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Nutraceutical Effect of Trace Elements as Additional Injectable Doses to Modulate Oxidant and Antioxidant Status, and Improves the Quality of Lamb Meat

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Abstract

Our study aimed to evaluate whether zinc, copper, selenium, and manganese subcutaneous mineral application (trace elements) reduced mortality, improved performance, and modulated oxidant and antioxidant balance in lamb meat, thereby improving its quality. We divided the 110 newborn Lacaune lambs into two groups: non-treated (control), and treated (application of minerals) with three doses of 0.33 mL/kg of body weight mineral complex on days of life 1, 30, and 60. All animals were weighed on day of life 1, 30, 60, 90, and 150. At the end of the experiment, 12 animals were slaughtered for physical and chemical analysis of meat, oxidant, and antioxidant status, and for allometric analysis. Mineral-application animals had greater live-weight ($P < 0.05$) on days of life 60 and 90. There was an increase in fat thickness ($P = 0.004$); pH levels ($P = 0.002$) were lower in mineral-application animal meat than in that of the control group. Meat was paler (according to lightness (L color)) in the control group ($P = 0.04$). Weight loss from cooking was greater in control animals ($P = 0.004$). Shear strength values were lower in the meat of treated lambs ($P = 0.008$) suggesting that mineral application was associated with increased meat tenderness. In addition, catalase and superoxide dismutase activities were higher ($P = 0.01$) in mineral-treated animals, associated with a reduction in reactive oxygen species levels ($P < 0.01$), and lipid peroxidation products ($P = 0.02$). These data suggest that mineral application modulated oxidant and antioxidant status, reflecting better meat quality.

Keywords Performance · Lipid oxidation · Reactive oxygen species · Antioxidant system · Mineral

Introduction

With the increasing demand for sheep meat, sheep breeding has become an increasingly attractive pursuit in the agricultural economy [27]. Because they are considered fast-cycling animals with accelerated metabolism, sheep are susceptible to various diseases that compromise the entire production cycle, depreciating value of the final product, i.e., lamb meat [25]. Mineral deficiencies cause substantial morbidity in these animals, manifesting as decreased productivity, growth impairment, poor weight gain, susceptibility to disease, and decreased fertility [25]. Minerals such as manganese, zinc, selenium, and copper are required in various quantities by these animals, and all are essential for maintaining proper physiological function. Deficiencies of one or more limit animal performance [25].

The micronutrient content of animal meat varies, depending on muscle type, age, species, nutritional status, breed, genetics, sexual maturity, and presence of a suitable production system [33]. Quality sheep meat (especially lamb) is characterized by a combination of attributes including flavor, juiciness, texture, softness, and appearance [39, 46]. It is essential to implement new alternatives, aiming for greater productivity and quality, to satisfy the increasingly demanding consumer [16]. Retailers view meat color as a primary consideration for

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consumers who prefer bright red color (oxymyoglobin) in fresh meat and avoid brown color (metmyoglobin) [47]. The pH of sheep meat ranges from 5.5 to 5.8; however, higher values (≥ 6.0) may be found in animals with depleted muscle glycogen prior to slaughter. This may influence the quality of the final product [16].

Oxidative stress caused by heat stress and incorrect management reduced meat quality, principally texture and protein contents [49]. For this reason, several interventions have been implemented to reduce animal deaths and aid production of good quality product for consumers, including the use of mineral products in the form of injectable micronutrient solutions. The latter are increasingly employed in conjunction with initial immunization in young animals in order to prevent and treat micronutrient deficiencies. These products may prevent or minimize oxidative stress during the weaning period [40], consequently improving meat quality [23]. Khan et al. [23] demonstrated that supplementation with sodium selenite, a compound used in the present study, was capable of improving meat quality via prevention of meat oxidation and augmentation of antioxidant status in breast muscle of broiler chickens. In addition, Holen et al. [21] reported that zinc, another compound used in the present study, improved carcass composition and meat quality in pigs housed under crowded conditions. Recently, Soldá et al. [44] demonstrated that injectable mineral application based on copper and sodium selenite reduced lipid oxidation and improved antioxidant capacity in dairy cows during the transition period, improving animal health. Paz Matias [32] suggested that zinc supplementation improved SOD activity, thereby boosting the antioxidant system. This occurs because zinc is a cofactor of the enzyme, and is necessary for its proper function.

Thus, our hypothesis was that mineral application based on zinc, copper, selenium, and manganese would improve meat quality via reduction of oxidative processes and increase of antioxidant levels that characterize the nutraceutical effect of the minerals. This nutraceutical term derives from the junction of "nutrients" and "pharmaceuticals," because the nutrients, in this case minerals, have proven ability to provide health benefits, including prevention and/or therapy. The objective of this study was to evaluate whether additional injectable doses of minerals (zinc, copper, selenium, and manganese) in lambs reduced mortality, improved performance, and modulated oxidant and antioxidant status, with consequent improvements in meat quality.

Materials and Methods

Product

The commercial product used in this study was Adaptador® (Biogen). The product included copper edetate (1.0 g), zinc edetate (4.0 g), manganese edetate (1.0 g), and sodium selenite (0.5 g) per 100 mL. Based on the information given was calculated that the product contained 1.6 mg Zn/mL, 1.9 mg Cu/mL, 1.6 mg Mn/mL, and 2.3 mg Se/mL.

Animals

The experiment was conducted on a commercial farm in the municipality of Chapecó (latitude 27° 05' 47" S; longitude 52° 37' 06" W) between September 2017 and February 2018. We studied 110 male newborn Lacaune lambs weighing approximately 3 kg. The animals were randomized into two groups, a non-treated group (control, 55 animals), and a group given mineral supplementation (treated, 55 animals); in addition, the lambs were sub-grouped by chronological order of birth. Both groups were raised simultaneously. Adaptador was injected at 0.33 ml/kg of body weight (BW) subcutaneously on day of life (DOL) 1 (hours after birth) and the injection was repeated on DOLs 30 and 60. It is important to note that the animals were weighed and that the dose of minerals was calculated individually, i.e., applied volumes differed between animals.

If we consider the average body weight of each group in the three periods, approximately, at DOL 1 (mean 3.0 kg BW/animal), DOL 30 (mean 8.4 kg BW/animal), and DOL 60 (mean 17.4 kg BW/animal), the calculated dose of minerals was Zn 1.6, 4.5, 9.3 mg; Cu 1.9, 5.3, 11.0 mg; Mn 1.6, 4.5, 9.3 mg; and Se 2.3, 6.5, 13.4 mg, respectively. Note that the injection has a nutraceutical role, i.e., providing additional benefit beyond fulfilling basic requirements.

Animals from both groups received colostrum in the first hours of life. Subsequently, the lambs were allocated at five per stall and fed sheep's milk until DOL 7, followed by artificial and sheep's milk on DOL 8–20, artificial milk on DOL 21–45 (weaning) and commercial concentrate with 20% crude protein (CP) on DOL 8–45 (Table 1).

The feeding regimen was determined according to life stage of the animal (Table 1), and diet was formulated according to their physiological needs up to 150 days [30]. The animals received daily concentrate, hay, and silage (Table 1). The measures of dry matter, crude protein, and minerals (copper, zinc, selenium, and manganese) were determined in silage, hay, and concentrate by a near-infrared spectroscopy method in a commercial laboratory [37, 38] (Table 2).

After weaning, 20 lambs were placed in two stalls (control and treatment groups: 10 animals each) in order to evaluate feed intake for five consecutive days in three periods (days 60–64, 90–94, and 150–154 of the experiment). The remaining animals were kept in a collective pen, receiving the same diet as the others. Animals were offered feed at 2% live weight/animal twice a day (8:00 AM and 6:00 PM). Leftovers were quantified.

Table 1 Ingredients and diet used to feed lambs from birth to slaughter. Animals from both groups received the same diet throughout the experimental period

Ingredients	Age of animals (days)				
	1 to 7	8 to 20	21 to 45	46 to 90	91 to 150
Ewe milk (mL/animal/day)	500	200	–	–	–
Artificial milk (mL/animal/day)	–	300	500	–	–
Commercial concentrate (g/animal/day)*	–	140	300	–	–
Concentrate (g/animal/day) #	–	–	–	400	600
Corn silage (kg/animal/day)	–	–	–	1.00	1.50
Hay (kg/animal/day)	–	–	0.12	0.25	0.30
& Ingredients of the concentrate					
Ground corn (%)	–	–	–	61.0	70.0
Soybean meal (%)	–	–	–	25.0	25.0
Powdered milk (%)	–	–	–	10.0	–
$Mineral/vitamin (%)	–	–	–	4.0	3.0
Ammonium chloride (%)	–	–	–	–	1.0
Calcitic limestone (%)	–	–	–	–	1.0
Calculated mineral composition of total diet+					
Calcium (g/kg)	2.39	2.22	3.60	5.48	8.38
Phosphorus (g/kg)	1.72	1.49	2.23	3.18	4.58
Copper (mg/kg)	1.79	4.80	9.45	0.00	0.00
Iron (mg/kg)	30.2	23.02	37.55	22.75	25.26
Manganese (mg/kg)	6.44	13.15	25.61	24.8	27.9
Selenium (mg/kg)	0.50	0.23	0.31	0.35	0.40
Zinc (mg/kg)	25.0	19.78	30.25	48.0	54.0

* Specific commercial concentrate (pelleted) for lambs: crude protein (min 210 g/kg), crude fat (min 30 g/kg), crude fiber (max 100 g/kg), acid detergent fiber - ADF (max 120 g/kg), ash (max 100 g/kg), calcium (min 8 g/kg, and max 12 g/kg), phosphorus (min 6 g/kg), *Saccharomyces cerevisiae* (min 1.5×10^7 CFU/kg), vitamin A (min 15,000 IU/kg), vitamin D3 (min 2250 IU/kg), vitamin E (min 50 IU/kg), copper (min 30 mg/kg), iron (min 100 mg/kg), iodine (min 1.8 mg/kg), manganese (min 80 mg/kg), selenium (min 0.6 mg/kg), cobalt (min 1 mg/kg), zinc (min 80 mg/kg), growth promoter additive (Lasalocid) (45 mg/kg)

Concentrate (mash) produced on the farm with ingredients described in this table (&), using Y-type mixer

$ Premix composition (Nucleus®): phosphorus (min 55 g/kg), calcium (min. 215 g/kg, max 225 g/kg), sulfur (min 12 g/kg), sodium (min 80 g/kg), cobalt (min 60 mg/kg), chromium (min 12 mg/kg), iron (min 1420 mg/kg), iodine (min 100 mg/kg), magnesium (min 14 mg/kg), manganese (min 1550 mg/kg), selenium (min 22 mg/kg), vitamin A (min 20,000 IU/kg), vitamin D (min 40,000 IU/kg), vitamin E (min 550 IU/kg), and fluorine (max 550 mg/kg)

+ The levels of minerals in the diet were calculated according to requirements of the NRC [30]

Blood Collection

Whole blood from 10 animals per group was collected from the jugular vein, being the same animals used in the three collection periods (DOL 1, 30 and 60). Tubes without anticoagulant were used to obtain serum after centrifugation (3500 RPM × 10 min) for measurement of minerals, biochemical and immunological variables. All samples were stored at − 20 °C until analysis.

Mineral Analysis in Serum

Concentrations of selenium, cooper, manganese, and zinc were measured at DOL 1, 30, and 60 in blood serum, and samples were decomposed accordingly to Flores et al. [15]. 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).

Total Antioxidant and Cytokine Levels

Total antioxidant levels in serum were measured through the ferric reducing ability of plasma (FRAP) in serum of according to the technique described by Benzie and Strain [5] and the results were expressed in μmol/L. Serum concentrations of interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) were determined using the commercial immunoassay Quantikine®, according to manufacturer's recommendations (R & D Systems, Minneapolis, MN, USA). The presence and

Table 2 Chemical composition of ingredients used in lamb diets

Chemistry composition	Silage	Hay	*Concentrate (8–45 days)	#Concentrate (46–150 days)
Dry matter (%)	29.8	86.2	88.0	87.5
Crude Protein (%)	7.78	14.5	20.8	16.4
Copper (mg/kg)	nd	nd	30.0	nd
Zinc (mg/kg)	0.15	nd	80.2	89.8
Selenium (mg/kg)	0.03	nd	0.61	0.66
Manganese (mg/kg)	24.1	nd	81.0	46.5

not-detected (nd)

*Commercial concentrate

Concentrate produced with ingredients described in Table 1

concentration of cytokine were determined by color intensity measured spectrophotometrically in a micro ELISA reader. Results were expressed in pg/mL.

Growth Performance

All animals in the experiment were weighed on DOL 1, 30, 60, 90, and 150 using a digital scale.

Allometric Analysis, Slaughter and Muscle Collection

Twelve animals were slaughtered (control ($n = 6$) and treated ($n = 6$)), with all animals at 158 days of age. Note that the age (DOL 158 ± 0) and mean body weight of each group (control ($n = 48$), 35.8 ± 5.67 kg and treated ($n = 52$), 38.1 ± 4.73 kg) were used to define slaughtered animals. First, the animals were weighed on the day of slaughter. Then, the pelvic limb height, perimeter, thoracic limb height, body length in vivo, length of lamb carcass, depth of thorax, length of pelvic limb, length of thoracic limb, pelvic limb width, perimeter of the testicle, and distal extremity of the limbs (legs) were evaluated (cm) with a measuring tape. After slaughter, the "Green viscera" (digestive system) were removed and quantified (kg), sequentially, and we weighed (kg) the heart, spleen, internal fat, testicle, penis, skin of lamb (skin and wool), lung, kidneys, head, urinary bladder, and liver.

Carcass weight, fat thickness, and ribeye (depth and width) were also measured in slaughtered animals. Subsequently, the carcasses were divided longitudinally. The longissimus dorsi muscle at the 13th thoracic vertebra was collected for meat analysis, as well as for oxidant and antioxidant status described below. In the muscle collected, we determined meat pH using a portable equipment with penetration electrode (Hanna, HI 99163).

Meat Analysis

The longissimus dorsi muscle was refrigerated at 6 °C for 24 h, and was used for color determination, water retention capacity (WRC), cooking weight loss (CL) and shear strength (SS) determinations. Loin eye area (LEA) was calculated according to Yamamoto et al. [51]. Color was calculated as follows: L* (luminosity), a* (intensity of red) and b* (intensity of yellow), using a colorimeter (Minolta CR-200). Determination of meat pH was also performed, using the same methodology previously described for the muscle. WRC was determined according to Hamm's methodology, adapted by Yamamoto et al. [51]. To determine CL during cooking, samples were weighed before and after cooking on a portable grill (Grill Mondial Due Grill Smart Pretoforno), preheated to 170 °C until achieving 75 °C internal temperature. Subsequently, we measured areas of portions of cooked samples and measured SS using a texture analyzer coupled to a Warner-Bratzler device [51].

Determination of Oxidant/Antioxidant Status in Meat

A muscle fragment of approximately 20 g was removed and was frozen for oxidant and antioxidant analysis. The sample was homogenized in buffer solution containing 10 mM Tris-HCl, pH 7.4 on ice and was centrifuged at 9000*g* for 15 min at 4 °C. Aliquots were stored at − 20 °C until utilization. Protein levels in the pancreas were standardized between 1.4 and 1.8 for biochemical tests.

Lipid peroxidation in meat was evaluated by measuring levels of malondialdehyde (MDA), using the measurement of thiobarbituric acid reactive substances (TBARS) levels [31]. Results were expressed in nanomoles MDA per g of tissue (nmol MDA/g). Oxidation of 2′−7′-dichlorofluorescein determined the index of peroxide produced by cellular components, according to the modified method of Colpo et al. [10] for determining ROS levels in meat [19, 28], being the results expressed as U DCF/mg of protein. Briefly, 50 μL of supernatants (S1) from muscle was incubated with 12 μL DCF and 1 mM CF at 37 °C for 1 h in the dark. Fluorescence was determined using 488 nm for excitation and 520 nm for emission. Fluorescence measurements were normalized to time 0

values; rates of increased fluorescence reflected ROS levels. Results were expressed as U DCFA mg^{-1} protein.

SOD activity in meat was determined spectrophotometrically by measuring inhibition of the autocatalytic rate of adrenochrome [26]. Results were expressed as U SOD/mg protein. CAT activity in meat was evaluated using the methodology of Nelson and Kiesow [29] that determines the decomposition rate of H_2O_2. CAT results were expressed as U CAT/mg protein.

Statistical Analysis

Results from each group were presented as mean and standard deviation. For weight, biochemical, and immunologic analyses, related variables for each group and day of observation were tested for normality using the Shapiro-Wilk test. Skewness, kurtosis, and homogeneity were evaluated by the Levene test, or log transformation when needed. There was a need for transformation of the following variables: FRAP in serum, as well as ROS, TBARS, CAT, and SOD in meat. Then, with all the variables with normal distribution, a *t* test was used to analyze all parameters, i.e., between groups (controlling for data dependency due to dependence in time), and over time for weight (DOL 1, 30, 60, 90, and 150), FRAP, and cytokines (DOL 1, 30, and 60). Chi-squared analysis was used to evaluate mortality data. Significant difference was assumed if $P < 0.05$. The statistical analyses were performed using R-language, v.3.1 (R Development Core Team 2012).

Results

Mineral Concentrations in Serum

Serum selenium, copper, zinc, and manganese results showed in Fig. 1. In the treated group, there was an increase in levels of selenium and copper (DOL 60) and zinc (DOL 30 and 60) compared to control. Generally, and numerically, the four minerals were higher in treated animals than in control animals (DOL 30 and 60). In the control animals, a reduction of the four minerals occurred after weaning (at 45 days), an effect that was not observed in the group supplemented for three minerals, since they maintained their levels of copper, selenium, and zinc (DOL 60).

Total Antioxidants and Cytokines

On DOL 30 and 60, FRAP levels were higher ($P < 0.05$) in animals from the treated group than in the control group (Table 3). On DOL 60, TNF-α and IL-1 levels were also higher ($P < 0.05$) in the treated group than in the control group (Table 3). Over time, the FRAP, TNF, and IL-1 levels increased in both groups (Table 3).

Growth Performance

There was a significant difference between the groups at DOL 60 and 90, where the treated group gained more weight (Fig. 2). Weight gain was significantly greater in the treatment group on DOL 60 and 90. Despite the fact that there was no significant difference at 150 days ($P > 0.05$), the animals in the treated group had 8.7% higher weight compared to the control group.

Feed intake did not differ between groups in the three periods ($P > 0.05$). The daily leftovers were 8.5% and 9.2% (days 60–64 of the experiment), 10.4% and 10.1% (days 90–94 of experiment), and 11.4% and 11.2% (days 150–154 of the experiment) to groups control and treated, respectively.

There was no difference between groups for mortality data ($P > 0.05$). Mortality was 12.75% (7/55) and 5.45% (3/55) in the control and treatment groups, respectively. The animals exhibited hyperthermia and diarrhea followed by dehydration, weight loss, and weakness, ultimately resulting in death in 2–5 days. Hyperthermic animals were given the antibiotics but without therapeutic success. The period from the appearance of clinical signs to death ranged from 2 to 5 days.

Allometric Evaluations

Allometric measurements are displayed in Table 4. No differences between groups were observed for any allometric evaluation ($P > 0.05$).

Chemical and Physical Meat Analysis

Chemical and physical analyses are presented in Table 5. Fat thickness was higher in the treated group than in the control group ($P < 0.01$). pH levels ($P < 0.01$) were lower in the treated group than in the control group. Regarding color, the only difference between groups was observed in L, returning higher values in control animals than in treated animals ($P < 0.05$). Cooking weight loss was higher in control animals than in treated animals ($P < 0.01$). The shear strength was lower in the treated group than in the control group ($P < 0.01$).

Oxidant and Antioxidant Status

Antioxidant enzyme activity in meat is shown in Fig. 3. The treated group had lower values of ROS ($P < 0.001$) and TBARS ($P < 0.05$) compared with the control group (Fig. 3b). CAT and SOD activities differed between groups ($P < 0.01$), with higher levels in the treated group (Fig. 3c, d).

Discussion

According to the literature, trace elements are essential for multiple organic processes, including skeletal development, immune responses, and productive and reproductive performance in animals [48]. Unfortunately, cattle diets do not always contain adequate amounts of trace elements to meet metabolic demands. In addition, although cattle may receive mineral supplementation ad libitum, it is not always enough to block the mineral antagonists that may be present in the diet. The use of parenteral minerals (subcutaneous or intramuscular) may be beneficial for preventing negative interactions that may occur during the digestion and mineral absorption processes or to augment the mineral status of an animal before periods of greatest need (i.e., growth, lactation, and reproduction). After parenteral injection, the elements circulate and are incorporated into the cells as needed, the rest being filtered by the liver, where minerals are required to maintain proteins for long-term use, or where they are excreted from the body [45]. In this way, minerals can be better utilized by the animal. In the present study, parenteral mineral supplementation improved performance in the growth phase during the lambs' suckling phase. In addition, supplementation improved the quality of the meat, reflected in the reduction of oxidation levels. This result was similar to that observed by Do Carmo et al. [13] in cattle supplemented with minerals. According to the authors [13], mineral supplementation may be considered a novel approach to improving animal performance and beef quality. In the present study, we did not observe a significant difference in lamb mortality with application of minerals, unlike the results reported by Zakaria et al. [53] and by Kralik and Kralik [24] in chickens supplemented with minerals. Salim et al. [34] reported that organic mineral supplementation based on Zn stimulated the development of the immune system, possibly reducing mortality rates. In addition, the presence of selenium may contribute to the reduction of mortality by

Table 3 Mean and standard deviation of ferric reducing ability of plasma (FRAP), tumor necrosis factor (TNF-α), and interleukin 1 (IL-1) in serum of treated ($n = 10$) and control ($n = 10$) animals on days of life 1, 30, and 60

Variable	Days	Mean ± standard deviation		
		Treated	Control	p value
FRAP (μmol/L)	1	60.9 (10.3)^{b}	63.5 (8.6)^{b}	> 0.05
	30	128.7(16.4)^{a}	95.6 (18.9)^{a}	< 0.05
	60	118.3 (17.5)^{a}	88.4 (12.2)^{a}	< 0.001
	p value	< 0.001	< 0.001	
TNF-α (pg/mL)	1	47.6 (9.4)^{b}	50.4 (11.6)^{c}	> 0.05
	30	143.10 (37.2)^{a}	152.20 (26.7)^{a}	> 0.05
	60	125.17 (12.5)^{a}	96.15 (21.9)^{b}	< 0.05
	p value	< 0.001	< 0.001	
IL-1 (pg/mL)	1	21.9 (10.90)^{b}	28.8 (4.70)^{c}	> 0.05
	30	91.1 (14.8)^{a}	80.7 (13.2)^{a}	> 0.05
	60	79.0 (8.7)^{a}	60.7 (11.0)^{b}	< 0.001
	p value	< 0.001	< 0.001	

$p \leq 0.05$ on the same line shows difference between groups. Similarly, $p \leq 0.05$ in the same column shows difference between moments, a repeated analysis over time (illustrated with different subscript letters)

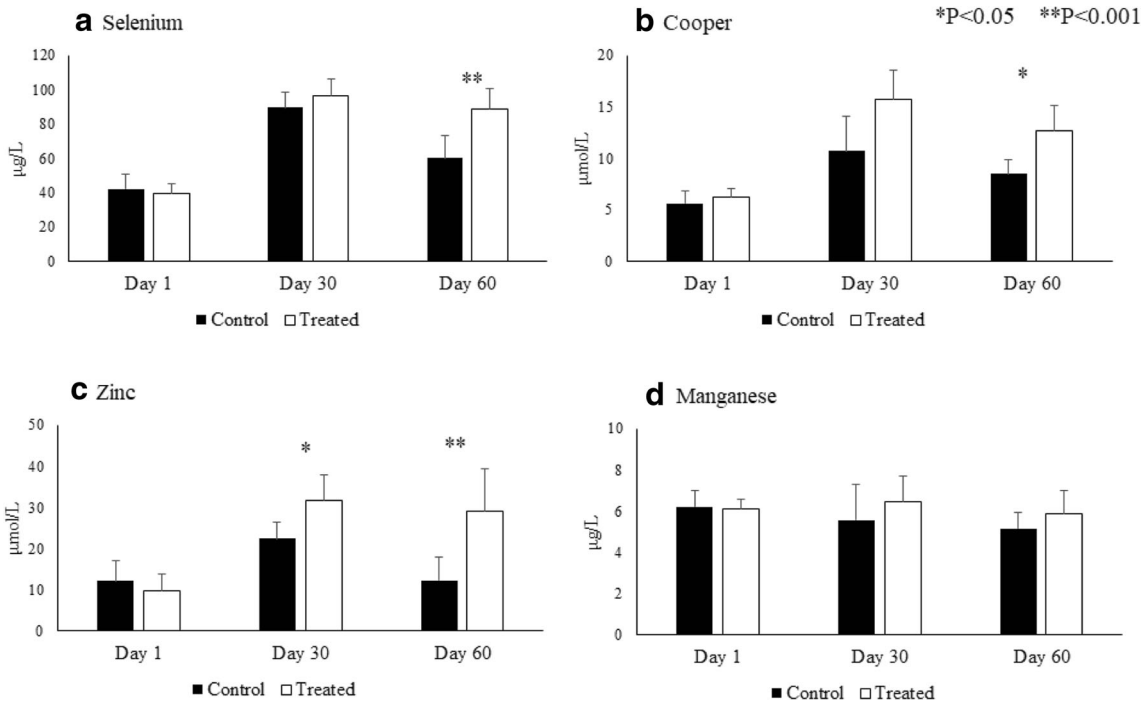

Fig. 1 Serum levels of minerals **a** selenium, **b** copper, **c** zinc, and **d** manganese in lambs of the control and treated groups

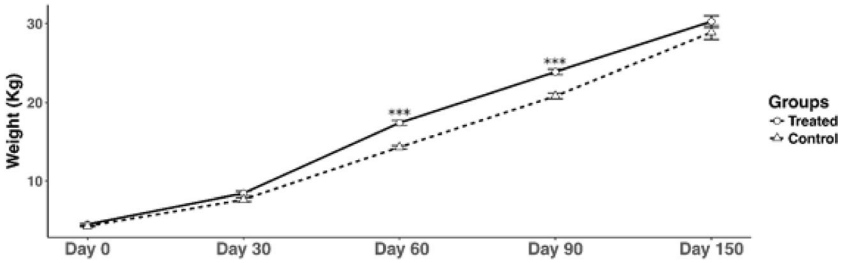

Fig. 2 Distribution by group of weight in lambs treated with minerals. Each day of sampling the mean and standard deviation of each variable are represented. *** represents significant differences between two groups in DOL 60 and 90 ($p < 0.001$)

improving the humoral and innate immune response in sheep [18]. Based on these results, we hypothesized that mortality reduction of lambs would occur due application of organic minerals, mediated by the activation and modulation of the immune system, as observed in this study, with increased pro-inflammatory cytokines (TNF-α and IL-1) and total antioxidants (FRAP). Although not statistically significant, mortality reduction occurred numerically in the lambs that received additional doses of minerals.

Previously published results of this study showed that the animals in the treated group had a higher number of lymphocytes at 45 days of age [9]. These results influenced the choice of proinflammatory cytokines analyzed here. According to the literature, IL-1 and TNF are produced intensely by macrophages; however, they are also expressed by B [11] and T lymphocytes [43]. Therefore, the higher number of lymphocytes may explain the higher levels of IL-1 and TNF in these animals in the current study at day 60 of the experiment.

Table 4 Distribution of the animal measurements for treated and control groups to allometric evaluations

Variable	Groups (mean and standard deviation)		*p* value
	Treated	Control	
Body weight on day of slaughter (kg)	37.9 (4.27)	36 (5.02)	> 0.05
Pelvic limb height (cm)	59.6 (2.50)	58. (3.10)	> 0.05
Weight in slaughterhouse (kg)	14 (1.62)	13.8 (2.03)	> 0.05
Perimeter (cm)	74.5 (2.78)	73.0 (11.74)	> 0.05
Thoracic limb height (cm)	65.0 (2.45)	61.5 (4.09)	> 0.05
Body length - in vivo (cm)	56.5 (4.59)	56.6 (3.33)	> 0.05
Length of lamb carcass (cm)	50.3 (2.14)	53.1 (2.86)	> 0.05
"Green viscera" (digestive system) (kg)	10.6 (1.20)	10.9 (1.26)	> 0.05
Heart (kg)	0.1 (0.02)	0.1 (0.02)	> 0.05
Spleen (kg)	0.05 (0.01)	0.06 (0.02)	> 0.05
Internal fat (kg)	0.5 (0.14)	0.7 (0.25)	> 0.05
pH	6.6 (0.32)	6.5 (0.13)	> 0.05
Testicle weight (kg)	0.1 (0.06)	0.19 (0.08)	> 0.05
Perimeter of the testicle (cm)	20.5 (3.70)	21.3 (3.67)	> 0.05
Distal extremity of the limbs (Legs) (cm)	0.7 (0.07)	0.77 (0.12)	> 0.05
Penis (kg)	0.07 (0.02)	0.06 (0.02)	> 0.05
Skin of lamb (skin and wool) (kg)	2.6 (0.29)	2.7 (0.61)	> 0.05
Lung (kg)	0.6 (0.09)	0.60 (0.12)	> 0.05
Kidneys (kg)	0.08 (0.01)	0.09 (0.01)	> 0.05
Head (kg)	1.7 (0.18)	1.6 (0.15)	> 0.05
Urinary bladder (kg)	0.02 (0.02)	0.03 (0.02)	> 0.05
Liver (kg)	0.6 (0.11)	0.7 (0.10)	> 0.05
Depth of thorax (cm)	23.7 (0.71)	23.6 (0.75)	> 0.05
Length of pelvic limb (cm)	35.3 (1.19)	34.6 (1.46)	> 0.05
Length of thoracic limb (cm)	12.8 (1.79)	12.3 (0.82)	> 0.05
Pelvic limb width (cm)	8.9 (0.60)	9.8 (1.14)	> 0.05

There were no statistical differences in these analyses between groups ($p > 0.05$). As central tendency measurement, we show the mean and dispersion by standard deviation

Table 5 Distribution of the animal measurements for treated and control groups for meat quality analyses of lambs

Variables	Groups (mean and standard deviation)		*p* value
	Treated	Control	
Color: a	17.9 (1.26)	17.4 (0.95)	> 0.05
Color: b	7.8 (1.37)	6.4 (0.81)	> 0.05
Color: L	40.4 (1.98)	43.2 (2.32)	< 0.05
Fat thickness (mm)	1.9 (0.58)	1.3 (0.21)	< 0.01
Ribeye - depth (cm^2)	58.0 (4.21)	55.7 (4.63)	> 0.05
Ribeye - width (cm^2)	28.1 (2.08)	28.2 (1.75)	> 0.05
pH	5.4 (0.04)	5.6 (0.03)	< 0.01
Loss of water by cooking (LC) (%)	0.06 (0.01)	0.09 (0.02)	< 0.01
Water retention capacity (WRC) (g water/g dry matter)	4.0 (0.22)	3.9 (0.11)	> 0.05
Shear strength (SS) (kgf/cm^2)	4.4 (0.34)	5.2 (0.46)	< 0.01
Carcass weight (kg)	14.7 (1.49)	13.8 (1.62)	> 0.05

Significant difference between groups ($p \leq 0.05$). As central tendency measurement, we show the mean and dispersion by standard deviation

Mineral supplementation in the lambs did not alter the allometric analysis; however, it promoted significant differences in some chemical and physical characteristics of the meat. The fat content was greater, and pH was lower in mineral-treated animals. According to Siqueira et al. [41, 42], subcutaneous fat directly contributes to meat softness by acting as an insulator, preventing sudden cooling of the carcass, thereby shortening sarcomeres and making the meat harder. The high fat content in mineral-treated animals may be responsible for the tenderness of their meat. Garcia et al. [16] reported that the final pH in sheep meat varied from 5.5 to 5.8. However, higher values (≥ 6.0) could be found in cases of depletion of muscle glycogen deposits. This may negatively influence the quality of the final product. Treated animals had lower pH than that reported in the literature (5.4). However, since the 1990s, investigators have argued that meat quality at moderately low final pH (5.4) characterized normal, usually soft meat [7, 12].

The intensity of meat color was determined by the total concentration and structure of myoglobin, which was itself affected by ante-mortem factors such as species, gender, and age of the animal, and by post-mortem factors such as anatomical region, temperature, and pH [36]. L* indicated the luminosity of the meat [36]. Luminosity values were lower in the treated group than in the control group, suggesting that the meat became paler in control animals, associated with improvement of antioxidant/oxidant status [23]. However, there were no changes in color values a* and b*. Xu et al. [50] and Zakaria et al. [53] reported that animal diets containing Zn did not change factors associated with L* or a* and b* color values. These differences may be related to the dosing and the timing of supplementation, as well as to the difference of species studied (chickens as opposed to lambs). The low pH in the treatment group associated with lower L* is desirable, as is the red color, an important factor for retail customers [39]. Xu et al. [50] showed that pigs supplemented with zinc showed improvement in pH-associated meat quality, similar to the effect observed in the present study. However, Zakaria et al. [53] reported that zinc did not alter the pH of chicken breast 24 h post-mortem. Taken together, these results suggest that supplementation with zinc and other microminerals may improve meat quality in lambs. Cooking weight loss and shear strength were lower in the treatment group. According to the softness classification, described by Boleman et al. [6] values from 2.3 to 3.6 correspond to very soft meat, 4.1 to 5.4 moderately soft, and 5.9 to 7.2 to slightly soft. By this measure, lamb meat in the treatment group can be classified as moderately soft, with mean values between 2.79 and 3.53. This suggests that greater forces were required to disrupt meat samples from the control group. Zakaria et al. [53] demonstrated that a diet containing zinc produced more succulent breast meat. The mechanisms involved in this process remain unknown, but we speculate that they may involve the diminution of oxidative reactions in post-mortem meat; this is because the nutraceutical effects of the minerals used in this study have potent antioxidant effects, thus protecting from free radicals in cells during the transition period from muscle to meat, as well as during meat storage.

The treated group had lower ROS and TBARS levels than did the control group. According to Schneider and Oliveira [35], excessive production of ROS can lead to oxidative stress with subsequent harmful effects on the animal. Increases in ROS and TBARS levels may lead to oxidation of proteins, lipids, and nucleic acids, with consequent loss of function and tissue damage [20]. Excessive levels of ROS and other free radicals cause lipid peroxidation, as observed in the present study with the increase in MDA levels. MDA accumulation indicates oxidative stress and tissue damage, possibly contributing to changes in weight [22]. In addition, high levels of

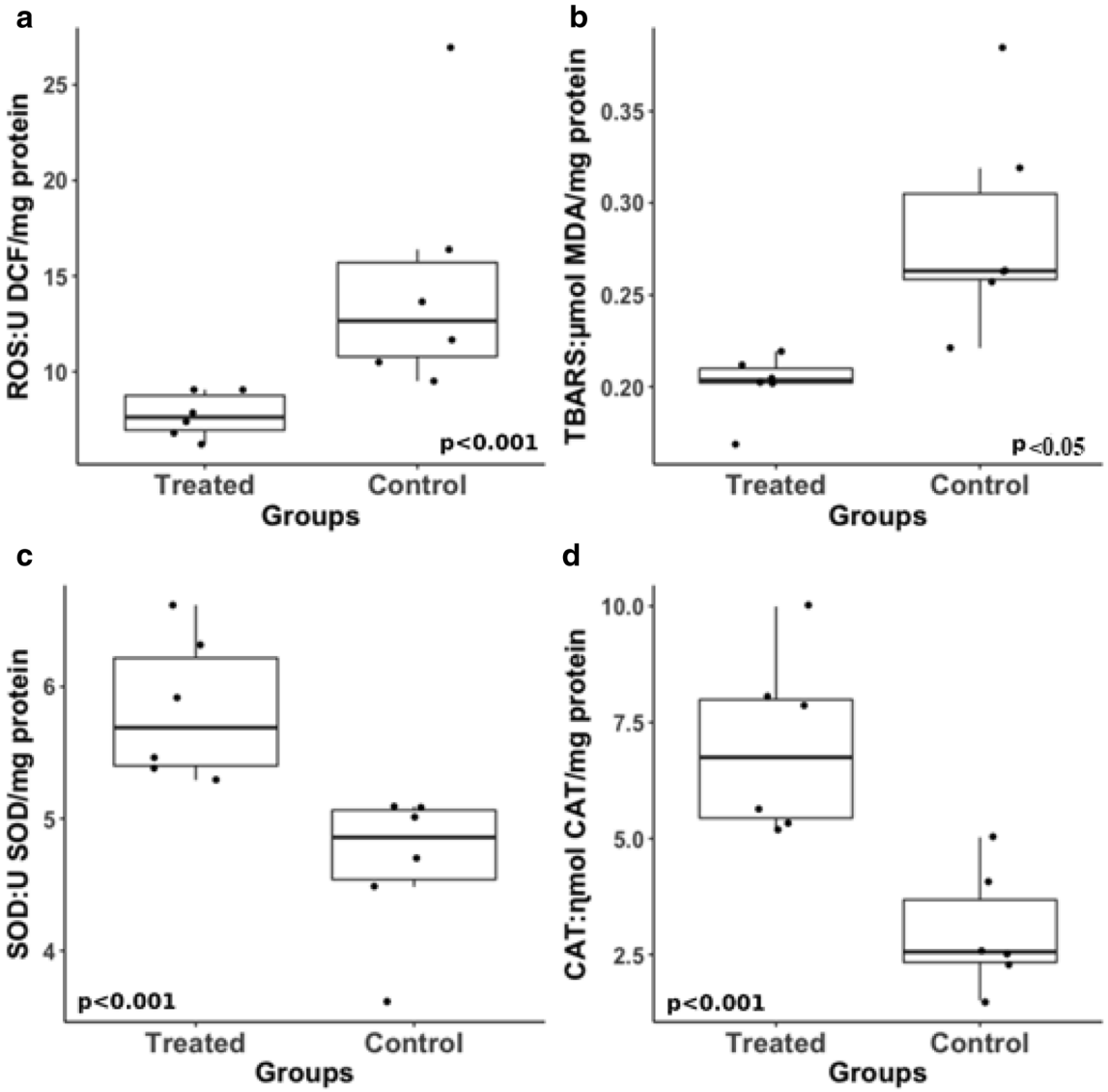

Fig. 3 Distribution of biochemical variables for each group in meat. **a** A significant difference between groups ($p < 0.001$) was observed, i.e., higher reactive oxygen species [ROS] in the control group, **b** and followed the same tendency with the control group having more thiobarbituric acid reactive substances [TBARS] ($P < 0.05$); **c**, **d** the treated group show higher values ($p < 0.001$) for both enzymes (catalase [CAT] and superoxide dismutase [SOD]). The dots represent the individual observations; regular box-and-whisker bars are depicting groups of numerical data through their quartiles. Box plots may also have lines extending vertically from the boxes (whiskers) indicating variability outside the upper and lower quartiles; the center line is always the median value

ROS and increased lipid peroxidation may impair the activity of antioxidant enzymes such as CAT and SOD, as observed by Baldissera et al. [3]. Damage to CAT activity may lead to increased levels of hydrogen peroxide, a molecule with pro-oxidative and pro-inflammatory effects [2]. SOD inhibition may increase levels of hydroxyl radicals, in turn leading to impaired performance and risk of mortality [4]. In the present study, supplementation with organic minerals produced lower values of ROS and TBARS, suggesting reduced radical formation and lipid peroxidation, similar to results reported by Soldá et al. [44] in mineral-supplemented cows. For example, a study conducted by Yeo and Kang [52] demonstrated that sodium selenite reduced ROS content through elevated glutathione peroxidase (GPx) activity and stimulation of selenoprotein P via redox regulation, decreasing free-radical-mediated lipid peroxidation. It is important to highlight that sodium selenite was not immediately used for synthesis of selenoprotein, i.e., it was converted to hydrogen selenite that acted as a central pool of selenium for specific integration of selenium in selenoproteins, contributing to protective effects against oxidation [8]. Using organic compounds, studies have suggested that zinc edetate inhibited lipid peroxidation, as observed in the present study, through improvement of GPx activity and consequent reduction of ROS content [14, 17]. In summary, organic and/or inorganic compounds may prevent lipid peroxidation via enhancement of the antioxidant system,

as evaluated in the present study by stimulation of SOD and CAT activities. The finding of higher SOD and CAT activity in treated lambs may be accounted for by the presence of copper, which is a component of the SOD molecule, and zinc, which is a cofactor of the CAT enzyme. Recall that these enzymes participate in regulation of free radicals and non-radical species that are associated with oxidative reactions [1]. In addition, it is important to emphasize that improvement of antioxidant/oxidant status was directly associated to improvement of meat quality, as observed by Khan et al. [23]. Xu et al. [50] demonstrated that Zn supplementation in swine improved SOD anti-oxidant activity.

Conclusions

In lambs, among the nutraceutical effects of zinc, manganese, copper, and selenium, we highlighted the stimulation of important pro-inflammatory cytokines to the benefit of animal defense and modulated oxidative reactions via activation of the antioxidant system. Consequently, there was a greater weight gain in the initial phase of life, as well as improved physical characteristics of the meat. These changes are desirable as they contribute to animal health and offer the final consumer a better product. Although not significant, there were smaller lambs in the treated group, an economically positive effect on the farmer. Therefore, in general, we conclude that the subcutaneous application of minerals provides additional benefit beyond fulfilling the basic requirements for lamb production.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethics Committee This study was approved by the Ethics Committee of Use of Animals (CEUA) of Universidade do Estado de Santa Catarina (UDESC), protocol number 7398301116, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

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