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Nutraceutical effect of vitamins and minerals on performance and immune and antioxidant systems in dairy calves during the nutritional transition period in summer

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ABSTRACT

We aimed to determine whether the use of injectable vitamins and minerals improves growth performance and immune and antioxidant responses in dairy calves during pre- and post-weaning period in summer. Twenty dairy calves (45 days of age) were randomized to two groups (10 each): control group (CON) and treated group [TREAT; injection providing 0.20, 0.80, 0.20, 0.10, 35 and 1 mg/kg of copper, zinc, manganese selenium, and vitamins A and E, during two periods (15 days pre- and 15 days post-weaning)]. The animals were weighed and blood samples were collected on days 1, 15, 30 and 45 of the study. Levels of serum copper, selenium, zinc, and manganese were measured on day 1; and the results showed that calves were not deficient in these minerals. The TREAT group had greater BW gain during the final third of the experiment. There was an increase in total leukocyte numbers as a result of elevation in neutrophil counts (day 45) and monocytes (days 30 and 45) in the TREAT group. This group also had lower reactive oxygen species (ROS) content (days 15, 30 and 45) and lipid peroxidation (LPO; days 15 and 45). Furthermore, the TREAT group had greater antioxidant capacity against peroxyl radicals (ACAP; days 15 and 30), activities of the enzymes glutathione peroxidase (GPx; days 15, 30 and 45) and superoxide dismutase (SOD; day 15), concentrations of total serum proteins (day 30), serum globulin (days 15 and 30), ceruloplasmin (day 15), tumor necrosis factor-alpha (TNF-α), interleukin-1, (IL-1; days 30 and 45) and interferon gamma (IFNγ; day 45), compared to CON group. High respiratory rates during hot times of the day in all study calves was suggestive of heat stress. Taken together, the data suggest that mineral and vitamins injections increased the growth performance and boosted the antioxidant and immunological systems of dairy calves during the diet transition period in summer.

1. Introduction

The initial phase of cattle life is critical because their immune system is immature shortly after birth; nevertheless, the animal requires rapid development of the immune system in order to respond the infections and to optimize growth (Besser and Gay, 1994; Botteon et al., 2008). Therefore, these animals are dependent on the immunity acquired from colostrum to generate immune responses, despite the fact that they are capable of mounting some immune responses in utero.

Another challenge for calves is weaning. During this critical period, there are drastic changes related primarily to the transition from a liquid to a solid diet. Reduction in the levels of supplied dry matter and greater ruminal activity can affect animal development (Campos and Lizieire, 2000). According to the literature, this phase generates stress, a factor often associated with reduced resistance to diseases (Grandin and Gallo, 2007). In addition, possible changes of facilities and management (e.g., dehorning) commonly practiced during this period, can render the animal more vulnerable (Campos and Lizieire, 2000).

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Table 1
Calf diet during the experimental period, as chemical composition of hay and commercial concentrate used in animal feeding.

Ingredients	Day 1–15 of experiment (preweaning)	Day 15–30 of experiment (post-weaning)	Day 30–45 of experiment (post-weaning)
Milk cow (liter/animal/day)	4.0	0	0
Commercial concentrate* (grams/animal/day	400	600	800
Hay[#] (grams/animal/day)	*Ad libitum*	*Ad libitum*	*Ad libitum*

Chemical composition,[a], [#]	Hay[#]	Concentrate[a]
Dry matter (%) in dry matter	95.5	89.0
Mineral (%) in dry matter	7.5	7.3
Crude protein (%) in dry matter	12	16
Ethereal extract (%) in dry matter	1.4	2.8
Neutral detergent fiber (%) in dry matter	61	49.4
Acid detergent fiber (%) in dry matter	36.2	10.8
Mineral composition,[a], [#]		
Copper (mg/kg)	nd	37.6
Zinc (mg/kg)	nd	136.2
Selenium (mg/kg)	nd	0.85
Manganese (mg/kg)	nd	41.8

Note: not-detected (nd).
[a] Commercial concentrated guaranteed levels: calcium (Min/Max) 10–15 g/kg; phosphorus (Min) 5000 mg/kg; zinc (Min) 140 mg/kg; cobalt (Min) 2 mg/kg; copper (Min) 40 mg/kg; sulfur (Min) 2000 mg/kg; iodine (Min) 2 mg/kg; magnesium (Min) 1500 mg/kg; manganese (Min) 40 mg/kg; selenium (Min) 1 mg/kg; sodium (Min) 2000 mg/kg; Fluorine (Min) 100 mg/kg; virginiamycin (Min) 50 mg/kg; Vitamin A (Min) 10,000 IU/kg; Vitamin D3 (Min) 2000 IU/kg; and Vitamin E (Min) 50 IU/kg.

The metabolic stresses associated with weaning can be severe, compromising the antioxidant system (Sundrum, 2015) and generating oxidative stress. This biochemical event is characterized by an imbalance between the production of reactive oxygen species (ROS) and the exhaustion or activation of the animal's antioxidant system (Persson et al., 2014; Vedovatto, 2018) possibly favoring deleterious effects on cells, tissues and consequently the onset of diseases (Burke et al., 2009). Intake of a diet rich in antioxidants had beneficial effects on animal health (López-Alarcón and Denicola, 2013).

According to researchers, supplementation with minerals plays an important role in cattle by stimulating the immune system (Rink, 2000), because many minerals are enzymatic cofactors (Filappi et al., 2005). Recently, studies showed that injectable minerals would be a suitable method to improve mineral utilization by animals, and this may be a promising alternative to improve animal performance (Collet et al., 2017). In addition, vitamins A and E were identified as antioxidants (Sies, 1991). These are important for promoting good health, and are key factors for calf-rearing and future performance (Weiss, 2002).

Calves exhibit poor growth performance, low immunity, increased respiratory rates, and greater susceptibility to diseases attributable to reduced feed intake during the summer months (West, 2003; Tao and Dahl, 2013; Kargar et al., 2018). The consequences of thermal stress are economic losses associated with reduced weight gain, and increased mortality and morbidity (Roland et al., 2016). In a recent study, researchers observed that chromium supplementation in liquid and solid feeds improved growth performance in summer-exposed calves as a result of reduced respiration rate and increased feed intake (Kargar et al., 2018). Studies from our research group showed that supplementation with minerals and/or vitamins in calves (Glombowsky et al., 2018; Tomasi et al., 2018; Volpato et al., 2018) and lambs (Cazarotto et al., 2018) are beneficial to the health and growth performance of the animals during the nursing phase. We also observed similar results when minerals (zinc and selenium) were given during the transition period from liquid to solid diet (weaning) in calves (Volpato et al., 2018). Our hypothesis was that the combination of injectable minerals and vitamins would increase growth performance, and would augment immunological and antioxidant responses in dairy calves even in hot seasons. Therefore, the objective of this study was to determine whether the subcutaneous application of minerals and vitamins has nutraceutical effects on antioxidants and consequent growth performance of dairy calves during the diet transition period (weaning) in summer.

2. Materials and methods

2.1. Products

The commercial product used contained vitamins A and E (Adaptador® Vit; Biogénesis Bagó, Buenos Aires, Argentina), and the minerals zinc, copper, selenium, and manganese (Adaptador® Min; Biogénesis Bagó). The dose used was 1 mL/50 kg of body weight, according to the manufacturer's recommendations. The dose of Adaptador® Min provided 0.20 mg/kg of copper, 0.80 mg/kg of zinc, 0.20 mg/kg of manganese and 0.10 mg/kg of selenium. The dose of Adaptador® Vit provided 35 mg/kg of vitamin A and 1 mg/kg of vitamin E. On experimental days 1 (15 days pre-weaning = 45 days of age) and 30 (15 days post-weaning = 75 days of age), was applied the supplement subcutaneously, with the two products being applied alone and in different places. In the control group, the same management and application procedure was performed; however, the same volumes of the treated group were applied with only saline solution (0.9% NaCl) and mineral oil for purposes of the placebo effect.

2.2. Animals and experimental design

The study was carried out in a commercial farm in the western region of Santa Catarina state, Brazil, using 20 Holstein heifer calves (45 ± 2 days of age) divided into two groups (10 animals each): control group (CON) and treated group (TREAT). The groups were randomized in 20 pens, in order to randomize the effect of the environment inside the shed during lactating phase. The animals were housed in individual pens at the beginning of the experiment because they were nursing (between 45 and 60 days of age); however, at weaning (60 days of age), they were allocated to four collective pens with five calves each, separated by groups. The four pens were located next to each other, in the middle of the shed, and the groups were distributed in the following order: CON-TREAT–CON–TREAT. Before the study, the animals received colostrum (4 L/animal until 6 h after birth), transition milk (4 L/animal/day until 4 days after birth) and milk (4 L/animal/day until 60 days after birth). Up to 45 days after birth, the calves received water, hay (*Cynodon* spp.) and concentrate ad libitum (Table 1). Forty-five days after birth, the study started (day 1),

and calves received milk [only from day 1 to day 15 (weaning); 4 L/animal/day], concentrate (day 1–15: 400 g/animal/day; day 15–30 of 600 g/animal/day; day 30–45: 800 g/animal/day), water and hay (*Cynodon* spp.) ad libitum (Table 1). The liquid and solid feeds were provided in two meals (08:00 h and 16:00 h). Dehorning and immunization were not done until the end of the study to avoid affecting the serum variables.

The experiment was carried out in the south of Brazil, in a shed without air conditioning, with lateral openings. The experiment took place during the summer months (January and February) and the temperature was measured inside the building during the day using an inside digital thermometer. Data of ambient temperature in the municipality of the experiment were obtained from a meteorological station located 3.8 km away from the experimental shed; the minimum and maximum temperatures recorded were 29 °C and 37 °C, respectively. The hottest temperature occurred around 14:30 h throughout the experimental period, and temperatures registered inside the shed at this time of day temperatures were as high as 39.8 °C.

During the hottest times of the day, all animals increased their breathing rates and panting in order to dissipate the heat. At the end of the experiment (day 42 of the experiment), between 08:00 h and 09:00 h and between 14:00 h and 15:00 h, the respiratory movements per minute were recorded for all heifers.

2.3. Sample collection

The animals were weighted and feces and blood samples were collected on days 1 (15 days pre-weaning), 15 (weaning day: calves at 60 days of age), 30 (15 days post-weaning) and 45 (30 days after weaning). Blood samples were collected from jugular veins into two vacuum tubes, with and without anticoagulant (EDTA 10%). The samples were maintained in an isothermal box at 10 °C until the time of the laboratory analysis. Samples collected in tubes without anticoagulant (clotted blood) were centrifuged at 7000 rpm for 10 min to separate serum. The animals were weighed using a tape measure, a method of weighing the animals indirectly using thoracic perimeter (Reis et al., 2008). We also performed fecal score analyses (Larson et al., 1977) on feces collected from the rectal bulb. Fecal samples were kept in isothermal boxes at 10 °C for further parasitological analysis. Concentrate and hay samples were collected from the total diet provided (concentrate and hay) for all calves in the three periods (Table 1). We measured dry matter, ash, ether extract and crude protein, following a method described by Silva and Queiroz (2006). In addition, neutral detergent fiber and acid detergent fiber were analyzed following the methodology described by Van Soest (1994). Minerals (copper, zinc, selenium and manganese) were determined in hay and concentrate by a near-infrared spectroscopy method in a commercial laboratory (Shankar, 2015, Table 1).

2.4. Serum concentrations of minerals

Serum concentrations of selenium, copper, manganese and zinc were determined on day 1 of the experiment. In the analyses, specific tests were used, as described below. Selenium levels were measured using the hydride generation atomic absorption spectrometry technique (HG-AAS) (PerkinElmer Model 3030), using chemicals of analytical grade from Merck (Darmstadt, Germany; Cazarotto et al., 2018; Flores et al., 2001). Thus, 1 mL of HNO3 and 250 µL H_2O_2 were added to 500 µL of serum into 15 mL vials. The vials were warmed in a commercially-available microwave for 5 min. Milli-Q water was added until 10 mL final volume and the solution was analyzed. Copper, manganese, and zinc concentrations in digested samples were determined using ICP-OES. Operational conditions used for these minerals determinations were those recommended by the instrument manufacturer. Aqueous calibration standards were prepared by sequential dilution of a stock solution of copper, manganese, and zinc (10 µmol/L, Spex CertiPrep, Metuchen, NJ, USA). Standards were prepared daily at concentrations of 5, 10, 15, 20, 40, 60, and 80 µmol/L Cu, Mn, and Zn. Sample digests were diluted in order to present suitable concentrations for measurements. Recovery tests were also performed for sample digests. After each ten measurements, two standard Cu, Mn, and Zn solutions were analyzed to check the slope of calibration curve. In case of a slope difference of higher than 10%, the calibration curve was prepared again using all standards. All samples were assayed in triplicate.

2.5. Hematological analysis

Blood stored in EDTA tubes was used to perform complete blood counts. The erythrocyte count, total leukocytes and hemoglobin concentration were measured using semi-automatic equipment (CELM model CC530). For the determination of hematocrit values, we used capillary tubes, centrifuged for 1 min at 1400 rpm. At sampling, blood smears were performed and stained with commercial dye (*Panótipo Rápido*) to perform leukocyte differential counts using a light microscope at 1000× magnification (Feldman, 2000), with 100 cells randomly identified per slide. Knowing the number of total leukocytes (µL), differential leukocytes (%) were calculated using absolute numbers of neutrophils, lymphocytes, monocytes and eosinophils. Counts were expressed per microliter (µL) of blood.

2.6. Analysis of oxidant/antioxidant status

Serum ROS levels were determined using the 2′,7′-dichlorofluorescein (DCF) oxidation method described by LeBel et al. (1992). Fluorescence was measured using excitation and emission wavelengths of 485 and 538 nm, respectively. A calibration curve was established with standards of DCF (0.1 nM–1 µM), and results were expressed as U DCF/mg of protein.

The methodology of lipid peroxidation (LPO) was based on Hermes-Lima et al. (1995) with some modifications by the authors, called FOX based on the oxidation of Fe(II) under acidic conditions. The Fox method measures lipid peroxides, one of the principal products of lipid peroxidation. For LPO measurements, $FeSO_4$ (1 mM), H_2SO_4 (0.25 M), xylenol orange (1 mM, Sigma) and MilliQ water were sequentially added. Samples or methanol (blanks) were added and incubated for 30 min. Thereafter, absorbance (550 nm) was determined and cumene hydroperoxide (CHP; Sigma) was employed as a standard. LPO was expressed as µmol CHP/mg protein.

Total antioxidant capacity against peroxyl radicals (ACAP) was determined according to the method described by Amado et al. (2009). This method consists of determining the antioxidant capacity of tissues using a fluorescent substrate (2′,7′ dichlorofluorescein diacetate - H2DCF-DA) and the production of peroxyl radicals by thermal decomposition of ABAP (2.2′-azobis 2 methylpropionamidine dihydrochloride). The fluorescence was determined using a microplate reader (Spectramax I3), at 37 °C (excitation: 485 nm; emission: 530 nm) with readings at every 5 min over 30 min. The results were expressed as relative area (the difference between the area with and without ABAP divided by the area without ABAP), and levels were expressed as UF/mg protein.

The activity of superoxide dismutase (SOD) was determined according to the auto-oxidation principle of pyrogallol, inhibited in the presence of SOD. The optical density change was determined kinetically for 2 min at 420 nm, at 10-s intervals according to the methodology described by Beutler (1984). The activity was expressed as U SOD/mg of protein.

The activity of glutathione peroxidase (GPx) was measured indirectly by monitoring the oxidation rate of NADPH at 340 nm using cumene hydroperoxide (CuOOH), according to Wendel (1981). The enzymatic activity was expressed as U GPx/mg protein.

The protein concentrations in serum were determined using the Coomassie blue method following the methodology described by Read and Northcote (1981), using bovine serum albumin as a standard.

Table 2
Serum levels of minerals copper, zinc, selenium and manganese in calves of the control and treated groups on days 1 of the experiment (45-day-old animals = 15 days pre-weaning).

Mineral	Control (n =10)	Treated (n =10)	[a]P-value	[b]values (min. – max.)
Copper (μmol/L)	14.8 ± 3.9	15.1 ± 2.8	0.841	2.91 to 19.65
Zinc (μmol/L)	25.1 ± 5.4	23.8 ± 4.7	0.802	5.46 to 36.70
Manganese (μmol/L)	0.85 ± 0.05	0.87 ± 0.07	0.910	0.40 to 1.00
Selenium (μg/L)	80.4 ± 7.0	76.0 ± 10.3	0.751	51.0 to 85.0

[a] No statistical difference between groups ($P > 0.05$).
[b] Studies have reported minimum and maximum levels for cattle for minerals: copper and zinc (Pavlata et al., 2005), selenium (Villard et al., 2002), and manganese (Jokubauskienė et al., 2010). Note: Pavlata et al. (2005) defined as copper and zinc deficiency in blood serum concentrations of the respective element below 12 μmol/L.

2.7. Proteinogram

For protein fractionation, polyacrylamide gel electrophoresis with sodium dodecyl sulphate (SDS-PAGE) was performed according to a technique described by Fagliari et al. (1998) using mini-gels (10 × 10 cm). The gels were stained with Coomassie blue and photographed to identify and quantify protein fractions using Labimage1D software (Loccus Biotechnology). Standards containing fractions with molecular weights between 10 and 250 kDa (Kaleidoscope - BIORAD) were used as reference for the identification of protein fractions.

2.8. Cytokines

Cytokine quantification (tumor necrosis factor-alpha - TNF-α, interleukin-1, IL-1 and interferon gamma – IFNγ) was assessed by ELISA using commercial Quantikine immunoassay kits according to the manufacturer's instructions. Briefly, 96-well microplates were sensitized with primary antibody at room temperature for 30 min; samples were added and incubated for 30 min at 37 °C. After washing, secondary antibodies conjugated with peroxidase were added to each well and incubated. The concentration of the cytokines was determined by the intensity of the color measured spectrophotometrically using a microplate reader.

2.9. Fecal score and parasitological examination

The occurrence of diarrhea was observed daily following the methodology described by Larson et al. (1977), which is based on fecal score and fluidity: (1) normal; (2) soft; (3) runny and (4) watery. For the determination of parasitological infection, the technique described by Monteiro (2010) was used with subsequent reading using light microscopy. Fecal consistency and parasitic infection were evaluated only to monitor the health of calves because our goal was to use only healthy calves to evaluate the effect of minerals and vitamins.

2.10. Statistical analysis

Data were analyzed using descriptive statistics for contingency of information and for further assumptions that were presented as descriptive (mean and standard deviation) for blood cell parameters: hematocrit, erythrocyte count, hemoglobin, leukocytes, lymphocytes, monocytes and eosinophils. The second set of data were for GPx and SOD activities, followed by biochemical components: ROS, LPO, and ACAP. The third group of parameters measured were proteinogram (total protein, globulin, albumin, ceruloplasmin, and immunoglobulins) and cytokines (TNF-α, IL-1, and IFNγ). Finally, we took measurements associated with animal weight: body weight and weight gain. The Chi-square test was used to evaluate fecal score. Each calf was considered an experimental unit. For each group (CON and TREAT) and day of observation (days 1, 15, 30, and 45), all parameters were tested for normality using the Shapiro-Wilk test. Skewness, kurtosis and homogeneity were evaluated using the Levene test, or log transformation when needed. Two-way ANOVA was performed using repeated measurements to test for differences in the parameters over time (considering blocks of groups CON and TREAT) and compared between groups (controlling data dependency due to dependence in time). Significant difference was set at $P < 0.05$. Statistical manipulations were performed using R-language, v 3.1 (R Development Core Team, 2012).

3. Results

3.1. Serum mineral concentrations

On day 1 of the experiment, there were no significant differences between groups; furthermore, values of copper, zinc, manganese and selenium were within the reference values for calves with no deficiencies in these minerals (Villard et al., 2002; Pavlata et al., 2005; Jokubauskienė et al., 2010, Table 2).

3.2. Growth performance and clinical signs

There were no significant differences in fecal parameters (color, fluidity, odor or consistency) between groups ($P = 0.854$; data not shown). However, it should be noted that the vast majority (more than 95%) of the animals had feces with scores within the normal range. Feces of runny and (4) watery consistency were not observed, and feces with soft consistency (but with healthy aspect) were observed rarely in both groups at approximately 48 h after the withdrawal of milk as feed, lasting 2–3 days. On the day of blood collection, all calves had normal feces. All animals were negative for parasites during the experimental period.

The TREAT group had greater BW gain during the final third of the experiment (days 15–45; and days 30–45 of the experiment) compared to the CON group (Table 3). Over time, the BW of animals from both groups increased (Table 3).

The respiratory rates increased during the hottest times of the day, i.e. in the morning (0800 h–0900 h). We observed that 26 and 40 breaths/minute (mean CONT was 28.5, and TREAT was 29.2) and in the afternoon (1400 h–1500 h) varied between 39 and 52 breaths/minutes (mean CONT was 46.6, and TREAT was 43.8). There was no difference between groups ($P > 0.05$) at both moments.

3.3. Hemogram

The results of hematological analyses are shown in Table 4. No differences were observed between groups and over time in terms of erythrocyte numbers, hematocrit and hemoglobin concentrations. A greater number of total leukocytes ($P = 0.050$) was observed in the animals of the TREAT group on day 45, as a consequence of the increased neutrophil counts ($P = 0.023$). The number of monocytes was greater in the TREAT group on days 30 and 45 ($P = 0.045$ and 0.037, respectively). The number of lymphocytes and eosinophils did not differ between groups (Table 4).

Table 3
Body weight and weight gain of dairy calves that received mineral and vitamin applications (treated group) by the subcutaneous route on days 1 (15 days pre-weaning) and 30 days (15 days post-weaning) of the experiment.

Variable	Day	Control (n = 10)	Treated (n = 10)	P-value
Body weight (kg)	1	58.6 (8.9)[c]	61.3 (9.4)[c]	**0.847**
	15	72.3 (15.5)[bc]	72 (13.2)[bc]	**0.923**
	30	90.4 (17.4)[ab]	91.2 (15)[b]	**0.904**
	45	108.7 (14)[a]	120.7 (16.7)[a]	**0.187**
P-value		**0.001**	**0.001**	
Weight gain (kg)	1–15	13.7 (4.6)	10.7 (4.2)	**0.845**
	1–30	31.8 (8.7)	29.9 (8.1)	**0.695**
	1–45	50.1 (8.2)	59.4 (7.3)	**0.085**
	15–30	18.1 (5.3)	19.2 (4.1)	**0.745**
	15–45	36.4 (8.9)	48.7 (7.0)	**0.050***
	30–45	18.5 (4.6)	29.5 (7.6)	**0.024***

Note: results presented in mean and standard deviation. P ≤ 0.05 (*) on the same line shows the differences between groups. P ≤ 0.05 in the same column shows the differences over time in each group, the differences being represented by different letters.

Table 4
Hemogram of dairy calves that received mineral and vitamin applications (treated group) by the subcutaneous route on days 1 (15 days pre-weaning) and 30 days (15 days post-weaning) of the experiment.

Variable	Day	Control (n = 10)	Treated (n = 10)	P-value
Erythrocytes (x10^6 µL)	1	4.2 (1.08)	4.6 (0.78)	**0.598**
	15	4.25 (0.92)	4.12 (0.56)	**0.746**
	30	5.24 (0.75)	4.52 (0.64)	**0.114**
	45	4.59 (0.85)	5.34 (0.80)	**0.775**
P-value		**0.285**	**0.062**	
Hemoglobin (g/dL)	1	7.94 (1.27)	9.08 (0.57)	**0.201**
	15	8.68 (0.72)	9.27 (1.02)	**0.463**
	30	9.34 (0.83)	8.60 (0.97)	**0.495**
	45	9.63 (1.17)	9.47 (0.57)	**0.802**
P-value		**0.294**	**0.376**	
Hematocrit (%)	1	32.1 (5.1)	36.4 (3.8)	**0.408**
	15	32.7 (4.0)	32.9 (5.6)	**0.864**
	30	37.9 (4.5)	34.3 (4.5)	**0.653**
	45	36.4 (4.0)	35.8 (3.5)	**0.906**
P-value		**0.571**	**0.789**	
Leukocytes (x10^3 µL)	1	8.35 (1.9)	9.68 (2.2)[ab]	**0.569**
	15	9.63 (2.0)	8.41 (1.9)[b]	**0.528**
	30	10.0 (3.1)	9.12 (3.8)[ab]	**0.635**
	45	9.18 (1.2)	11.6 (1.8)[a]	**0.050***
P-value		**0.208**	**0.044***	
Neutrophils (x10^3 µL)	1	3.95 (1.22)	4.35 (1.4) [a]	**0.756**
	15	4.81 (1.42)	3.89 (1.50) [b]	**0.305**
	30	4.42 (1.83)	5.29 (2.51) [ab]	**0.652**
	45	3.97 (1.30)	6.13 (2.17)[a]	**0.023***
P-value		**0.598**	**0.001***	
Lymphocytes (x10^3 µL)	1	4.03 (0.98)	5.02 (1.73)	**0.422**
	15	4.48 (1.46)	4.31 (1.43)	**0.654**
	30	5.41 (1.60)	3.43 (1.30)	**0.235**
	45	4.79 (1.40)	4.62 (1.12)	**0.854**
P-value		**0.198**	**0.365**	
Monocytes (x10^3 µL)	1	0.25 (0.20)	0.21 (0.24)	**0.758**
	15	0.14 (0.21)	0.18 (0.19)	**0.651**
	30	0.08 (0.13)	0.35 (0.12)	**0.045***
	45	0.17 (0.11)	0.41 (0.14)	**0.037***
P-value		**0.625**	**0.064**	
Eosinophils (x10^3 µL)	1	0.08 (0.11)	0.08 (0.21)	**0.901**
	15	0.14 (0.14)	0.01 (0.05)	**0.352**
	30	0.16 (0.08)	0.07 (0.08)	**0.436**
	45	0.25 (0.23)	0.15 (0.25)	**0.621**
P-value		**0.456**	**0.652**	

Note: results presented in mean and standard deviation. P ≤ 0.05 on the same line shows the differences between groups. P ≤ 0.05 in the same column shows the differences over time in each group, the differences being represented by different letters.

Over time, the number of leukocytes and neutrophils increased only in the TREAT group (P = 0.044 and 0.001, respectively). The other hemogram variables did not differ over time in either group.

3.4. Oxidant and antioxidant status

Oxidative and antioxidant status results are shown in Fig. 1. The TREAT group showed lower levels of ROS on days 15, 30 and 45 (P = 0.041, 0.001 and 0.001, respectively), as well as lower levels of LPO on days 15 and 45 (P = 0.050 and 0.040, respectively). ACAP levels in the TREAT group had greater values on days 15 and 30 (P = 0.001 and 0.001, respectively). The GPx activity was greater on days 15, 30 and 45 in the TREAT group (P = 0.001, 0.001 and 0.050, respectively), while SOD activity was greater only on day 15 of the experiment (P = 0.036).

Over time, there was a reduction in ROS levels in the TREAT group, i.e. days 1, 15 and 30 to day 45 (Fig. 1). LPO levels were lower in both groups, i.e. day 1–45 (Fig. 1). The application of minerals and vitamins generated an increase of ACAP, SOD and GPx over time (Fig. 1) that did not occur in the CON group.

3.5. Proteinogram

Total protein levels were greater (P = 0.001) in the TREAT group on day 30, as a consequence of the increase in globulin levels on days 15 and 30 (P = 0.020 and 0.035, respectively; Fig. 2). Levels of albumin did not differ between groups and over time (Fig. 2). The proteinogram revealed that globulins were elevated due to increased levels of IgA (days 15 and 30; P = 0.050 and 0.044, respectively), IgG heavy chain (days 15, 30 and 45; (P = 0.031, 0.001 and 0.013, respectively) and ceruloplasmin (day 15; P = 0.001) (Fig. 2).

Over time, only animals in the TREAT group showed increased total protein (day 1–30), globulin (day 1–30), IgG heavy chains (day 1–45), IgA (days 15 and 30 to day 45) and ceruloplasmin (day 15–45) levels (Fig. 2). In CON animals, there were no differences over time.

3.6. Cytokines

Results of cytokine levels are shown in Fig. 3. In the calves in the TREAT group, we observed that there were increases in serum TNF-α and IL-1 levels on days 30 (P = 0.001 and 0.001, respectively) and 45 (P = 0.001 and 0.001, respectively) of the experiment, as well as IFNγ on day 45 (P = 0.027). Over time, only animals in the TREAT group showed increases in TNF-α (day 1 to days 30 and 45), IL-1 (day 1 to days 30 and 45) and IFNγ (days 1 and 15 to day 45) levels (Fig. 3).

4. Discussion

The application of injectable minerals and vitamins ensures a single dose of known treatment without oscillations, as in voluntary intake models (Arthington et al., 2014). The components injected are available directly into the bloodstream without encountering antagonism or other interactions (Abuelo et al., 2014; Arthington et al., 2014). The application of injectable minerals showed beneficial health results for calves in the weaning process (Sheffy and Schultz, 1979; Arthington et al., 2014), as we observed in the present study. Importantly, the calves used in this study were healthy, free of endoparasites, and were not trace mineral deficient blood mineral levels within reference values (Villard et al., 2002; Pavlata et al., 2005; Jokubauskienė et al., 2010); therefore, the results discussed below regarding the immune and antioxidant system were due to the nutraceutical effects of minerals and vitamins.

Minerals are fundamental in processes such as growth (Spears, 2000), and vitamin deficiencies are associated with lower growth rates (Weiss, 2005). In the present study, weight gain did not differ between groups at days 1–45 of the experiment, possibly as a consequence of the

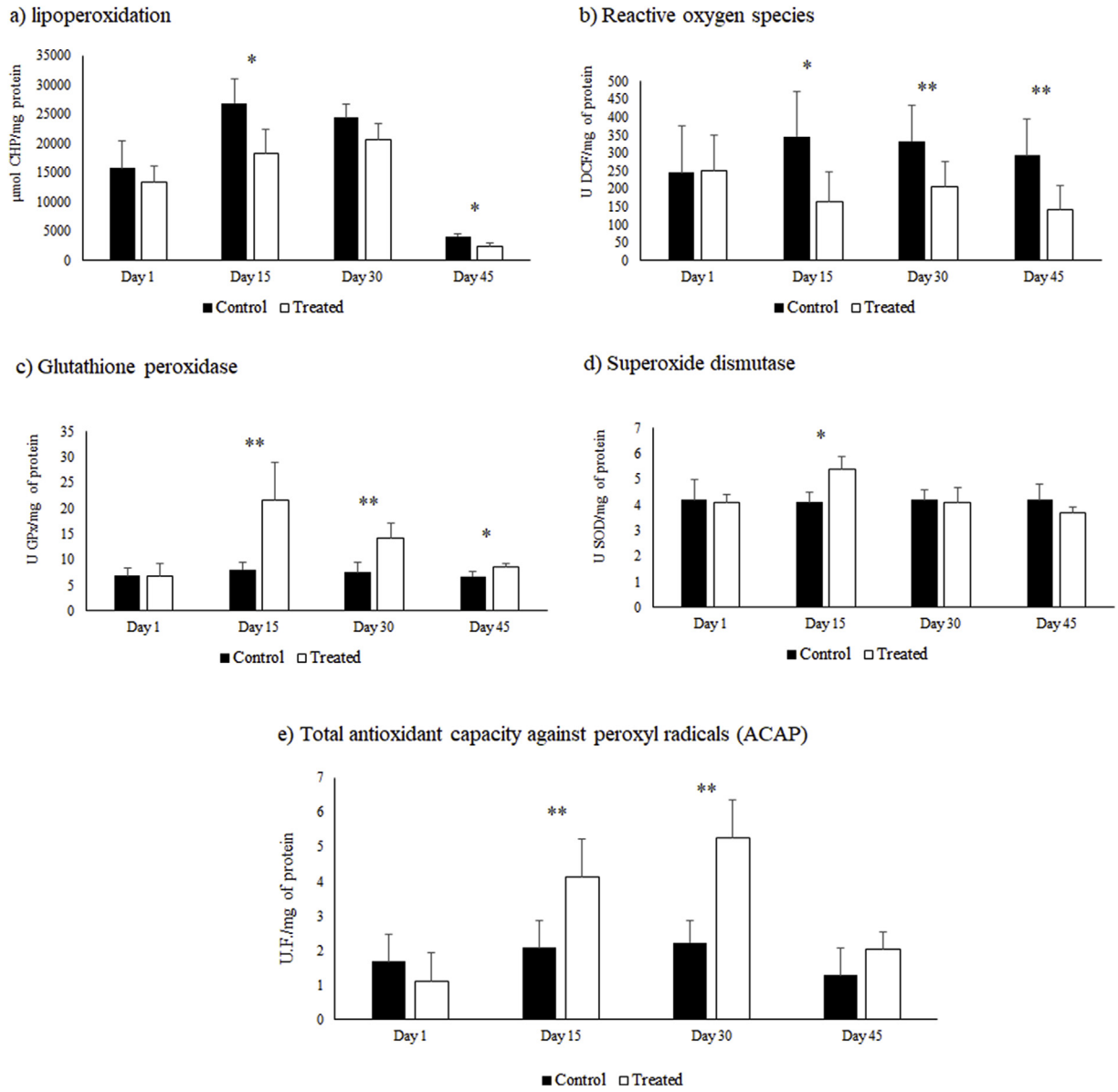

Fig. 1. Lipoperoxidation (a: LPO), reactive oxygen species (b: ROS), glutathione peroxidase (c: GPx), superoxide dismutase (d: SOD), and total antioxidant capacity against peroxyl radicals (e: ACAP) in the serum of dairy calves receiving subcutaneous vitamins and mineral on days 1 (15 days pre-weaning) and 30 (15 days post-weaning) of experiment. Note: results presented as mean and standard deviation. *P ≤ 0.05 or **P ≤ 0.001 shows the difference between groups.

few health challenges faced by calves, as previously described in other studies (Roberts et al., 2016). However, in the final third of the experiment, there was greater body weight gain in the animals in the TREAT group (days 15–30 and days 30–45), when the most critical period in the food transition had passed. Teixeira et al. (2014) observed no effect on growth performance in nursing dairy calves, and these authors cited a number of factors that may play a role in calf performance attributable to the major health challenges faced by animals at this stage of life. In a study carried out by our research group, there was a tendency toward greater weight gain in calves during the weaning period (60 days of age) in animals receiving injectable sodium selenite and vitamin A and E during the nursing phase (Volpato et al., 2018). Furthermore, when the authors measured body weight of 210-day-old calves, they found greater weights in the supplemented group (Weiss, 2005).

The increase in total leukocytes can be explained by the action of copper, which stimulates neutrophil and monocyte responses (Maggini et al., 2008) and of zinc, a cofactor in more than 300 enzymes including those involved in the synthesis of DNA and RNA, responsible for replication and proliferation of immune cells (Andriguetto et al., 1999; Spears, 2000; Roberts et al., 2016). The lower lipid peroxidation levels may also have contributed to increased leukocyte levels (Vedovatto, 2018), because immune cells contain membranes with polyunsaturated fatty acids, and these polyunsaturated fatty acids are sensitive to lipid peroxidation by ROS and other free radicals; reduction in lipid damage may indicate minor damage and relative protection of the cells (Spears and Weiss, 2008). Vitamin E protects cell membranes from lipoperoxidation (Andrieu, 2008), acting in synergy with selenium (Mehdi and Dufrasne, 2016).

Vitamin A acts by neutralizing ROS molecules (Sies, 1991). The enzymatic antioxidant system is stimulated by the minerals Zn, Cu and Mn, components of SOD enzymes (Marklund, 1980) and by selenium, an essential component of the GPx enzyme (Volpato et al., 2018). This explains the activation of SOD and GPx activity over time, reflecting an increase in total antioxidant capacity (ACAP), as well as lower levels of ROS. Other studies have shown that minerals cause decreases in ROS

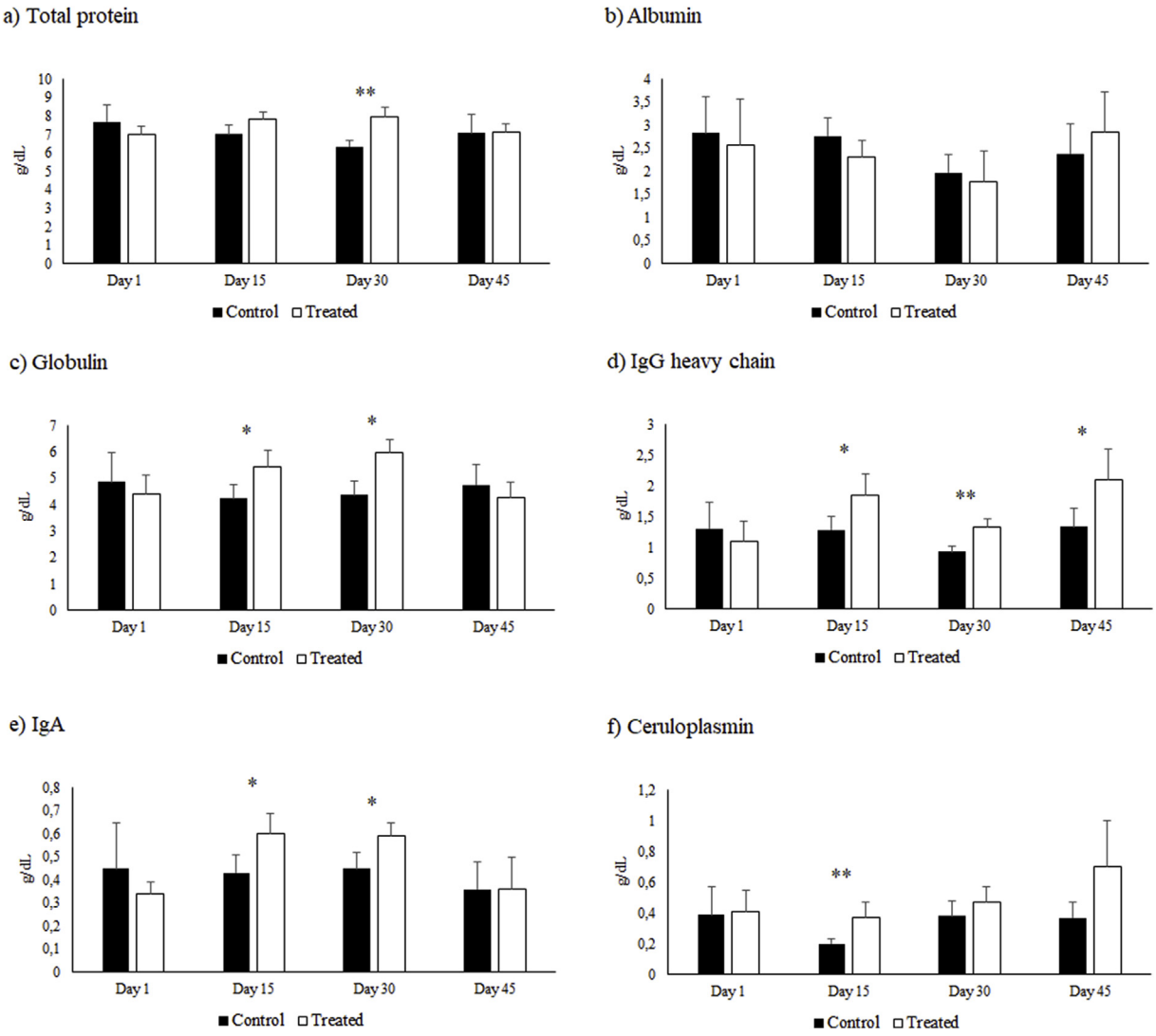

Fig. 2. Total protein (a), albumin (b), globulin (c), IgG heavy chain (d), IgA (e) and ceruloplasmin (f) in serum of dairy calves that received mineral and vitamin applications (treated group) by subcutaneous route on days 1 (15 days pre-weaning) and 30 days (15 days post-weaning) of experiment. Note: results presented as mean and standard deviation. *P ≤ 0.05 or **P ≤ 0.001 shows the difference between groups.

levels (Santos et al., 2019), as well as increases in antioxidant enzyme activity in calves (Glombowsky et al., 2018; Tomasi et al., 2018), cows (Machado et al., 2014) and lambs (Cazarotto et al., 2018), corroborating to our results.

We observed that the greater serum concentration of total proteins was due to increased globulin levels, the basis of the humoral immune response that guarantees specificity in the action of antibodies (Fernández-Cruz et al., 2009). Similar results were observed by Tomasi et al. (2018) and Volpato et al. (2018) who used minerals or a combination of minerals and vitamins in nursing calves. The increase in globulin levels was caused by elevated levels of IgA and IgG heavy chain, both of which are important to the immune system (Murata et al., 2004), as well as ceruloplasmin, the main copper-chelating protein in the circulation (Roeser et al., 1970; Sheffy and Schultz, 1979) that prevents copper from participating in ROS-producing reactions (Weiss, 2002; Schneider and Oliveira, 2004). Zinc acts on protein production and formation of disulfide bonds, present in the structure of antibodies (Charlton and Ewing, 2007). Deficiencies in selenium and copper levels are associated with reduced antibody production and responses to infections (Sheffy and Schultz, 1979; Spears, 2000); therefore, this additional injectable dose in calves is important because it may have two important roles as an additional or primary supply to overcome basic deficiencies. Increased ceruloplasmin and IgG heavy chains were also observed by Volpato et al. (2018), using a protocol of association of sodium selenite and vitamins A and E. In the present study, we observed a significant increase in serum levels of TNF-α and IL-1 on days 30 and 45 of experiment, as well as in IFNγ on day 45 in animals supplemented with minerals, in disagreement with results reported by Jiao et al. (2018). According to those authors, copper and zinc reduced intestinal levels of pro-inflammatory cytokines (IL-1 and TNF-α) that could be considered an improvement in the immune system because of a reduction in pro-inflammatory mediators during the weaning period. Nevertheless, it is important to highlight that slow augmentation of IL-1 and TNF-α levels may be positive responses because these cytokines are involved in control of infectious diseases (Fishman, 1996). As observed in this study, serum levels of IL-1, TNF-α and IFNγ showed small increases, possibly indicating control of infectious diseases.

In summary, the dairy calves used in this study had no mineral deficiencies (zinc, copper, selenium and manganese) and had no weight gain differences. All animals remained apparently healthy during the experiment, except for one episode of pasty stools in fewer than 5% of the calves (both groups) after withdrawal of the milk from the diet. Based on our data, we conclude that mineral complexes, as well as

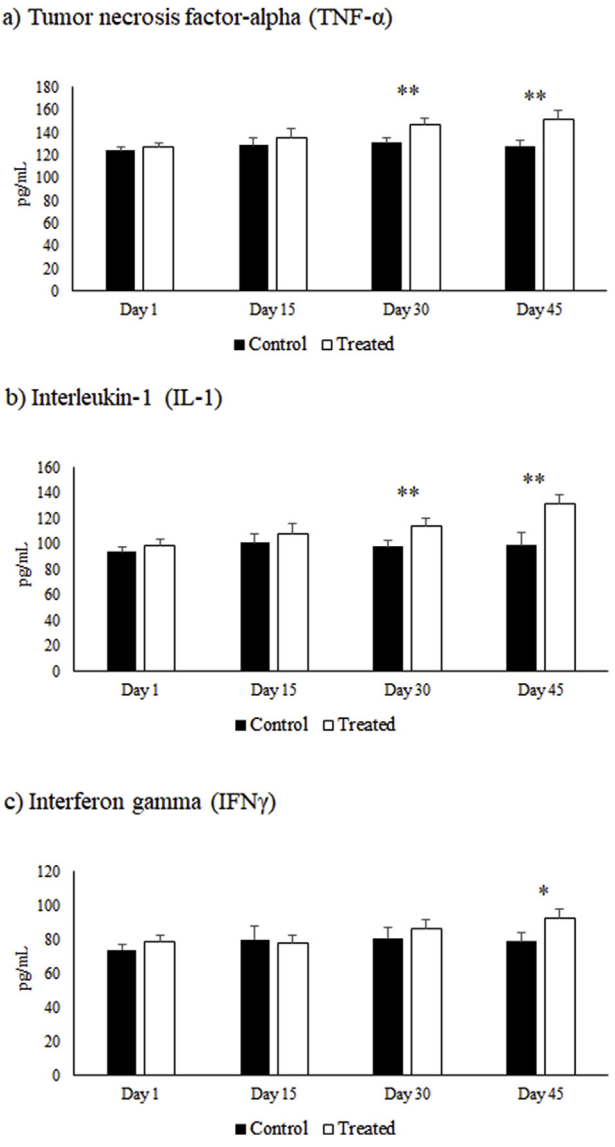

Fig. 3. Cytokine (tumor necrosis factor-alpha (a: TNF-α), interleukin-1 (b: IL-1), and interferon gamma (c: IFNγ) levels in serum samples of calves. Note: results presented as mean and standard deviation. *P ≤ 0.05 or **P ≤ 0.001 shows the difference between groups.

vitamins A and E, have nutraceutical effects on nursing dairy calves in the transition period from infancy to weaning, demonstrated by the fact that these supplements increased variables related to immunity and antioxidant status. There were possible beneficial effects on the animals in the form of greater post-weaning weight gain.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethics committee

All procedures this project were approved by the *Comitê de Ética do Uso de Animais na Pesquisa* (CEUA) of the *Universidade do Estado de Santa Catarina*, under the protocol number 6965281117, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA).

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References

Abuelo, A., Hernández, J., Benedito, J.L., Castillo, C., 2014. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J. Anim. Physiol. Anim. Nutr. 99, 1003–1016.
Amado, L.L., Garcia, M.L., Ramos, P.B., Freitas, R.F., Zafalon, B., Ferreira, J.L.R., Monserrat, J.M., 2009. A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Sci. Total Environ. 407, 2115–2123.
Andrieu, S., 2008. Is there a role for organic trace element supplements in transition cow health? Vet. J. 176, 77–83.
Andriguetto, J.M., Perly, L., Minardi, I., Gemael, A., Flemming, J.S., Souza, G.A., Bona Filho, A., 1999. Nutrição Animal. 3ed. Nobel, Sao Paulo.
Arthington, J.D., Moriel, P., Martins, P.G.M.A., Lamb, G.C., Havenga, L.J., 2014. Effects of trace mineral injections on measures of performance and trace mineral status of pre- and postweaned beef calves. J. Anim. Sci. 92, 2630–2640.
Besser, T.E., Gay, C.C., 1994. The importance of colostrum to the health of the neonatal calf. Vet. Clin. N. Am. Food Anim. Pract. 10, 107–117.
Beutler, E., 1984. Superoxide dismutase. In: Beutler, E. (Ed.), Red Cell Metabolism. A Manual of Biochemical Methods.
Botteon, R.D.C.C.M., Botteon, P.D.T.L., Júnior, J.D.C.B.S., Pinna, M.H., Lóss, Z.G., 2008. Freqüência de diarréia em bezerros mestiços sob diferentes condições de manejo na região do médio Paraíba Rio de Janeiro e Minas Gerais. Braz. J. Vet. Res. Anim. Sci. 45, 153–160.
Burke, N.C., Scaglia, G., Boland, H.T., Swecker Jr., W.S., 2009. Influence of two-stage weaning with subsequent transport on body weight, plasma lipid peroxidation, plasma selenium, and on leukocyte glutathione peroxidase and glutathione reductase activity in beef calves. Vet. Immunol. Immunopathol. 127, 365–370.
Campos, O.F., Lizieire, R.S., 2000. Desaleitamento precoce e alimentação de bezerras. Simpósio sobre manejo e nutrição de gado de leite. pp. 1–20.
Cazarotto, C.J., Boito, J.P., Gebert, R.R., Reis, J.H., Machado, G., Bottari, N.B., Morsch, V.M., Schetinger, M.R.C., Doleski, P.H., Leal, M.L.R., Baldissera, M.D., Da Silva, A.S., 2018. Metaphylactic effect of minerals on immunological and antioxidant responses, weight gain and minimization of coccidiosis of newborn lambs. Res. Vet. Sci. 121, 46–52.
Charlton, S.J., Ewing, W.N., 2007. The Minerals Directory. Context Products Ltd 5a-5f.
Collet, S.G., Demeda, M.A., Taffarel, G.V., Taffarel, L., Girardini, L.K., Nesi, C.N., do Rego Leal, M.L., 2017. Effect of injectable trace mineral supplement and vitamins A and E on production and milk composition of Holstein cows. Revista de Ciências Agroveterinárias (Journal of Agroveterinary Sciences) 16, 463–472.
Fagliari, J.J., Santana, A.E., Lucas, F.A., Campos, E., Curi, P.R., 1998. Constituintes sangüíneos de bovinos recém-nascidos das raças Nelore (Bos indicus) e Holandesa (Bos taurus) e de bubalinos (Bubalus bubalis) da raça Murrah. Arq. Bras. Med. Vet. Zootec. 50, 253–262.
Feldman, B.F., 2000. Schalmís Veterinaty Hematology, vol. 5. Lippincott Williams and Wilkins, Philadelphia, pp. 1221p.
Fernández-Cruz, E., Alecsandru, D., Ramon, S.S., 2009. Mechanisms of action of immune globulin. Clin. Exp. Immunol. 157, 1–2.
Filappi, A., Prestes, D., Cecim, M., 2005. Suplementação mineral para bovinos de corte sob pastejo. Revisão. Veterinária Notícias Veterinary News 11, 91–98.
Fishman, R.H.B., 1996. Slow-release TNF receptor controls inflammatory disease. The Lancet 348, 166–167.
Flores, E.M.M., Saidelles, A.P.F., Barin, J.S., Mortari, S.R., Martins, A.F., 2001. Hair sample decomposition using polypropylene vials for determination of arsenic by hydride generation atomic absorption spectrometry. J. Anal. Atomic Spectrom. 16, 1419–1423.
Glombowsky, P., da Silva, A.S., Soldá, N.M., Galli, G.M., Biazus, A.H., Campigotto, G., Bottari, N.B., Sousa, R.S., Brisola, M.C., Stefani, L.M., Baldissera, M.D., Leal, M.L.R., Morsch, V.M., Schetinger, M.R.C., Machado, G., 2018. Mineralization in newborn calves contributes to health, improve the antioxidant system and reduces bacterial infections. Microb. Pathog. 114, 344–349.
Grandin, T., Gallo, C., 2007. Cattle transport. Livestock handling and transport 3, 134–154.
Hermes-Lima, M., Willmore, W.G., Storey, K.B., 1995. Quantification of lipid peroxidation in tissue extracts based on Fe (III) xylenol orange complex formation. Free Radic. Biol. Med. 19, 271–280.
Jiao, L.F., Zhang, Q.H., Wu, H., Wang, C.C., Cao, S.T., Feng, G., Hu, C.H., 2018. Influences of copper/zinc-loaded montmorillonite on growth performance, mineral retention, intestinal morphology, mucosa antioxidant capacity, and cytokine contents in weaned piglets. Biol. Trace Elem. Res. 185, 356–363.
Jokubauskienė, V., Špakauskas, V., Matusevičius, A., Klimienė, I., Ružauskas, M., Žilinskaitė, M., 2010. Manganese, molybdenum and iron concentration in sera in–calf and milk cows under the influence of different factors. Vet. Zootech. 50, 71–72.
Kargar, S., Mousavi, F., Karimi-Dehkordi, S., Ghaffari, M.H., 2018. Growth performance, feeding behavior, health status, and blood metabolites of environmentally heat-loaded Holstein dairy calves fed diets supplemented with chromium. J. Dairy Sci.

101, 9876–9887.
Larson, L.L., Owen, F.G., Albright, J.L., Appleman, R.D., Lamb, R.C., Muller, L.S., 1977. Guidelines toward more uniformity in measuring and reporting calf experimental data. J. Dairy Sci. 60, 989–991.
LeBel, C.P., Ischiropoulos, H., Bondy, S.C., 1992. Evaluation of the probe 2', 7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem. Res. Toxicol. 5, 227–231.
López-Alarcón, C., Denicola, A., 2013. Evaluating the antioxidant capacity of natural products: a review on chemical and cellular-based assays. Anal. Chim. Acta 763, 1–10.
Machado, V.S., Oikonomou, G., Lima, S.F., Bicalho, M.L.S., Kacar, C., Foditsch, C., Felippe, M.J., Gilbert, R.O., Bicalho, R.C., 2014. The effect of injectable trace minerals (selenium, copper, zinc, and manganese) on peripheral blood leukocyte activity and serum superoxide dismutase activity of lactating Holstein cows. Vet. J. 200, 299–304.
Maggini, S., Wintergerst, E.S., Beveridge, S., Hornig, D., 2008. Contribution of selected vitamins and trace elements to immune function. Proc. Nutr. Soc. 67 (OCE1).
Marklund, S., 1980. Distribution of CuZn superoxide dismutase and Mn superoxide dismutase in human tissues and extracellular fluids. Acta Physiol. Scand. Suppl. 492, 19–23.
Mehdi, Y., Dufrasne, I., 2016. Selenium in cattle: a review. Molecules 21, 545.
Monteiro, S.C., 2010. Parasitologia Na Medicina Veterinária. São Paulo: Rocca, pp. 356.
Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet. J. 168, 28–40.
Pavlata, L., Podhorsky, A., Pechova, A., Chomat, P., 2005. Differences in the occurrence of selenium, copper and zinc deficiencies in dairy cows, calves, heifers and bulls. Veterinarni Medicina – Czech 50, 390–400.
Persson, T., Popescu, B.O., Cedazo-Minguez, A., 2014. Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? Oxid. Med. Cell Longev., 427318. https://doi.org/10.1155/2014/427318. 2014.
Read, S.M., Northcote, D.H., 1981. Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal. Biochem. 116, 53–64.
Reis, L.G., Melo Andrade Rodrigues Albuquerque, F.H., Dourado Valente, B., Araújo Martins, G., Teodoro, R.L., Dias Ferreira, M.B., Madalena, F.E., 2008. Predição do peso vivo a partir de medidas corporais em animais mestiços Holandês/Gir. Ciência Rural. 38, 778–783.
Rink, L., 2000. Zinc and the immune system. Proc. Nutr. Soc. 59, 541–552.
Roberts, S.L., May, N.D., Brauer, C.L., Gentry, W.W., Weiss, C.P., Jennings, J.S., Richeson, J.T., 2016. Effect of injectable trace mineral administration on health, performance, and vaccine response of newly received feedlot cattle. Prof. Anim. Sci. 32, 842–848.
Roeser, H.P., Lee, G.R., Nacht, S., Cartwright, G.E., 1970. The role of ceruloplasmin in iron metabolism. J. Clin. Investig. 49, 2408–2417.
Roland, L., Drillich, M., Klein-Jöbstl, D., Iwersen, M., 2016. Invited review: influence of climatic conditions on the development, performance, and health of calves. J. Dairy Sci. 99, 2438–2452.
Santos, D., Boito, J.P., Reis, J.H., Gebert, R.R., da Silva, A.S., 2019. Health benefits of subcutaneous zinc edetate and diphenyl diselenide in calves during the weaning period. An Acad. Bras Ciências 91, e20171042.
Schneider, C.D., Oliveira, Á.R.D., 2004. Radicais livres de oxigênio e exercício: mecanismos de formação e adaptação ao treinamento físico. Rev. Bras. Med. do Esporte 10, 308–313.
Shankar, V., 2015. Field characterization by near infrared (NIR) mineral identifiers- a new prospecting approach. Procedia Earth and Planetary Science 11, 198–203.
Sheffy, B.E., Schultz, R.D., 1979. Influence of vitamin E and selenium on immune response mechanisms. In: Federation Proceedings. vol. 38. pp. 2139–2143.
Sies, H., 1991. Oxidative stress: from basic research to clinical application. Am. J. Med. 91, S31–S38.
Silva, D.J., Queiroz, A.C., 2006. Análise de alimentos: métodos químicos e biológicos. Editora UFV. Viçosa. 3, 235p.
Spears, J.W., 2000. Micronutrients and immune function in cattle. Proc. Nutr. Soc. 59, 587–594.
Spears, J.W., Weiss, W.P., 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Vet. J. 176, 70–76.
Sundrum, A., 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. Animals 5, 978–1020.
Tao, S., Dahl, G.E., 2013. Invited review: heat stress effects during late gestation on dry cows and their calves. J. Dairy Sci. 96, 4079–4093.
Teixeira, A.G.V., Lima, F.S., Bicalho, M.L.S., Kussler, A., Lima, S.F., Felippe, M.J., Bicalho, R.C., 2014. Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on immunity, health, and growth of dairy calves. J. Dairy Sci. 97, 4216–4226.
Tomasi, T., Volpato, A., Pereira, W.A.B., Debastiani, L.H., Bottari, N.B., Morsch, V.M., Schetinger, M.R.C., Leal, M.L.R., Machado, G., Da Silva, A.S., 2018. Metaphylactic effect of minerals on the immune response, biochemical variables and antioxidant status of newborn calves. J. Anim. Physiol. Anim. Nutr. 102, 819–824.
Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant. vol. 2 Cornel University Press, New York.
Vedovatto, M., 2018. Microminerais injetáveis pós-natal sobre a atividade antioxidante, sistema imunológico, saúde e desempenho de cabritos Boer no período pré-desmame. 143f. (Tese de doutorado). Programa de Pós-graduação em Ciência Animal. Universidade Federal de Mato Grosso do Sul. Campo Grande, MS, Brazil.
Villard, D., Arthur, J.R., Gonzalez, J.M., Pallares, F.J., 2002. Selenium status in cattle: interpretation of laboratory results. Bovine Pract. 36, 73–80.
Volpato, A., Da Silva, A.S., Crecencio, R.B., Tomasi, T., Fortuoso, B.F., Ribeiro, M.P., Morsch, V.M.M., 2018. A prophylactic protocol to stimulate the immune response also control infectious disease and, consequently, minimizes diarrhea in newborn heifers. Microb. Pathog. 121, 262–268.
Weiss, W.P., 2002. Antioxidant Nutrients and Milk Quality. Department of Animal Sciences. The Ohio State University, Wooster, pp. 44691.
Weiss, W.P., 2005. Antioxidants nutrients, cow health and milk quality. In: Dairy Cattle Nutrition Workshop. Department of Dairy and Animal Sciences, Pennsylvania State University, pp. 11–18.
Wendel, A., 1981. Glutathione peroxidase. In: Methods in Enzymology. vol. 77. pp. 325–333.
West, J.W., 2003. Effects of heat-stress on production in dairy cattle. J. Dairy Sci. 86, 2131–2144.