GSH-Px. The covariance structures were selected according to the lowest Akaike information criterion. The binary data of cumulative diarrhea incidence was analysed using the GLIMMIX procedure of SAS with Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. The diarrhea incidence was tested for fixed effect of treatment using sex and kid (treatment) as random effect. Means were separated using PDIF and all results were reported as LSMEANS followed by SE. The SAS commands used in this study are presented in the supplementary material. Significance was defined when $P ≤ 0.05$, and tendency when $P > 0.05$ and ≤0.10.

**RESULTS**

Effects of treatment × day, and treatment were detected ($P < 0.01$) for plasma concentrations of SOD (Table 2). Kids assigned to ITM treatment had greater concentrations of SOD on d 7, 14, 28 and 56, when compared to kids assigned to Saline treatment (Fig.1). A tendency for a treatment effect ($P = 0.06$) but no effect of treatment × day ($P = 0.24$) was observed for plasma GSH-Px. Kids assigned to ITM treatment tended to have greater concentrations of plasma GSH-Px when compared to kids assigned to Saline treatment (Table 2).
No effect of treatment was detected \((P \geq 0.11)\) for eggs/oocists count of Strongyle, Strongyloides, *Trichuris*, *Eimeria* or total eggs/oocists count per gram of feces (Table 3). A tendency for treatment effect \((P = 0.06)\) was observed for the cumulative incidence of diarrhea on day 28. Further, a treatment effect \((P = 0.02)\) was observed on day 35 and 42, where kids assigned to ITM treatment had lower incidence on these days, when compared to kids assigned to Saline treatment (Fig. 2). No kid mortality was observed during the study.

No effect of treatment \(\times\) day or treatment was detected for BW \((P = 0.55)\). Similarly, no effect \((P = 0.40)\) of treatment was detected for ADG during the study (Table 4).

**DISCUSSION**

The ITM application increased the plasma concentration of SOD and GSH-Px in kids. Similar response was previously observed in cows (Vedovatto et al. 2019a, 2019b), calves (Glombowsky et al. 2018, Vedovatto et al. 2019c) and lambs (Cazarotto et al. 2018, 2019). This response is likely due to the structural formation of the superoxide dismutase, where Zn, Mn and Cu are constituent of the enzymes Cu/Zn-SOD and Mn-SOD. Further, Se is constituent of the GSH-Px (Sordillo & Aitken 2009) and increased enzymatic concentration observed in the current study should be expected with the increased systemic mineral concentration. Improving enzymatic concentration of SOD and GSH-Px is important, because these antioxidants enzymes can reduce the concentration of reactive species oxygen and consequently reduce damage caused by the oxidative stress in the cells (Cazarotto et al. 2019).

The ITM application increased the concentration of eosinophils in kids. A similar response was previously reported in calves (Vedovatto et al. 2019c) but not in lambs (Cazarotto et al. 2018). In the current study, the increased concentration of eosinophil observed in kids receiving ITM injection can be attributed to Se mitogenic properties, inducing the proliferation of eosinophils by changes on mitotic index (Hawkes et al. 2001). Another explanation may be related to a better control of the damage caused by oxidative stress.

### Table 2. Blood concentrations of antioxidants enzymes, leukogram, erytogram and platelets in Boer kids administered a single subcutaneous injection \((0.1\text{mL}/4.5\text{kg of body weight})\) of saline solution or injectable trace mineral (ITM)

| Items* |
|--------|
| Saline | ITM   | SEM | \(P\)-value |
|        |        |     |            |
| Antioxidants enzymes |
| Superoxide dismutase, U/mL | 1.84 | 3.01 | 0.12 | <0.01 | <0.01 |
| Glutathione peroxidase, nmol/min/mL | 1.87 | 2.17 | 0.13 | 0.24 | 0.06 |
| Leukogram |
| Leukocytes, \(\times 10^5\) cells/μL | 15.15 | 16.39 | 0.77 | 0.79 | 0.29 |
| Neutrophils, \(\times 10^5\) cells/μL | 7.48 | 8.38 | 0.61 | 0.92 | 0.34 |
| Lymphocytes, \(\times 10^5\) cells/μL | 7.06 | 7.76 | 0.38 | 0.25 | 0.23 |
| Monocytes, \(\times 10^3\) cells/μL | 0.30 | 0.22 | 0.14 | 0.62 | 0.69 |
| Eosinophils, \(\times 10^3\) cells/μL | 0.09 | 0.29 | 0.06 | 0.56 | 0.05 |
| Erytogram |
| Erythrocytes, \(\times 10^5\) cells/μL | 14.62 | 15.40 | 0.35 | 0.95 | 0.15 |
| Haemoglobin, g/dL | 10.06 | 10.40 | 0.24 | 0.66 | 0.34 |
| Haematocrit, % | 28.83 | 31.15 | 2.03 | 0.94 | 0.27 |
| MCV, fl | 20.11 | 19.47 | 0.35 | 0.84 | 0.23 |
| MCH, pg | 6.94 | 6.90 | 0.08 | 0.25 | 0.75 |
| MCHC, pg | 35.23 | 34.85 | 0.52 | 0.44 | 0.63 |
| Platelets, \(\times 10^3\) cells/μL | 129.06 | 123.76 | 8.15 | 0.11 | 0.65 |

* MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration; *b* saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15mg/mL of Zn, Mn, Se and Cu, respectively (Multimin® 90, Multimin, Fort Collins/CO, USA); Both treatments were administered on the right side of the neck of each kid.

### Table 3. Eggs per gram of feces in Boer kids administered a single subcutaneous injection \((0.1\text{mL}/4.5\text{kg of body weight})\) of saline solution or injectable trace mineral (ITM)

| Items |
|-------|
| Saline | ITM   | SEM | \(P\)-value |
| log_{10} of eggs/g of faeces |
| Strongyle | 1.78 (268.33) | 1.49 (151.79) | 0.30 | 0.47 |
| Strongyloides | 0.36 (2.33) | 0.22 (1.43) | 0.10 | 0.33 |
| Trichuris | 0.69 (4.66) | 0.52 (3.57) | 0.09 | 0.20 |
| Eimeria | 1.14 (13.01) | 0.85 (6.43) | 0.10 | 0.11 |
| Total | 2.10 (288.33) | 1.85 (163.21) | 0.21 | 0.28 |

*log_{10} of eggs/g of faeces; saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15mg/mL of Zn, Mn, Se and Cu, respectively (Multimin® 90, Multimin, Fort Collins/CO, USA); Both treatments were administered on the right side of the neck of each kid; Values in parentheses represent the original number of eggs per gram without being transformed to log_{10}.
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To these leukocytes due to the greater production of SOD and GSH-Px. 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 (Spears & Weiss 2008).

Kids assigned to ITM treatment did not have greater resistance to endoparasites, when compared to kids assigned to the Saline treatment. ITM injection in newborn lambs reduced the faecal number of *Eimeria spp.* oocysts, and the authors attribute the improvement in immune system caused by the ITM application (Cazarotto et al. 2018). In our study, we only apply the ITM on day 0, differing from the study of Cazarotto et al. (2018), that was applied on day 0 and again on day 30; it could explain the differences between studies.

In other study, the administration of oral copper capsules reduced the EPG count of *Haemonchus contortus* in sheep feces, but the authors attribute this result to a direct effect of Cu on the parasite, suggested that dietary Cu damages the parasite cuticle, resulting in its inability to maintain infection; rather than an indirect effect of improved immune system (Campigotto et al. 2019). Thus, in the current study, the improved concentration of eosinophils, did not reflect in better resistance to endoparasites in ITM kids.

In the current study, kids assigned to the ITM treatment had lower incidence of diarrhea, compared to kids assigned to Saline. Similarly, the application of ITM in calves on day 0 and 30 days 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 other studies, the ITM application at birth (Tomasi et al. 2018) or on day 2 and again on day 14 (Glombowsky et al. 2018) reduced the severity of the diarrhea, occurrence of pneumonia and the use of antibiotics in dairy calves. In the current study, ITM injection increased plasma concentrations of antioxidant enzymes which may have reduced the damage caused by the oxidative stress to the immune cells leading to a reduction in the incidence of diarrhea, by reducing the pathogenic bacteria infection in the gastrointestinal tract. To support this rationale, it has been previously demonstrated that the total count of bacteria in faeces, was lower in calves that received an ITM application (Glombowsky et al. 2018). Further, Zn supplementation exhibits therapeutic action by facilitating the transport of water and electrolytes across the intestinal mucosa, preventing villous atrophy and improving overall immunity, consequently, reducing the incidence of acute diarrhea (Tomkins et al. 1993).

The use of ITM improved the immune and antioxidant systems and the health of the kids. However, these outcomes did not lead to enhanced growth performance when compared to Saline injection. Similar responses were observed in previous studies using ITM, where ITM application also did not improve the growth of calves (Arthington et al. 2014, Teixeira et al. 2014, Glombowsky et al. 2018, Vedovatto et al. 2019c) as observed in the current study with kids.

**Table 4. Growth performance of Boer kids administered a single subcutaneous injection (0.1mL/4.5kg of body weight) of saline solution or injectable trace mineral (ITM)**

| Items                  | Treatments | SEM | P-value | P-value |
|------------------------|------------|-----|---------|---------|
|                       |            |     | Treatment × day | Treatment |
| Body weight, kg        |            |     | 0.55    | 0.65    |
| d 0                    | 3.97       | 3.90|         |         |
| d 28                   | 5.41       | 5.50|         |         |
| d 56                   | 7.61       | 7.89|         |         |
| Average daily gain, kg/d |            |     |         |         |
| d 0 to 28              | 0.051      | 0.057| 0.005   | 0.45    |
| d 28 to 56             | 0.078      | 0.085| 0.007   | 0.53    |
| d 0 to 56              | 0.065      | 0.071| 0.005   | 0.40    |

*Saline solution consisted of 0.9% NaCl, whereas ITM had 60, 10, 5, and 15mg/mL of Zn, Mn, Se and Cu, respectively (Multimin 90, Multimin, Fort Collins/CO, USA); Both treatments were administered on the right side of the neck of each kid.
CONCLUSIONS

To the knowledge of the authors, this is the first publication evaluating the effects of injectable trace minerals (ITM) in goats.

The application of ITM increased the levels of eosinophils, SOD and GSH-Px, decreased the incidence of diarrhea only in the midle of the experiment, but did not affected the EPG count and the growth of Boer kids.

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Conflict of interest statement.- The authors have no competing interests.

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