



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

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### 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 increase 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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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; Ortnho, 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

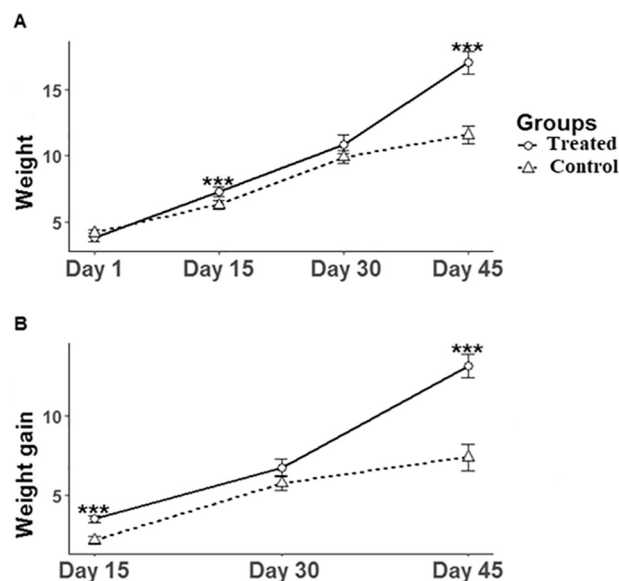
We used a total of 110 newborn lambs, Lacaune breed, weighing approximately 3 kg at birth. They were housed in pens (5 animals each) and divided into two groups ( $n = 55$  each): a control group and a treated group. Animals from both groups received colostrum in the first few hours of life. Thereafter, 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

**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 <sup>1</sup> (mL)	500	200	–
Replacer milk <sup>2</sup> (mL)	–	300	500
Concentrate <sup>3</sup> (g)	–	100	300

<sup>1</sup>Milk offered to lambs soon after ewe's milking. <sup>2</sup> 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. <sup>3</sup> 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. <sup>3</sup> 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.

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.

The diet of ewes in pre-calving was with concentrate (0.5 kg/day) and silage (3.0 kg/day). The ingredients present in concentrate were ground corn, soybean meal, calcitic limestone, sodium bicarbonate, and vitamin and mineral nucleus (calcium 195–220 g; phosphorus min. 39 g; sodium min. 75 g; sulfur min. 18 g; magnesium min. 12 g; cobalt min. 45 mg; iodine min. 65 g; manganese min. 1300 mg; selenium min. 15 mg; zinc min. 3500 mg; niacin min. 500 mg; vitamin A min. 316,000 mg; vitamin D3 min. 63,000 UI; vitamin E min. 650 UI; fluorine max. 390 mg in 1.0 kg of product). Animals were fed in collective stalls.

### 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).

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