# Efecto metafiláctico de los minerales sobre la respuesta inmunológica y antioxidante, el aumento de peso y la mitigación de los efectos de la coccidiosis en corderos recién nacidos

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*Research in Veterinary Science – Publicado el 26/09/2018*

## Resumen

El objetivo del estudio fue evaluar el efecto metafiláctico de los minerales sobre la respuesta inmunológica y antioxidante, así como también sobre la performance y la prevención de coccidiosis en corderos recién nacidos. Dividimos 110 corderos recién nacidos en dos grupos (55 por grupo): control (sin tratamiento) y tratado con dos dosis de 0,33 ml/kg de peso vivo de un complejo mineral conteniendo cobre, zinc, selenio y manganeso (ADAPTADOR® MIN de Biogénesis Bagó), los días de vida (DOL) 1 y 30. Se colectó sangre entera a los 1, 15, 30 y 45 días de vida con el objetivo de medir enzimas antioxidantes, realizar análisis bioquímicos e inmunológicos y hemograma. Los animales tratados fueron más pesados ($P<0,05$) en comparación a los animales no tratados a los 15 y 45 días de vida, pero no a los 30 días debido a un brote de coccidiosis. La actividad de la enzima catalasa no difirió entre los grupos, mientras que las actividades de las enzimas superóxido dismutasa y xantina oxidasa fueron mayores ($P<0,05$) en los corderos tratados comparado con los animales controles. Los niveles séricos de proteínas totales y globulinas fueron mayores ($P<0,05$) en los animales tratados (DOL 15, 30 y 45). Un aumento significativo en el número de linfocitos (DOL 45), así como también en los niveles séricos de inmunoglobulinas (IgM e IgG), fueron observados en los animales tratados (DOL 15 y 30). Los niveles séricos de inmunoglobulinas permanecieron constantes durante todo el experimento en el grupo tratado, pero fluctuaron en el grupo control. Los niveles séricos de glucosa fueron mayores en el grupo tratado (DOL 15 y 30). Es posible concluir que la administración subcutánea de minerales posee efectos beneficiosos en corderos incrementando la respuesta inmunológica y antioxidante, lo cual se vio reflejado en un aumento de la ganancia de peso, que pudo haber mitigado el impacto de la coccidiosis.

Research in Veterinary Science 121 (2018) 46–52

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

# Metaphylactic effect of minerals on immunological and antioxidant responses, weight gain and minimization of coccidiosis of newborn lambs

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ARTICLE INFO

*Keywords:*
Lambs
Nutraceutical effect
Immunological response
Animal health
Antioxidant action
Coccidiosis

ABSTRACT

The aim of this study was to evaluate the metaphylactic effect of minerals on immunological and antioxidant responses, as well as performance and prevention of coccidiosis in newborn lambs. We divided 110 newborn lambs into two groups (55/group): control (untreated) and treated with two doses of 0.33 mL/kg of a mineral complex (zinc, copper, selenium, and manganese) on day of life (DOL) 1 and 30. Total blood was collected at DOL 1, 15, 30 and 45 to measure antioxidant enzymes, biochemical and immunology analyses, and haemogram. Treated animals were heavier ($P < .05$) than untreated lambs on DOL 15 and 45, but not on DOL 30 due to a coccidiosis outbreak. Catalase activity did not differ between groups, while superoxide dismutase and xanthine oxidase activities were higher ($P < .05$) in treated lambs compared with control animals. Serum levels of total protein and globulins were higher ($P < .05$) in treated animals (DOL 15, 30 and 45). A significant increased on the number of lymphocytes (DOL 45), as well as on seric levels of immunoglobulins (IgM and IgG) was observed in treated animals (DOL 15 and 30). Serum Ig levels remained constant throughout the experiment in the treated group, but fluctuated in the control group. Serum glucose levels were greater in treated animals (DOL 15 and 30). It is possible to conclude that subcutaneous administration of minerals has beneficial effects on lambs by increasing antioxidant and immunological defenses, reflected by greater weight gain, which could mitigate the impact of coccidiosis.

## 1. Introduction

Mineral supplementation of sheep is an important practice from a practical and economic point of view, especially regarding animal productivity. In general, this type of supplementation is an indispensable component of several production systems throughout Brazil (Silva et al., 2000). Optimal sheep mineral supplementation is essential for several vital functions, including digestion, respiration, circulation, and enzymatic reactions (Ortunho, 2013). Mineral deficits may cause many problems, including decrease in productivity, poor growth and weight gain, in addition to higher susceptibility to diseases and infertility. Approximately 5% of animal body weight is mineral, which can vary with age, species, breed and individual characteristics (Martin, 1993; Ortunho, 2013). Some minerals are required in major quantities and are called macro-minerals (calcium, phosphorus, sodium, chlorine, magnesium, manganese, potassium and sulfur); those required in smaller quantities (zinc, iron, copper, selenium, cobalt, manganese and fluoride) by the animals body are known as micro-minerals (Martin, 1993). All these minerals are essential in order to maintain all vital physiological functions and deficiencies may limit animal survival and performance.

Delays on body development and high mortality rates during the first few weeks of live may cause huge economic losses to farmers. These problems might be associated with improper animal handling, lack of adequate food, or poor facilities to raise these animals from birth to weaning (Martin, 1993). When exposed to this environment, animals are susceptible to various pathogens. Since 1985, several studies have demonstrated the importance of mineral supplementation for

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https://doi.org/10.1016/j.rvsc.2018.09.003
Received 30 April 2018; Received in revised form 12 September 2018; Accepted 26 September 2018

optimization of tissue structure, ionic homeostasis, acid-base equilibrium, and enzymatic systems (NRC, 1985); however, at this early stage of life, the consumption of solid diets is low, and therefore, lambs dependent on the minerals present in the colostrum and the milk of their mothers. In the literature, high deficiency of selenium was found in lactating ewes compared to non-lactating females of other species (Khan et al., 2010), suggesting that lambs may suffer of mineral deficiencies in the first days of life when they consume only milk. Studies showed that zinc deficiency (Courdouhji et al., 1991) and copper deficiency (Sol and Hagendijk, 1995) negatively affects the health and performance of lambs, as well as immune response.

Therefore, the development of strategies to deal with these problems is essential in order to raise healthy and productive herds and the oral supplementation or injectable application of minerals may mitigate several risks surrounding newborn lambs. Minerals are essential to the immunological and inflammatory responses and protect the animals against performance impairments (Garg et al., 2008; Ortunho, 2013). Recent studies have shown that the administration of mineral complexes to calves activates the immunological and antioxidant responses, as well as reduces health problems, including diarrhea (Glombowsky et al., 2018; Tomasi et al., 2018). However, we found no records on the metaphylactic or nutraceutical effect of mineral supplementation for lambs. Thus, the aim of this study was to evaluate the metaphylactic effect of minerals (zinc, copper, selenium, and manganese) on the immunological and antioxidant responses, as well as its impact on weight gain and prevention of coccidiosis of newborn lambs.

## 2. Materials and methods

### 2.1. Products

A commercial product (Adaptador MIN®, Biogen) was used to evaluate the effect of mineral supplementation in newborn lambs. This product (100 mL) is composed of copper edetate (1 g), zinc edetate (4 g), manganese edetate (1 g), and sodium selenite (0.5 g).

### 2.2. Animals

We used a total of 110 newborn lambs, Lacaune breed, weighing approximately 3 kg at birth. They were housed in pens (5 animals each) and divided into two groups ($n = 55$ each): a control group and a treated group. Animals from both groups received colostrum in the first few hours of life. Thereafter, newborns were fed milk from their mothers until day of life (DOL) 7. After this period, the animals received also artificial milk (Table 1). A dose of 0.33 mL/kg mineral compound was administered subcutaneously on DOL 1 (until 2 h post-birth) and DOL 30. This commercial product has no indication for sheep, and we used the same dose indicated for calves as recommended by the manufacturer in order to satisfy their physiological needs and according to the literature (Glombowsky et al., 2018; Tomasi et al., 2018). Animals were weighed on DOL 1, 15, 30, and 45. Table 1 also shows the measures of minerals (copper, zinc, selenium and manganese) determined in the concentrate by the Near Infrared Spectroscopy (NIRS) method in a commercial laboratory (Shankar, 2015), and feeding of lambs.

**Table 1**
Ingredients used to feed lambs a different phases of life.

| Ingredients | Age (days) | | |
|---|---|---|---|
| | 1 to 7 | 8 to 20 | 21 to 45 |
| Natural milk[1] (mL) | 500 | 200 | – |
| Replacer milk[2] (mL) | – | 300 | 500 |
| Concentrate[3] (g) | – | 100 | 300 |

[1]Milk offered to lambs soon after ewe's milking. [2] Replacer milk prepared by mixing 1.0 kg of powder to 4 L of water (according to manufacturer's instructions), followed by heating at 80 °C and offered to lambs at 37 °C. [3] Nutritional composition of concentrate: 20% of crude protein; 3% of ethereal extract; 10% of fibrous material, 12 g of calcium; 6 g of phosphorus, and 72% of total digestible nutrients. [3] Levels of selenium, zinc, copper and manganese in the concentrate was 0.61; 80.2; 30.0; and 81.0 mg/kg, respectively.

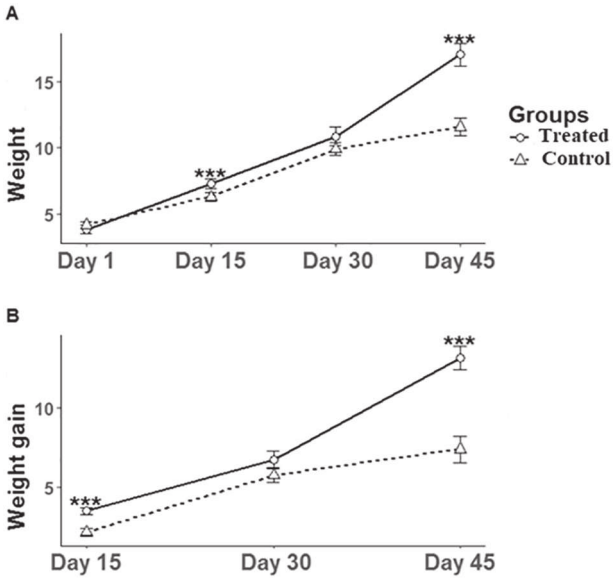


**Fig. 1.** Body weight (kg) [A] and weight gain (kg) [B] of treated ($n = 55$) and untreated lambs (control; n = 55) on days 1, 15, 30 and 45 of life. Asterisks indicate significant differences between groups (***$P < .05$). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

### 2.3. Sample collection and blood analyses

Total blood from 10 animals per group was collected by the jugular vein in tubes containing EDTA for complete blood counts, and also in tubes containing sodium citrate that were used to measure antioxidant enzymes. Blood collected without anticoagulant was used to obtain serum (3500 RPM for 10 min) for biochemical analyses and IgM and IgG quantification. All samples were stored at −20 °C until analysis.

### 2.4. Hematological analyses

The number of erythrocytes, total leukocytes, and hemoglobin concentration were performed using a semi-automated blood cell counter (CELM CC530), and for hematocrit a micro centrifugation method (Feldman et al., 2000) was used. Blood smears were prepared and stained according to Romanowski's method for microscopic examination to perform cell morphology and leukocyte differentiation (Feldman et al., 2000).

**Table 2**
Mean and standard deviation of blood components: total erythrocytes, hematocrit, hemoglobin concentration, total leukocytes, lymphocytes, neutrophils, monocytes and eosinophils of treated ($n$ = 10) and untreated (control; n = 10) lambs on days 1, 15, 30 and 45 of age.

| Variable | Days | Mean ± standard deviation | | |
|---|---|---|---|---|
| | | Treated group | Control group | *$p$-value |
| Erythrocytes (x$10^6$ µL) | 1 | 5.13 (0.25)$^{b}$ | 5.23 (0.40) $^{c}$ | 0.19 |
| | 15 | 8.51 (1.89) $^{a}$ | 9.10 (1.67) $^{ab}$ | 0.46 |
| | 30 | 8.40 (1.22) $^{a}$ | 8.30 (0.75) $^{b}$ | 0.84 |
| | 45 | 9.78 (1.09) $^{a}$ | 9.63 (0.41) $^{a}$ | 0.69 |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Hematocrit (%) | 1 | 33.33 (3.06) $^{ab}$ | 30.00 (1.00) $^{b}$ | 0.96 |
| | 15 | 39.60 (5.21) $^{a}$ | 43.10 (7.20) $^{a}$ | 0.23 |
| | 30 | 26.15 (1.96) $^{c}$ | 25.17 (2.56) $^{c}$ | 0.35 |
| | 45 | 30.54 (3.19) $^{bc}$ | 29.59 (1.57) $^{b}$ | 0.41 |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Hemoglobin (g/dL) | 1 | 8.83 (0.70) $^{c}$ | 8.87 (0.90) $^{b}$ | 0.76 |
| | 15 | 10.75 (1.28) $^{ab}$ | 11.10 (1.45) $^{a}$ | 0.62 |
| | 30 | 9.68 (1.07) $^{bc}$ | 9.04 (0.75) $^{b}$ | 0.14 |
| | 45 | 11.13 (1.18) $^{a}$ | 10.89 (0.53) $^{a}$ | 0.56 |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Leukocytes (x$10^3$ µL) | 1 | 8.33 (1.21) | 8.73 (1.03) $^{b}$ | 0.68 |
| | 15 | 11.44 (3.78) | 12.20 (3.66) $^{a}$ | 0.65 |
| | 30 | 8.94 (1.50) | 8.59 (3.61) $^{ab}$ | 0.78 |
| | 45 | 9.59 (0.83) | 8.83 (0.74) $^{b}$ | 0.04* |
| p-value$^{\&}$ | | 0.08 | 0.01 | |
| Lymphocytes (x$10^3$ µL) | 1 | 3.93 (0.51) $^{b}$ | 4.10 (0.85) | 0.78 |
| | 15 | 4.65 (1.82) $^{ab}$ | 4.93 (1.82) | 0.73 |
| | 30 | 5.29 (1.70) $^{ab}$ | 5.42 (1.48) | 0.50 |
| | 45 | 6.17 (0.50) $^{a}$ | 4.71 (0.33) | 0.001* |
| p-value$^{\&}$ | | 0.001 | 0.27 | |
| Neutrophil (x$10^3$ µL) | 1 | 4.00 (1.18) $^{ab}$ | 3.97 (0.85) $^{b}$ | 0.97 |
| | 15 | 5.88 (2.51) $^{a}$ | 6.37 (2.23) $^{a}$ | 0.64 |
| | 30 | 2.52 (1.43) $^{bc}$ | 2.40 (1.44) $^{c}$ | 0.85 |
| | 45 | 2.36 (0.51) $^{c}$ | 2.89 (0.65) $^{bc}$ | 0.04* |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Monocytes (x$10^3$ µL) | 1 | 0.30 (0.20) $^{c}$ | 0.33 (0.25) $^{c}$ | 0.86 |
| | 15 | 0.54 (0.42) $^{bc}$ | 0.59 (0.46) $^{bc}$ | 0.80 |
| | 30 | 1.10 (0.57) $^{a}$ | 1.44 (0.21) $^{a}$ | 0.06 |
| | 45 | 0.85 (0.23) $^{ab}$ | 1.00 (0.35) $^{ab}$ | 0.29 |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Eosinophils (x$10^3$ µL) | 1 | 0.17 (0.12) | 0.10 (0.10) | 0.49 |
| | 15 | 0.35 (0.28) | 0.32 (0.27) | 0.81 |
| | 30 | 0.28 (0.10) | 0.26 (0.13) | 0.63 |
| | 45 | 0.21 (0.07) | 0.24 (0.10) | 0.41 |
| p-value$^{\&}$ | | 0.45 | 0.24 | |

*P < 0.05 represents significant differences between groups. $^{\&}$ Similarly, P < 0 .05 in the same column shows difference between moments after repeated analysis over time (illustrated with different subscript letters). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

### 2.5. Serum biochemistry

Serum levels of total protein, glucose, albumin, and urea were measured using commercial kits (Analisa®) and a semi-automated analyzer (BioPlus-2000®). Serum levels of globulins were obtained by the formula: total protein – albumin.

### 2.6. Antioxidant enzymes: catalase, superoxide dismutase and xanthine oxidase

Catalase (CAT) activity in total blood was measured according to the method described by Nelson and Kiesow (1972) and the results were expressed in U CAT/ mg of protein. Superoxide dismutase (SOD) activity was measured and quantified according to the technique described by McCord and Fridovich (1969) and the results were expressed in U SOD/ mg of protein.

Serum xanthine oxidase (XO) activity was determined using the method described by Westerfeld and Richert (1949). The reaction mixture contained 1 mM of xanthine as substrate and 50 mM of phosphate buffer (pH 7.4). This mixture was incubated with approximately 0.5 mg of protein at 37 °C for 60 min in a final volume of 0.5 mL. The rate of urate formation from xanthine degradation was determined by measuring absorbance at 290 nm. Activity was expressed as U/L.

### 2.7. Immunoglobulins

Immunoglobulins (IgG and IgM) were quantified using ELISA commercial kits (eBIOSCIENCE, San Diego, USA), according to the manufacturer's.

### 2.8. Determination of seric concentrations of minerals

The seric concentrations of selenium, cooper, manganese and zinc were determined (DOL 1, 15 and 45) as described by Flores et al. (2001) using the Hydride Generation Atomic Absorption Spectrometry (HG–AAS) technique and chemicals of analytical grade (Merck, Darmstadt). Thus, 250 µL of $HNO_3$ and 62.5 µL of $H_2O_2$ were added to 125 µL of serum. Milli-Q water was added to achieve the final volume and the solution was analyzed by Inductively Coupled Plasma–optical Emission Spectrometry (ICP-OES).

### 2.9. Sampling and fecal analyses

Fecal samples (DOL 15, 30 and 45) from all animals were collected from the rectal ampulla to count for the number of oocysts per gram of feces (OOPG) using the McMaster technique (Gordon and Whitlock, 1939) with sucrose solution.

### 2.10. Statistical analysis

Data was analyzed using the descriptive statistics for contingency of information and for further assumptions, which were presented as descriptive (mean and standard deviation) for blood cell parameters: hematocrit, erythrocyte count, hemoglobin, leukocytes, lymphocytes, monocytes, and eosinophils. The second group of parameters measured were immunoglobulins (G and M) and XO. The third set of data was for CAT and SOD activities, followed by biochemical components: total protein, urea, glucose, albumin, and globulin. Finally, measurements associated with animal weight: body weight and weight gain. For each group and day of observation (DOL 1, 15, 30, and 45), all parameters were tested for normality using the Shapiro-Wilk test. Skewness, kurtosis and homogeneity were evaluated by the Levene test, or log transformation when needed. A $t$-test was used to analyse all parameters, i.e. between groups (controlling data dependency due to dependence in time), and over time for weight (DOL 1, 15, 30, and 45). Significant difference was set at $P < .05$. Statistical manipulations were performed using R-language, v.3.1 (R Development Core Team 2012).

## 3. Results

### 3.1. Weight gain

Treated animals showed higher body weight and weight gain ($P < .05$) on days 15 and 45 of age compared with untreated lambs (Fig. 1-A-B). Over time, body weight increased in both groups ($P < .001$).

### 3.2. Parasitological examination

On DOL 15, all animals were negative for oocysts of *Eimeria* spp. On

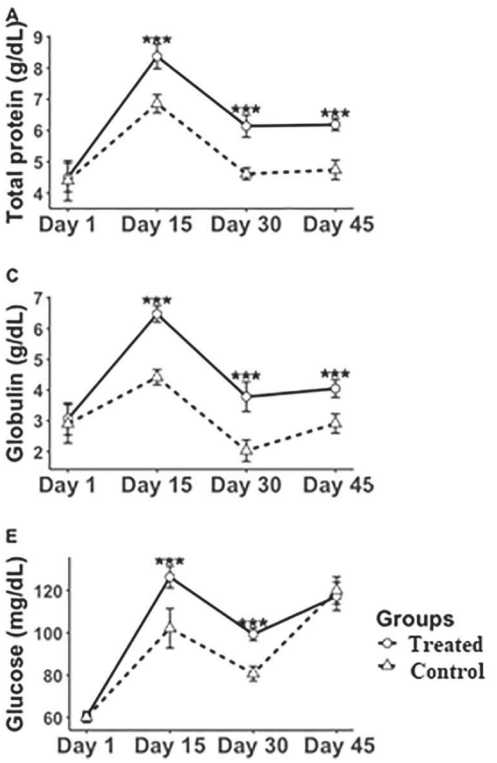


**Fig. 2.** Seric levels of total protein (A), albumin (B), globulin (C), urea (D) and glucose (E) of treated ($n$ = 10) and untreated (control; n = 10) lambs on days 1, 15, 30 and 45 of age. Asterisks indicate significant differences between groups (***P < .05). Note: treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

DOL 30, the number of *Eimeria* spp. oocysts was higher ($P < .05$) in the control group (2800 ± 1435 OOPG) compared with the treated group (750 ± 574 OOPG). Most animals showed signs of diarrhea, and thus, all animals were treated with toltrazuril at DOL 31 (Baycox Ruminantes®) at a dose of 5 mg/kg. At DOL 45 all animals were negative for any type of oocysts.

### 3.3. Blood counts

No difference was observed between groups regarding red blood cells, hematocrit, and hemoglobin concentration ($P > .05$ – Table 2); however, over time, these variables differed in both groups (P < .001). In summary, there was an increase in these variables on days 15 and 45 of life compared to day 1 in both groups. In general, there was a reduction in some erythrogram variables on day 30 of age (Table 2), a period that coincided with natural coccidial infection.

Total leukocytes on DOL 45 were significantly higher in treated animals compared with untreated lambs ($P < .05$), due to an increased number of lymphocytes (Table 2). The number of neutrophils was lower in treated lambs (DOL 45). The other white cells (eosinophils and monocytes) did not differ between groups (P > .05, Table 2). In summary, over time there were changes in the leukogram of lambs, except for the number of eosinophils (Table 2). We would like to draw attention to lymphocyte counts that increased over time in treated lambs ($P < .05$; Table 2).

### 3.4. Serum biochemistry

Serum levels of total proteins and globulins were higher ($P < .05$) in treated animals (DOL 15, 30, and 45) compared with control (Fig. 2 A, C), while serum albumin levels were lower in the treated group (DOL 15) (Fig. 2-B). Treated animals showed constant serum levels of urea throughout the experiment, but it was lower on DOL 30 and higher on DOL 45 in the control group (Fig. 2-D). Serum levels of glucose were higher in treated animals on DOL 15 and 30 compared with the control group ($P < .05$, Fig. 2-E). Over time, all biochemical variables increased ($P < .05$) in both groups comparing to DOL 1 to DOL 15 of age. However, no differences were found on total protein and globulins on day 1 to day 30 and 45 only in the control group, but there was a reduction from day 15 to 30 and 45 in both groups ($P < .05$).

### 3.5. Antioxidant enzymes and xanthine oxidase activity

The activity of antioxidant enzymes was shown in Fig. 3. CAT activity did not differ between groups ($P > .05$, Fig. 3-A). Superoxide dismutase and xanthine oxidase activities were higher ($P < .05$) on DOL 15, 30, and 45 in treated compared with untreated lambs (Fig. 3 B, C). In general, CAT, SOD and XO activities increased in both groups (P < .05). However, at the first moment, the XO activity was lower on DOL 1 to 15.

### 3.6. Immunoglobulins

Elevated levels of IgM and IgG on DOL 15 were observed in mineral treated compared to untreated lambs (Fig. 4). However, IgM levels decreased (P < .05) on DOL 30 in treated animals compared with control lambs, while IgG levels increased (Fig. 4-B). Levels of IgM increased over time (DOL 1 to DOL 15 and 30), and subsequently it reduced on DOL 15 and 30 to DOL 45). Over time, IgG levels increased in both groups (P < .05).

### 3.7. Mineral in serum

Selenium (DOL 45) and zinc (DOL 15 and 45) levels in serum were higher in treated compared to untreated lambs (P < .05; Table 3). Cooper and manganese levels in serum did not differ between groups (P > .05). Numerically (P > .05), all four minerals showed higher levels in treated animals (DOL 15 and 45). In summary, the levels of all minerals increased over time (P < .05), with the exception of manganese that did not differ over time untreated lambs (P > .05).

## 4. Discussion

The metaphylactic effects of minerals in newborn lambs remain

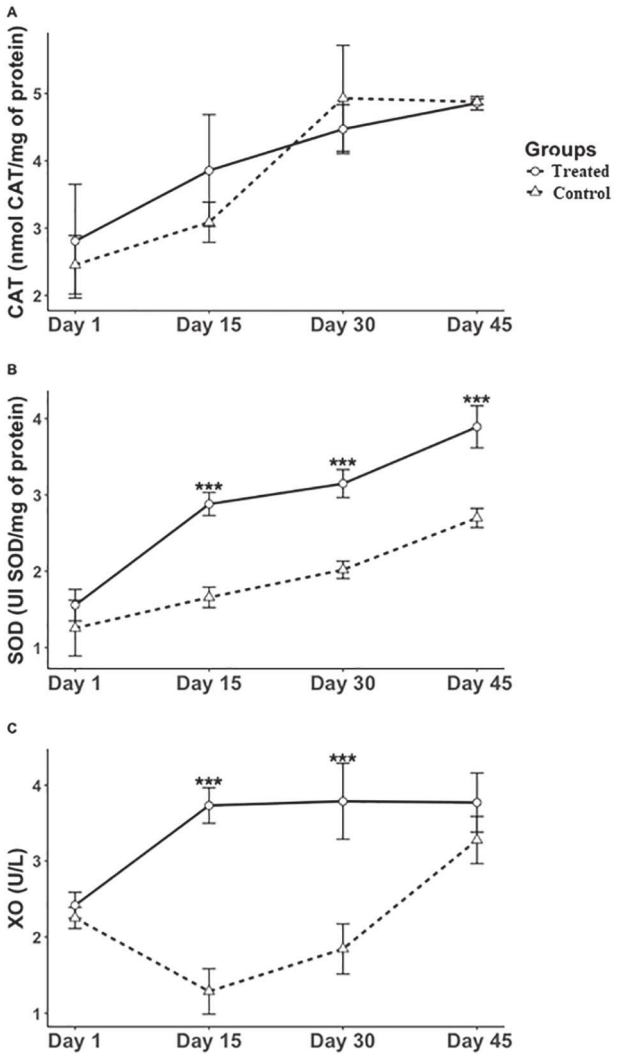


**Fig. 3.** Superoxide dismutase (SOD) [A], catalase (CAT) [B] and xanthine oxidase (XO) [C] activities in treated (n = 10) and untreated (control; n = 10) lambs on days 1,15,30 and 45 of age. Asterisks indicate significant differences between groups (***P < .05). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

poorly understood. However, Arthington et al. (2014) demonstrated that the administration of a mineral complex in beef cattle based on zinc, manganese, copper, and selenium stimulated humoral responses and increased mineral deposition in the liver. In the present study, a mineral complex based on zinc, manganese, copper, and selenium stimulated the immune and antioxidant responses. These results are similar to those observed by Soldá et al. (2017) while studying cows injected with mineral complex during the transitional period. The use of minerals (subcutaneously or intramuscularly) might be beneficial to prevent some negative interactions that may occur during digestion and absorption of minerals and to increase mineral levels at times of greater demand (i.e., growth, lactation and reproduction). After parenteral injection, these elements circulate in the animal's body and may be incorporated into the cells as needed, excedent amounts are bounded to liver proteins for further use or filtered and excreted by this organ (Suttle, 2010). In this way, minerals can be better utilized by the animal.

Mineral application in lambs did not alter blood counts, but increased the number of total leukocytes on DOL 45 as a result of increased lymphocytes, and this increase can be attributed to selenium mitogenic property, i.e., selenium induces the proliferation of peripheral blood lymphocytes by changes on the mitotic index (Hawkes et al., 2001). Also, a study conducted by Prasad (2008) reported that zinc is capable to induce T-lymphocytes proliferation by the inhibition of interleukin-2, since this interleukin is an inhibitor of the cellular cycle of the immune cells, such as lymphocytes. There were also increased serum levels of IgM and IgG probably a consequence of increased lymphocyte counts, since immunoglobulins are molecules synthesized by lymphocytes (Fernández-Cruz et al., 2009). Moreover, increased seric IgM and IgG levels can be attributed to copper, since a study conducted by Makhlouf et al. (1998) demonstrated its ability to stimulate the synthesis of antibodies and the formation of immune complexes, which consequently, rises the immunoglobulin levels. Immunoglobulin G is found in large quantities in blood and extracellular fluids, serving essential functions in antibody-mediated defense, as well as in the neutralization of toxins, immobilization of bacteria, sensitization of natural killer cells, and activation of the complement system (Tizard, 2013). Lambs supplemented with minerals showed elevated serum levels of total proteins and globulins, suggesting an activation of the immune response. On the other hand, we found that untreated animals were more affected by coccidiosis due to poor immune responses. Mineral supplementation for lambs was able to enhance their immune responses, meliorating their overall health, explaining their greater weight gain.

Treated animals showed better metabolic stability throughout the experiment compared to untreated lambs. Interestingly, untreated animals showed relatively higher serum levels of albumin on DOL 15, which may be interpreted as a compensatory mechanism in response to loss of other proteins, since albumin is involved in several vital functions, including blood homeostasis and coagulation (Bern et al., 2015). Lambs in the control group showed fluctuations in serum urea levels, suggesting a higher susceptibility to metabolic disorders and other risks to the animal's health (Peixoto and Osório, 2007). In the current study, the control group showed only minor weight gain and serum levels of glucose were greater in treated animals, suggesting better carbohydrate availability. These findings may suggest that the mineral application improved the digestion and absorption of nutrients, mainly carbohydrates. In addition, increased serum glucose levels may be considered beneficial overall for several metabolic functions, including ATP synthesis (Alberts et al., 2002).

SOD and XO activities increased in treated animals compared with control. This may be attributable to the role of copper and zinc in the SOD molecule. This enzyme participates in the control of free radicals, molecules that mediate oxidative stress (Andrade and Marreiro, 2011). Although the treatment did not alter CAT activity, a study conducted by Soldá et al. (2017) showed that mineral complex supplementation augments the antioxidant system of cows during the transitional period. This effect may be due to improved CAT activity. This enzyme is also an important antioxidant associated with the decomposition of hydrogen peroxide (Van der and Janssen-Heininger, 2014). XO activity is associated with catalysis of purine proteins (adenine, guanine and hypoxanthine) to uric acid, the final product of purine metabolism (Stangassinger et al., 1995). Augmentation of serum XO activity contributes to increased levels of uric acid, a molecule that also shows antioxidant properties (Ames et al., 1981). All these effects benefit animal's health by preventing or dimishing oxidative stress (Birten et al., 2012). Therefore, mineral application may help lamb's health indirectly by enhancing some anti-oxidant activities.

Eimeriosis is a common disease in newborn lambs (Urquhart et al., 1996), principally in intensive systems (Lima, 2004). In lambs, this disease is usually associated with severe diarrhea, fever, anorexia, weight loss, decreased wool quality, and death (McDougald, 1979). Farms that do not employ coccidiostatics in animal feed, commonly treat affected animals with toltrazuril due to f its high efficacy, as observed in this study. Both groups showed diarrhea, but treated animals shed lower amounts of *Eimeria* spp. oocysts. This can be explained by an enhancement of the immune system by zinc, copper and selenium, since

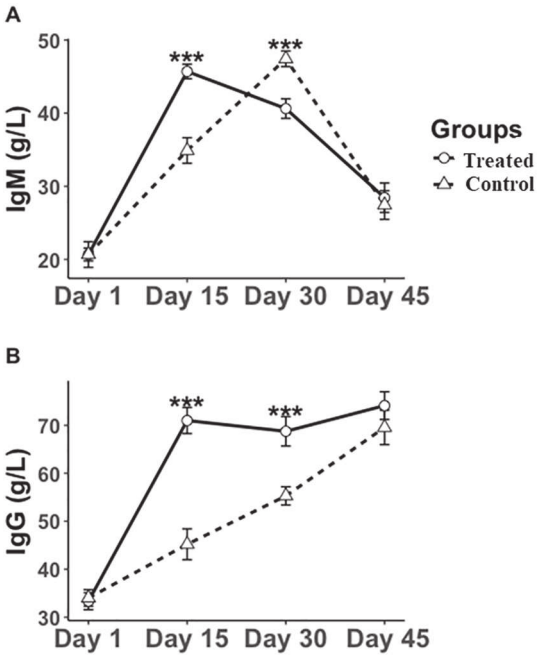


**Fig. 4.** Seric levels of immunoglobulins: IgM (A) and IgG (B) in treated (n = 10) and untreated (n = 10) lambs on days 1,15, 30 and 45 of age. Asterisks indicate significant differences between groups (***P < .05). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

**Table 3**
Mean and standard deviation of minerals (selenium, cooper, manganese and zinc) used for treated (n = 10) and untreated control; (n = 10) lambs on days 1, 15 and 45 of age.

| Mineral | Days | Mean ± standard deviation | | |
|---|---|---|---|---|
| | | Treated | Control | *p-value |
| Selenium (μg/L) | 1 | 39.7 ± 5.6$^{c}$ | 42.0 ± 8.4$^{b}$ | 0.65 |
| | 15 | 89.0 ± 6.2$^{b}$ | 81.0 ± 12.1$^{a}$ | 0.59 |
| | 45 | 103.5 ± 9.5$^{a}$ | 85.5 ± 13.9$^{a}$ | 0.01* |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Cooper (μmol/L) | 1 | 6.2 ± 0.9$^{b}$ | 5.6 ± 1.2$^{b}$ | 0.72 |
| | 15 | 14.7 ± 4.3$^{a}$ | 11.1 ± 5.1$^{a}$ | 0.41 |
| | 45 | 13.4 ± 3.6$^{a}$ | 10.0 ± 1.9$^{a}$ | 0.19 |
| p-value$^{\&}$ | | 0.001 | 0.001 | |
| Manganese (μg/L) | 1 | 6.1 ± 0.5$^{b}$ | 6.2 ± 0.8 | 0.84 |
| | 15 | 7.8 ± 0.7$^{a}$ | 6.9 ± 1.3 | 0.31 |
| | 45 | 7.9 ± 1.4$^{ab}$ | 6.7 ± 1.0 | 0.18 |
| p-value$^{\&}$ | | 0.03 | 0.52 | |
| Zinc (μmol/L) | 1 | 9.80 ± 4.0$^{b}$ | 12.1 ± 5.2$^{b}$ | 0.68 |
| | 15 | 28.9 ± 3.3$^{a}$ | 21.0 ± 6.1$^{a}$ | 0.01* |
| | 45 | 39.0 ± 11.1$^{a}$ | 24.2 ± 10.5$^{a}$ | 0.01* |
| p-value$^{\&}$ | | 0.001 | 0.001 | |

*P < .05 represents significant differences between groups. $^{\&}$ Similarly, P < .05 in the same column shows significant differences between moments after repeated analysis over time (illustrated with different subscript letters). Note: Treated lambs received minerals (zinc, copper, selenium, and manganese) subcutaneously on days 1 and 30 of age.

they are important minerals to stimulate the immunological response, leading to greater protection against aggressive infectious agents such as *Eimeria* spp. In addition, Cu is involved in antibody synthesis and secretion (mainly IgG), cellular immunity, and inflammatory responses (Saker, 2006); as well as zinc and copper by playing an important role in the redox metabolism, suggesting an antioxidant effect, mainly due to the activation of some enzymes, such as superoxide dismutase (Overbeck et al., 2008). Similarly, selenium helps to maintain many defense mechanisms, including antibody production, cell proliferation, cytokine production, prostaglandin metabolism, in addition to proper function of immune cells in the innate response (Smith et al., 1984; Hoffmann, 2007), as well as to enhance protection of cell membranes from oxidative damage (McDowell, 1992). Therefore, the mechanism involved in minimizing the negative effects of coccidiosis in lambs was indirect, i.e., the application of mineral caused a nutraceutical effect seen by a greater inflammatory and antioxidant responses, thus, protecting them from an exacerbated infection by *Eimeria* spp.

Based on these evidences, we conclude that the subcutaneous use of mineral complex based on zinc, manganese, copper and selenium in newborn lambs increases their concentration in the blood, and indirectly these minerals mediate the activation of the antioxidant system and enhance their immune responses. As a consequence, there was a better response of the animals against *Eimeria* spp., and the excretion of oocysts and clinical signs were minimized in treated animals. Moreover, mineral complex applied subcutaneously to lambs may favor the metabolism of proteins and carbohydrates, leading to increased body weight gain.

## Ethics committee

This study was approved by the Ethics Committee of Use of Animals (CEUA) of Universidade do Estado de Santa Catarina (UDESC), under protocol number 7398301116.

## Acknowledgment

We thank CAPES, CNPq and UDESC (PROMOP) for financial support.

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