Effects of injectable mineral supplementation on health, metabolic stress, and performance in Holstein cows during the transition period

Raquel Souza Marques
University of Sao Paulo: Universidade de Sao Paulo
Filipe Aguera Pinheiro
University of Sao Paulo: Universidade de Sao Paulo
Clara Satsuki Mori
University of Sao Paulo: Universidade de Sao Paulo
Susan Suárez-Retamozo
Universidad Peruana Cayetano Heredia
Rodrigo Almeida
Federal University of Parana: Universidade Federal do Parana
Bruno Sivieri Lima
Virbac Group: Virbac SA
Luc Durel
Virbac Group: Virbac SA
Viviani Gomes
viviani.gomes@usp.br

Universidade de São Paulo: Universidade de Sao Paulo https://orcid.org/0000-0002-4553-2276

Research Article

Keywords: ketosis, hypocalcemia, metritis, metabolism, immunity

Posted Date: February 10th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-5841480/v1

Abstract

Metabolic distress in dairy cows during the transition period is associated with a high incidence of infectious diseases and reduced performance. This experimental field trial evaluated the use of injectable mineral supplementation (IMS) to prevent metabolic distress in Holstein cows during the transitional period. The IMS group (n = 189) received three injections (10 mL) of a multi-mineral supplement (Fosfosal, Virbac Brazil, São Paulo, Brazil) at days − 14, 0 (calving day) and + 14 days after parturition. The non-supplemented group (NIMS; n = 123) received three placebo injections. Productive, health, and reproductive performances were assessed along with metabolic distress biomarkers in a subset of cows (NIMS, n = 32; and IMS, n = 34). IMS cows had lower incidences of persistent hypocalcemia and metritis, along with reduced haptoglobin, higher glucose, and lower beta-hydroxybutyric acid (BHB) concentrations. The IMS group had higher enzyme activities for glutathione peroxidase (GPx), reduced glutathione (GSH), and higher immunoglobulin G (IgG) concentrations. Mineral supplementation did not affect milk production, somatic cell count (SCC), or reproductive performance. In conclusion, IMS-supplemented cows showed improved immunity, characterized by an anti-inflammatory profile, higher IgG concentrations, reduced lipid metabolism, and oxidative stress, positively affecting their overall health.

1. Introduction

The transition period in dairy cows involves a sequence of events that ultimately lead to a series of occurrences connecting the metabolic profile, immune response, and oxidative stress in a scenario of health or disease (Sordillo and Mavangira 2014). It has been observed that around 75% of the incidence rate of diseases such as mastitis, metritis, ketosis, milk fever, and displaced abomasum concentrates during the early lactation period (LeBlanc et al. 2006). The greatest risk of infectious and metabolic disorders occurs within the first 10 days after calving (Ingvartsen et al. 2003). During this period, cows undergo several changes that require adjustments to their metabolic and endocrine states to meet their new physiological needs. These changes lead to a considerable increase in oxygen consumption, resulting in augmented production of reactive oxygen species (ROS) and nitrogen species (RNS). Antioxidant molecules must neutralize these reactive species to prevent oxidative stress (Sordillo and Aitken 2009). In this scenario, trace element (Zn, Mn, Cu, and Se), macromineral (Ca, P, K, and Mg), and vitamin requirements increase during the 3 weeks prepartum and 3 weeks postpartum, especially around calving time (Abuelo et al. 2015). Minerals and vitamins are associated with metabolism, endocrine function, fertility, growth, and immune regulation in dairy cows (Enjalbert et al. 2006).

Including minerals in a transition dairy cow diet may not ensure their intake or absorption because dry matter intake (DMI) had already decreased during this period. Additionally, dietary and animal factors contribute to DMI variation among individuals and, hence, to the variation in the intake of minerals. In addition, dietary mineral supplements may not be properly absorbed because of their antagonistic interactions with other nutrients. Drinking water containing mineral antagonists can negatively affect mineral uptake in the digestive tract (Spears 2003). An injectable mineral solution is an alternative

method for delivering additional minerals during the transition period. Pogge et al. (2012) reported that using an injectable multi-mineral solution increased the liver concentrations of Cu and Se for at least 15 days, and increased plasma Zn and Mn levels for several hours. Mineral blends appear to play an important role in udder health (Machado et al. 2013; Pogge et al. 2012), stillbirth rate, and endometritis incidence (Machado et al. 2013).

We hypothesized that supplementing dairy cows with minerals during the transition period can provide short-term and long-term benefits on immunity, heath and performance. Therefore, the aim of this study was to evaluate the effects of intramuscular injections of a solution containing P, Mg, K, Cu, and Se on the health, performance, and metabolic stress of dairy cows during their transition period.

2. Materials and Methods

2.1. Animals and management

This experimental field trial was conducted according to the procedures approved by the Committee on Ethics in the Use of Animals of the Faculty of Veterinary Medicine and Animal Science at the University of São Paulo (USP) (protocol #3886141020). The animals utilized in this study were from a commercial herd located in São Pedro, São Paulo State, Southeastern Brazil (47°54'50"W, 22°32'55"S). The cows were enrolled in the trial from February 2021 to May 2021, and the follow-up period continued until December 2021. The farm had 400 milking Holstein cows. The cows were housed either in a free-stall cross-ventilation barn with robotic milking or in a free-stall barn with a rotary milking parlor and milked twice a day. On average, each cow produced 37,5 kg of milk per day, resulting in a total average daily production of 15,000 kg of milk. Animal diets were formulated by the farm's nutritionist following the recommendations of the National Academies of Sciences, Engineering, and Medicine (2021). The dietary compositions that met the prepartum and postpartum requirements are presented in Online Resource 1. The nutrient determinations are shown in Online Resource 2 and 3.

Intramammary dry cow therapy was administered between 70 and 60 days before the expected calving date. Dry cows were housed in specific lots inside a free-stall barn with a cross-ventilation system. The animals were vaccinated against clostridiosis (Fortress 7, Zoetis), neonatal diarrhea (ScourGuard 4KC, Zoetis), reproductive diseases (CattleMaster Gold, Zoetis), and *E. coli*-causing mastitis (JVAC, Boehringer Ingelheim). They were moved to a second free-stall barn with a ventilation tunnel approximately 30 days before the expected calving date, where they were maintained until calving. After calving, the cows were directed to a free-stall cross-ventilation barn with robotic milking or kept in a free stall with a rotary milking parlor, depending on the mammary gland health, cow adaptation to the robot, and milk production, among other factors. At this point, they were vaccinated against keratoconjunctivitis (Biokeratogen, Biogénesis Bagó) and mastitis (JVAC, Boehringer Ingelheim).

2.2. Composition of experimental groups and treatments

The animals were paired into groups for treatment (IMS = 187) and non-treatment (NIMS = 114) with injectable mineral supplementation according to the number of calves. The animals were further subdivided according to their parity into heifers (NIMS, *n* = 51; IMS, *n* = 67) and multiparous cows (NIMS, *n* = 72; IMS, *n* = 122). Cows in the NIMS group received 10 mL of 0.9% NaCl by intramuscular route at day − 14 ± 7 (D-14), calving (D1), and 14 ± 7 (D14) days in milk (DIM), and cows of the IMS group were dosed thrice, similarly to control group schedule, with 10 mL administered by intramuscular injection of a commercial multi-mineral supplement composed of 140 mg/mL of $C_3H_7Na_2O_6P$ sodium glycerophosphate (14 g), monosodium phosphate (20.1 g), $CuCl_2$ (0.4 g), KCl (0.6 g), $MgCl_2$(2.5 g), and Na_2SeO_3 (0.24 g), in water (100 mL) (Fosfosal, Virbac Brazil, SP, Brazil). The injectable mineral supplementation protocol was adapted from Machado et al. (2013).

2.3. Health monitoring

Diseases were identified and registered based on the farm protocols. Cows that failed to release the placenta within 24 hours post-calving were considered to have retained the placenta. Daily uterine inspection was performed to detect metritis during the first 21 days after DIM. Between 30 and 40 days after calving, the cows were subjected to gynecological evaluation using a vaginal speculum. The veterinarians responsible for reproductive management classified cows that exhibited serosanguineous, purulent, or fetid secretions as positive for endometritis. An ultrasonographic evaluation (Mindray, Shenzhen, China) was performed by the same veterinarian to confirm the rectal palpation findings, which included the presence of liquid content in the uterus.

Ketosis was diagnosed on-farm using a ketometer (KetoVet Brazil; TaiDoc Technology, Taiwan, China) on days 5 (D5) and 10 (D10) postpartum. Animals were classified as positive on D5 and D10, according to the following intervals: 0–1.1 mmol/L, no ketosis; 1.2–2.8 mmol/L, subclinical ketosis; ≥ 2.9 mmol/L clinical ketosis (McArt et al. 2012). Blood for the diagnosis of hypocalcemia was collected from the animals by puncturing the coccygeal vein or artery in plain tubes without anticoagulant on calving day (D1) and the fourth day postpartum (D4) to determine the total calcium concentration. The cutoff point adopted to classify animals with subclinical hypocalcemia (HSC) was < 2.15 mmol/L (Reinhardt et al. 2011).Classification in transient hypocalcemic; hypocalcemic, persistently hypocalcemic and delayed hypocalcemic was based in McArt and Neves (2020).

Postpartum subclinical mastitis screening was performed between the 6th and 7th day postpartum, using the California Mastitis Test (CMT). Cows with a score of 2 (++) or 3 (+++) were considered positive for mastitis, according to farm criteria. The cows were sampled aseptically for bacteriological analysis and antimicrobial susceptibility testing. The mastitis detection index was used as a mastitis indicator in a robotic milking system (VMS, DeLaval, International AB, Tumba, Sweden). Traditional milk cultures and the analysis of. antimicrobial susceptibility and resistance tests were performed based on National Mastitis Council (NMC) (1999) and the guidelines of the Clinical and Laboratory Standards Institute (2005).

2.4. Metabolic distress biomarkers

Subsets of NIMS (n = 32) and IMS (n = 34) paired cows were bled seven times during the transition period: 3 weeks before the expected calving date (M-3), 2 weeks before the expected calving date (M-2), 1 week before the expected calving date (M-1), the week of calving, between calving day and 5 days postpartum (M0), 1 week postpartum (M + 1), 2 weeks postpartum (M + 2), and 3 weeks postpartum (M + 3). Blood samples were collected from the coccygeal vein using the Vacutainer system (Becton Dickinson). Oxidative stress was assessed by collecting samples in heparin tubes (10 mL) immersed in crushed ice, while EDTA tubes (4 mL) (BD Vacutainer K2 Ethylenediaminetetraacetic acid - EDTA, 3.6 mg REF367841®; BD Diagnosis, Franklin Lakes, NJ, USA) were stored in a separate insulated box at 4°C for transportation from the farm to the laboratory.

Blood preparation start from two to three hours after samples collection due to the distance between farm and the laboratory. Blood in heparin was centrifuged at 1900 × g for 15 minutes in a refrigerated centrifuge at 4°C. Plasma was removed and stored in black microtubes (Fisherbrand HS4323K) at − 80°C for the analysis of TAS and TBARS. Cell fraction was washed by adding buffered saline solution (NaCl; Na_2HPO_4; NaH_2PO_4; Milli-Q H_2O) and centrifuged at 1900 × g for 15 minutes in a refrigerated centrifuge at 4°C. The leukocyte layer and the buffered saline solution were removed. This process was repeated until the leukocyte layer could not be distinguished or was reduced so that it did not interfere with removal of the red blood cell (RBC) mass. RBC stored in black microtubes was kept in the freezer at − 80°C for the future determination of GPx.

Blood in EDTA was added to tubes with distilled water and precipitant solution (HPO_3; EDTA $Na_2.H_2O$; NaCl; H_2O) after vortexing for homogenization for GSH analysis. The samples were rested for 5 minutes and then centrifuged at 1 900 × g for 5 minutes. After centrifugation, the intermediate layer was removed and stored in amber microtubes (Thermo Fisher Scientific, Ireland) and stored at − 80°C.

Samples for haptoglobin (Hp) and IgG determination were collected using tubes containing a clot activator (Vacutube, Labor Import, China) to obtain blood serum, which was then centrifuged at 1500 x g for 10 minutes and stored in duplicate at − 40°C. All analyses were conducted as single assays, except for the haptoglobin and IgG assays, which were performed in duplicate.

GSH levels were determined according to a previously described method (Beutler et al. 1963). Serum GPx activity (RS504 and RS505, Randox Brasil Ltda, Brazil) and TAS (NX2332, Randox Brasil Ltda, Brazil) determination were performed using commercial Randox test kits in an automated biochemical analyzer (Labmax 240 Premium, Labtest Diagnostica, Brazil). TBARS was assayed by dissolving 3% (w/v) 5-sulfosalicylic acid hydrate and thiobarbituric acid (TBA) solution at 0.67% in purified water at 95°C for 30 minutes. The pH 1.8 was adjusted from 1.8 to 2.0 using a 1 M sodium hydroxide solution. For TBARS quantification, 0.25 mL of serum or washed erythrocytes were added to a test tube containing 0.25 mL of 3% 5-sulfosalicylic acid hydrate, and vortexed for 10 seconds, centrifuged at 18 000 × g for 3 minutes, and left to rest for 15 minutes at 25°C. Subsequently, 0.25 mL of purified water (blank) or supernatant (samples) was diluted into 0.5 mL of 0.67% TBA solution. The mixture was heated at 95°C for 30 minutes and then cooled on ice for 10 minutes to stop further reaction. After blanks and samples were

equilibrated to room temperature, 300 µL were pipetted into a microplate well and absorbance was measured at 535 nm. The results are expressed as nM TBARS per milligram of Hb or total protein (nM/mg of Hb or total protein) in washed erythrocytes or serum samples, respectively. The MDA-TBA complex extinction coefficient of 156 000 M^{-1}/cm^{-1} at 25°C and 0.9 cm path length were used for the calculations.

IgG concentrations in serum samples were measured using an in-house sandwich ELISA, according to the procedures described by Gomes et al. 2023. The intra-test coefficient of variation was 10% and inter-test coefficient was 10% for IgG assays.

The concentration of haptoglobin was determined based on its ability to bind to hemoglobin (Ramos et al. 2021) using spectrophotometry. The intra-test coefficient of variation was 7.25% and inter-test coefficient was 8.19% for haptoglobin assays.

Serum concentrations of total bilirubin, direct bilirubin, cholesterol, glucose, triglycerides, total protein, non-esterified fatty acids (NEFA), beta-hydroxybutyric acid (BHB), albumin, and aspartate aminotransferase (AST) were determined in a Labmax 240 Premium automatic biochemical analyzer (Labtest Diagnostica). References to commercial kits are shown in Online Resource 4.

2.5. Data collection

Data collection began during the prepartum period, from 30 to 20 days before the expected calving date, and lasted up to 90 DIM. Milk yield in the rotary milking parlor, metritis and endometritis occurrence, and reproductive performance were extracted from Dairy Plan (GEA Farm Technologies, Canada) databases. Milk yield data from the milking robot were extracted using DeLaval VMSTM V300 software during the 1st week of lactation. The weekly average milk yield was recorded over 12 weeks, and individual somatic cell counts (SCC) were recorded monthly. Reproductive indices, such as days between the calving date and the 1st artificial insemination and the type of semen used (sexed or conventional), were recorded to verify whether they impacted the conception rate, as well as the outcomes of pregnancy checks to estimate pregnancy rates between experimental groups.

2.6. Statistical analysis

Analyses were conducted using the Statistical Analysis System for Windows software (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). Descriptive statistics for qualitative nominal data, such as the occurrence of diseases and pregnancy, were calculated using the FREQ procedure. Associations between experimental groups (NIMS and IMS) and disease incidence were evaluated using the Chi-square test or Fisher's exact test, with statistical significance set at $P \leq 0.05$. Fisher's exact test was applied when group sizes included fewer than five animals. Variables with $P \leq 0.05$ were further analyzed through binary logistic regression to estimate odds ratios (OR) and 95% confidence intervals (95% CI) using the LOGISTIC procedure.

Quantitative variables were assessed for normality using the SAS Guided Data Analysis function. Non-normally distributed data underwent logarithmic, square root, quadratic, or inverse transformations to achieve normal distribution. Fixed effects of treatment (control vs. IMS), evaluation time points, and their interaction were tested for significance. These effects were analyzed using the MIXED procedure, with a post hoc least significant difference (LSD) test applied. Covariance structures were evaluated, and models with the lowest Akaike Information Criterion (AIC) value were selected. Statistical differences were considered significant at $P < 0.05$. When a significant interaction between treatment and time was identified, differences between groups at each evaluation point were analyzed using Student's t-test for both prepartum and postpartum periods, with significance defined as $P \leq 0.05$.

When working with unequal numbers of experimental units across treatments, variance between treatments may differ. This was particularly relevant in the present study, as data from control group animals were lost due to issues in the farm's information system. To address this, the statistical model included a correction for variance heterogeneity by incorporating the **group = trt** statement in the REPEATED command (e.g., Welch's test).

3. Results

3.1. Descriptive statistics

The sample size varied during the study owing to the removal of cows, but the proportions of cows removed were similar between the experimental groups: 7.5% (14/187) and 4.4% (5/114) for the NIMS and IMS groups, respectively. The incidence of twin births, stillbirths, and the sex of the calves from the 301 experimental cows are avalilable in Online Resource 5.

3.2. Effect of injectable mineral supplementation on ketosis and hypocalcemia

Online Resource 6 shows the serum calcium levels on D1 (at calving) and D4 in Holstein cows treated or not treated with IMS. The results indicated that heifer cows from the IMS group had slightly higher ($P = 0.06$) calcium levels (8.23 ± 0.23 mmol/L) than those from the NIMS group (8.78 ± 0.18 mmol/L) on D4 after calving (Online Resource 6). The effects of intramuscularly injected multi-mineral supplementation on the incidence of ketosis and hypocalcemia are shown in Table 1. The incidence of persistent hypocalcemia (D1 and D4) was higher in NIMS for both multiparous category ($P = 0.04$) and for all cows ($P = 0.01$). The odds of developing persistent hypocalcemia in the NIMS group were 4.60 times higher in the multiparous group (95% CI: 1.105–19.186) and 3.13 times higher in the overall population (95% CI: 1.288–7.595). On the other hand, the incidence of late hypocalcemia (only D4) was lower ($P = 0.02$) in the NIMS group than in IMS for the multiparous category (OR = 0.30, 95% CI; 0.107–0.836).

Table 1. Effect of the injectable multi-mineral supplement on ketosis and hypocalcemia incidence rate in Holstein transition cows.

Metabolic Diseases	Category	Incidence % (*n*/total)		Odd Ratio (95% CI)	*P*-value
		NIMS	IMS		
Subclinical ketosis (D5)	Heifers	14.29 (7/49)	15.87 (10/63)	0.88 (0.310 - 2.517)	0.82
	Multiparous	43.06 (31/72)	29.73 (33/111)	1.79 (0.962 - 3.319)	0.07
	Total	31.40 (38/121)	24.71 (43/174)	1.39 (0.833 - 2.336)	0.21
Subclinical ketosis (D10)	Heifers	12.24 (6/49)	14.29 (9/63)	0.84 (0.276 - 2.535)	0.75
	Multiparous	43.06 (31/72)	32.73 (36/110)	1.55 (0.842 - 2.870)	0.16
	Total	30.58 (37/121)	26.01 (45/173)	1.25 (0.749 - 2.096)	0.40
Transitory hypocalcemia (D1)	Heifers	11.90 (5/42)	14.55 (8/55)	0.80 (0.240 - 2.629)	0.71
	Multiparous	29.41 (20/68)	28.00 (28/100)	1.07 (0.543 - 2.115)	0.84
	Total	22.73 (25/110)	23.23 (36/155)	0.97 (0.544 - 1.739)	0.92
Late hypocalcemia (D4)	Heifers	23.81 (10/42)	10.91 (6/55)	2.55 (0.845 - 7.712)	0.09
	Multiparous	7.35 (5/68)	21.00 (21/100)	0.30 (0.107 - 0.836)	0.02
	Total	13.64 (15/110)	17.42 (27/155)	0.75 (0.377 - 1.485)	0.41
Persistent hypocalcemia (D1 and D4)	Heifers	4.76 (2/42)	0 (0/55)	-	0.96
	Multiparous	20.59 (14/68)	8.00 (8/100)	4.60 (1.105 - 19.186)	0.04
	Total	14.55 (16/110)	5.16 (8/155)	3.13 (1.288 - 7.595)	0.01

Abbreviations: NIMS = Control group; IMS = Injectable mineral supplementation group, received three doses of 10 ml of intramuscular injections at 260±7 days of gestation, at calving, and 14±7 days in milk; D1 = Calving day; D4 = Fourth day postpartum; D5 = Fifth day postpartum; D10 = Tenth day postpartum

3.3. Effect of injectable mineral supplementation on uterine health and reproductive performance

The incidence rates of retained placenta (RP) and endometritis were similar between the IMS and NIMS groups; however, the incidence of metritis was higher in the NIMS group than in the IMS group in all

parity categories. NIMS has at least 2.10 (95% CI: 1.218–3.625) more chances (P = 0.01) to develop metritis than the IMS group (Table 2). The intervals between calving and 1st artificial insemination and pregnancy rates were similar between the experimental groups (Online Resource 7 and 8).

Table 2. Effect of injectable multi-mineral supplementation on uterine health of Holstein transition cows.

Condition	Parity	Incidence % (*n*/total)		Odd Ratio (95% CI)	*P*-value
		NIMS	IMS		
Retained placenta	Heifers	6.35 (4/63)	11.76 (6/51)	1.97 (0.524 - 7.386)	0.32
	Multiparous	11.11 (8/72)	7.02 (8/114)	1.66 (0.593 - 4.630)	0.34
	Total	11.38 (14/123)	6.78 (12/177)	1.77 (0.787 - 3.962)	0.17
Metritis	Heifers	24.00 (12/50)	11.11 (7/63)	2.53 (0.912 - 7.000)	0.07
	Multiparous	36.11 (26/72)	21.43 (24/112)	2.07 (1.072 - 4.008)	0.03
	Total	31.15 (38/122)	17.71 (31/175)	2.10 (1.218 - 3.625)	0.01
Endometritis	Heifers	18.37 (9/49)	19.05 (12/63)	0.96 (0.367 - 2.493)	0.93
	Multiparous	14.71 (10/68)	14.68 (16/109)	1.00 (0.426 - 2.357)	0.99
	Total	16.24 (19/117)	16.28 (28/172)	0.99 (0.528 - 1.885)	0.99

Abbreviations: NIMS = Control group; IMS = Injectable mineral supplementation group, received three doses of 10 ml of intramuscular injections at 260±7 days of gestation, at calving, and 14±7 days in milk.

3.4. Effect of injectable mineral supplementation on mastitis, somatic cell count, and milk production

Milk production, SCC, the incidence of mastitis, and antimicrobial use in the immediate postpartum period (from calving to D7) and across the first 90 DIM were similar between the groups (Online Resource 9 to 11).

3.5. Effect of injectable mineral supplementation on metabolic profile

The effects of mineral supplementation on metabolic biomarkers in transitional Holstein cows are shown in Table 3. It was possible to detect higher mg/dL, and all experimental cows treated with an injectable multi-mineral complex; 64.10 ± 0.93 mg/dL vs. 61.15 ± 0.63 mg/dL (P = 0.02), respectively, for IMS and NIMS all parity cows.

Table 3. Mean (±standard error) of metabolism biomarkers in Holstein cows supplemented (IMS) or not (NIMS) with an injectable mineral complex.

Parity	Biomarkers	Group		Treatment	Time	Treatment*Time Interaction
		NIMS	IMS			
		Mean ± SE	Mean ± SE			
Heifers	ALB	2.63 ± 0.03	2.50 ± 0.04	0.30	< 0.01	0.64
	AST	33.59 ± 1.03	36.35 ± 1.10	0.25	< 0.01	0.65
	BT	0.18 ± 0.01	0.18 ± 0.03	0.58	< 0.01	0.75
	BD	0.23 ± 0.01	0.20 ± 0.01	0.26	< 0.01	0.90
	COL	84.72 ± 2.56	90.17 ± 3.30	0.24	< 0.01	0.56
	GLI	62.20 ± 0.94	66.34 ± 1.80	0.05	< 0.01	0.47
	BHB (mmol/L)	1.35 ± 0.03	1.35 ± 0.03	0.97	< 0.01	0.74
	NEFA (mmol/L)	1.01 ± 0.06	0.77 ± 0.41	0.22	0.67	0.19
Multiparous	ALB	2.64 ± 0.03	2.66 ± 0.024	0.89	< 0.01	0.61
	AST	34.82 ± 0.85	37.50 ± 1.00	0.37	< 0.01	0.24
	BT	0.17 ± 0.01	0.23 ± 0.02	0.17	< 0.01	0.20
	BD	0.22 ± 0.01	0.25 ± 0.01	0.10	< 0.01	0.05
	COL	98.57 ± 3.12	94.52 ± 2.77	0.41	< 0.01	0.88
	GLI	60.29 ± 0.84	62.90 ± 1.05	0.07	< 0.01	0.34
	BHB (mmol/L)	1.26 ± 0.03	1.25 ± 0.03	0.48	< 0.01	0.01
	NEFA (mmol/L)	0.92 ± 0.06	0.93 ± 0.05	0.97	0.06	0.58
Total	ALB	2.63 ± 0.02	2.60 ± 0.02	0.67	< 0.01	0.87

AST	34.41 ± 0.64	37.10 ± 0.75	0.17	< 0.01	0.34
BT	0.18 ± 0.01	0.215 ± 0.02	0.40	< 0.01	0.61
BD	0.22 ± 0.01	0.24 ± 0.01	0.36	< 0.01	0.30
COL	92.30 ± 2.11	93.00 ± 2.13	0.79	< 0.01	0.73
GLI	61.15 ± 0.63	64.10 ± 0.93	0.02	< 0.01	0.20
BHB (mmol/L)	1.3 ± 0.02	1.28 ± 0.02	0.78	< 0.01	0.22
NEFA (mmol/L)	0.96 ± 0.04	0.88 ± 0.03	0.44	0.04	0.71

Abbreviations: NIMS = control group; IMS = Injectable mineral supplementation group, received three doses of 10 ml of intramuscular injections at 260±7 days of gestation, at calving, and 14±7 days in milk; ALB = Albumin; AST = Aspartate Aminotransferase; BT = Total Bilirubin; BD = Direct Bilirubin; COL = Cholesterol; GLI = Glucose; BHB = Beta-hydroxybutyrate; NEFA = Non-esterified fatty acids; Mean = mean between groups; SE = Standard error. MIXED-model procedure.

3.6. Effect of injectable mineral supplementation on oxidative stress

In multiparous cows, injectable multi-mineral complex treatment resulted in higher (P = 0.02) GPx (U/g Hb). In addition, GSH (mg/dL) levels were higher (P = 0.05) in IMS cows than in untreated cows across parities. An effect of time was detected for most variables, independent of the parity of the cows, except for GPx and GSH. Interactions between treatment and time were also detected (P < 0.05) in the multiparous category for TBARS (nM/mg) and TAS (mmol/L) (Table 4 and Figure 1). We detected higher values of TBARS in the NIMS (2.16 ± 0.27) than in the IMS group (1.60 ± 0.33) at week +1 postpartum (P = 0.02). In addition, the total antioxidant activity (TAS, mmol/L) was lower in the NIMS group than in the IMS group at weeks +2 and +3 postpartum.

Table 4. Mean values and standard errors (SE) for biomarkers of oxidative stress in Holstein cows treated or not with an injectable mineral supplement during the transition period.

Parity	Variables	Group		*P*< Treatment	*P*< Time	*P*< Treatment*Time
		NIMS	IMS			
		Mean ± SE	Mean ± SE			
Heifers	GPx (U/g Hb)	1228.4 ± 39.2	1224.1 ± 44.6	0.71	0.07	0.32
	TBARS (nM/mg)	2.65 ± 0.17	1.99 ± 0.14	0.31	< 0.01	0.70
	GSH (mg/dL)	3.46 ± 0.36	3.59 ± 0.32	0.22	0.20	0.70
	TAS (mmol/L)	1.01 ± 0.01	0.98 ± 0.01	0.23	< 0.01	0.16
Multiparous	GPx (U/g Hb)	801.9 ± 34.7	940.7 ± 30.0	0.02	0.13	0.74
	TBARS (nM/mg)	1.98 ± 0.13	1.85 ± 0.13	0.59	< 0.01	0.04
	GSH (mg/dL)	3.76 ± 0.38	3.47 ± 0.27	0.68	0.43	0.63
	TAS (mmol/L)	0.99 ± 0.01	0.98 ± 0.01	0.33	< 0.01	0.05
Total	GPx (U/g Hb)	955.5 ± 28.1	1004.8 ± 25.6	0.25	< 0.01	0.23
	TBARS (nM/mg)	2.29 ± 0.11	1.90 ± 0.10	0.24	< 0.01	0.49
	GSH (mg/dL)	3.44 ± 0.22	3.7 ± 0.18	0.05	0.02	0.61
	TAS (mmol/L)	1.00 ± 0.01	0.98 ± 0.01	0.10	< 0.01	0.48

Abbreviations: NIMS = Control group; IMS = Injectable mineral supplementation group; animals were injected 3 times, between D-30 to D-20 prepartum, on calving day and D+15 postpartum; GPx = Glutathione peroxidase; TBARS = Thiobarbituric acid; GSH = Glutathione reductase; TAS = Total antioxidant status; Mean = mean between groups; SE = Standard error.

*Differences were considered significant when $P \leq 0.05$.

Total animals per parity: Heifers: 25 (NIMS = 15 and IMS = 10); Multiparous: 41 (NIMS, *n* =17; IMS, *n* = 24); Total: 66 (NIMS, *n* = 32; IMS, *n* = 34)

3.7. Effect of injectable mineral supplementation on immune biomarkers

Significant differences were observed in IgG concentrations between the IMS and NIMS groups for all parity categories, with higher ($P < 0.01$) IgG levels in the IMS group. In contrast, the haptoglobin concentration was higher ($P < 0.05$) in the NIMS group for multiparous cows and for all parity orders. A time-related effect was observed for all variables and lactate levels. An interaction between treatment and time was detected for IgG concentration in all experimental animals included in this study (Table 5 and Figure 2).

Table 5. Mean values, standard error (SE) for serum IgG and haptoglobin in Holstein cows treated or not with an injectable mineral supplement during the transition period.

Parity	Variables	Group		Treatment	*P* < Time	Treatment*Time Interaction
		NIMS	IMS			
		Mean ± SE	Mean ± SE			
Heifers	IgG (mg/ml)	27.19 ± 1.53	33.32 ± 1.96	< 0.01	< 0.01	0.14
	HAP (mg/ml)	5.48 ± 0.52	4.00 ± 0.37	0.49	< 0.01	0.23
Multiparous	IgG (mg/ml)	28.09 ± 1.45	36.87 ± 1.69	< 0.01	< 0.01	0.27
	HAP (mg/ml)	6.15 ± 0.65	4.32 ± 0.30	0.03	< 0.01	0.10
Total	IgG (mg/ml)	27.68 ± 1.05	35.63 ± 1.30	< 0.01	< 0.01	0.03
	HAP (mg/ml)	5.84 ± 0.42	4.21 ± 0.23	0.05	< 0.01	0.11

Abbreviations: NIMS = Control group; IMS = Injectable mineral supplementation group, animals were injected 3 times, between D-30 to D-20 prepartum, on calving day and D+15 postpartum; IgG = Immunoglobulin G; HAP = Haptoglobin; Mean = Mean between groups; SE = Standard error.

Total animals per parity: Heifers: 25 (NIMS = 15 and IMS = 10); Multiparous: 41 (NIMS, $n = 17$; IMS, $n = 24$); Total: 66 (NIMS, $n = 32$; IMS, $n = 34$)

Discussion

The analysis indicated that the untreated and treated groups had similar incidence rates of ketosis. Calcium analysis did not show a consistent difference between the experimental groups; however, qualitative analysis revealed a 4.6 times higher likelihood of developing persistent hypocalcemia in the NIMS group. This finding agrees with the usual development of subclinical hypocalcemia around parturition, where the high and immediate calcium requirements for the production of colostrum and

transition milk, along with a decrease in DMI, result in a great calcium deficiency that can take days to settle (Mann et al. 2019).

No significant effect of IMS on lipolysis biomarkers was observed for either BHB or NEFA, regardless of parity. Qualitative analysis of NEFA using odds ratios also did not detect differences between the experimental groups.

Omur et al. 2016 administered vitamins (ADE) and trace elements (Cu, Se, Mn, and Zn) to ten multiparous Brown Swiss cows and found lower NEFA values in the treated group at all sampling points during the transition period. The study by Yazlık et al. 2021 found elevated concentrations of non-esterified fatty acids (NEFA) in animals that received supplementation with the same commercial trace mineral and vitamin product used by Omur et al. 2016, indicating some inconsistency in outcomes.

These different results for NEFA and BHB may be associated with the non-standardization of sampling time, the technique used for sample analysis, cow physiological factors during the transition period, or the supplementation of different commercial products with different mineral concentrations.

An interaction between treatment and time was detected in multiparous cows at the first week postpartum, observing lower BHB concentration for the IMS group than for multiparous cows (0.75 ± 0.08 vs. 1.10 ± 0.15 mmol/L). One possible explanation for this finding is that multiparous cows generally have higher milk yields; consequently, they have a greater metabolic challenge and more severe negative energy balance (Kreipe et al. 2011). Therefore, IMS may have increased the risk of older cows.

Our findings are consistent with those reported by Machado et al. 2014. These researchers evaluated subcutaneous mineral supplementation containing 300 mg of Zn, 50 mg of Mn, 25 mg of Se, and 75 mg of Cu in 250 multiparous cows at 230 and 260 days of gestation and 35 days postpartum. Among the findings of the study, serum BHB concentrations for the group that received mineral supplementation was 0.27 mmol/L while the control group was 0.41 mmol/L. BHB analysis is usually performed for ketosis control during the first week postpartum (McArt et al. 2012).

The effect of the treatment on glucose concentration was observed regardless of parity, with higher glucose concentrations observed in the treated cows. It is possible that the sources of phosphate in the commercial mineral supplements used in this study were different. Blood P levels are physiologically reduced during the postpartum period, and P concentrations may be even lower in high-producing dairy cows. Therefore, P supplementation may improve the energy balance (Grünberg and Constable 2009). In addition, Grünberg and Constable (2009) found that small reductions in cytosolic P levels after parturition may affect the liver metabolic activity. Bertoni et al. (2008) and Trevisi et al. (2012) observed that transition cows with better liver function had lower NEFA and BHB serum levels.

The effect of mineral supplementation was observed in multiparous cows, with increased levels of the antioxidant substance GPx in the supplemented group. Enzymatic antioxidants are pivotal in transforming H2O2 into less reactive forms for the organism, including O2 and H_2O. Previous studies

have revealed that increased serum Se concentrations are associated with increased GPx activity in cattle (Koller et al. 1984). Bittar et al. (2018) reported that mineral supplementation (Zn, Cu, Mn, and Se) containing Se could also increase Se, which is a cofactor of GPx and could explain the GPx increase in multiparous cows.

Interaction between treatment and time was detected for TBARS and TAS in multiparous cows, with higher TAS and lower TBARS values in IMS animals than in NIMS animals in the postpartum period. Soldá et al. (2017) reported significantly lower TBARS concentrations and higher catalase levels in animals that received mineral supplementation during the transition period. However, Silva et al. (2022) did not find differences in GPx and SOD antioxidant enzymes in dairy cattle supplemented with two applications of subcutaneous mineral complexes (300 mg Zn, 50 mg Mn, 25 mg Se, and 75 mg Cu); therefore, their findings differ from the results of our study. The time at which antioxidant enzymes were evaluated may have influenced the results, since in our study, we analyzed the transition period from prepartum to postpartum. In contrast, the cited authors evaluated the first 10 days after calving.

According to our findings and previously published results, older animals may have a reduced ability to respond to selenoproteins, such as GPx, as the ability of younger animals to respond seems more evident. Thus, although further studies are needed, this may justify the fact that Se supplementation may reduce oxidative stress in older cows by increasing antioxidant activity.

The number of different antioxidant components in the serum and tissues makes it relatively difficult to measure each antioxidant component separately. In addition, because there is cooperation among various antioxidants, examining a single antioxidant may not accurately reflect their combined action. Therefore, the measurement of TAS seems to be a suitable biochemical parameter for evaluating the overall antioxidant status resulting from antioxidant intake or production and consumption by increasing levels of oxidative stress (Nemec et al. 2000). In the present study, differences were observed in the TAS scores of the multiparous cows. Interaction analysis revealed differences between multiparous cows in the second and third weeks postpartum.

Abuelo et al. (2013) argued that the concentrations of antioxidants and pro-oxidants observed separately are not good indicators of oxidative stress because an imbalance between them defines oxidative stress. Although the focus of our study was not on oxidative stress, antioxidant enzymes and the lack of oxidizing substance analysis may have prevented us from observing the entire scenario.

Haptoglobin and IgG levels were measured as representatives of the innate and adaptive immune responses. Haptoglobin is an α2-globulin synthesized by the liver during the acute-phase response. In cattle, the circulating concentrations of haptoglobin are negligible in healthy subjects; however, they may increase by more than 100-fold during inflammatory and acute-phase reactions (Eckersall and Bell 2010). Therefore, haptoglobin has been used as a marker of stress or inflammation induced by infections, diseases, and trauma in bovine (Eckersall and Bell 2010). In our study, haptoglobin concentration was lower in the mineral supplementation group, both for multiparous cows and all cows, in agreement with Silva et al. (2022). These finding disagree with the results presented by Silva et al.

(2022), who did not found differences in haptoglobin levels between cattle supplemented and non-supplemented with mineral complexes during the transition period.

The effect of treatment with injectable minerals was observed for IgG in almost all parities evaluated in this study. However, the interaction between treatment and time was only observed when the total population were analyzed, independently of calving number, in which all time points showed higher IgG values in the mineral supplementation group than in the control group. Since IgG is important for viral and toxin neutralization, bacterial agglutination, and opsonization, in addition to neutralizing antibodies and complement activation, among other immune functions, the increase in serum IgG dosage may be related to the improvement of the cow's immune status during the study period (Abuelo et al. 2013).

The mechanisms by which trace minerals influence the immune response are not yet fully understood, though a recent review in humans underscores the necessity of adequate mineral intake—specifically magnesium, zinc, copper, iron, and selenium—for immune competence. Inadequate levels of these minerals can temporarily impair immune function and disrupt inflammation regulation (Stefanache et al. 2023).

Magnesium and selenium, in particular, influence the acute-phase response by reducing cytokine production in macrophages following toll-like receptor (TLR) stimulation, resulting in an anti-inflammatory modulation. Additionally, selenium acts as a crucial co-factor for antioxidant enzymes, protecting innate immune cells from damage caused by high concentrations of reactive oxygen species (Stefanache et al. 2023). This mechanism may explain the observed reduction in acute-phase proteins, such as haptoglobin, in cattle receiving injectable mineral supplementation compared to a placebo group.

In terms of adaptive immunity, both magnesium and selenium are essential for lymphocyte activation, growth, differentiation, and proliferation. Redox balance also plays a significant role in the differentiation of T helper cell subtypes, including Th1, Th2, Th17, Treg, and other T helper types. Higher selenium intake has been associated with increased IFN-gamma production, whereas lower selenium intake elevates IL-4 levels (Huang et al. 2012; Stefanache et al. 2023). Selenium levels may also influence B-cell-dependent antibody production in a pathogen-specific manner, though its effect on T-cell immunity is generally more consistent. Studies have documented an increase in antibody production in cattle vaccinated and supplemented with injectable minerals (Bittar et al. 2020; Mattioli et al. 2020; Rodrigues et al. 2023).

While the specific mechanisms by which copper supports immune system development and function are still unclear, its importance is well-established. Copper is necessary for the normal development and optimal performance of the immune system, and deficiencies have been linked to reduced effectiveness of both cellular and humoral immune responses (Pogge et al. 2012).

This research highlights the beneficial impact of injectable mineral supplementation on reducing postpartum ketosis and metritis incidence, alongside its effects on oxidative stress and both innate and

humoral immune responses. Future studies are needed to further investigate and clarify the specific roles of each mineral in bovine immunity during critical periods.

The results from this study indicated that IMS enhanced the metabolic and uterine health of Holstein cows during the transition period. The treated animals showed reduced oxidative stress owing to the increased enzymatic activity of GPx (multiparous cows) and GSH (all animals). The immunity of cows, characterized by an anti-inflammatory profile, was also improved, as evidenced by a decrease in the haptoglobin biomarker levels compared to the control group, and higher serum IgG concentrations at all time points in the group that received injectable mineral treatment.

Declarations

Acknowledgements

The authors are grateful to the owners and all employees of São Jorge Farm. The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) through the Postgraduate Program in Veterinary Clinic (PcVet) of the School of Veterinary Medicine and Animal Science of the University of São Paulo (USP); which partially financed this project, awarded to R.S.M. This research was conducted as part of the thesis titled “Evaluation of oxidative stress and immunity in Holstein cows supplemented with macro and trace minerals during the transition period” by R.S.M. to obtain the Master’s degree in Sciences.

Statement of Animal Rights

The studies have been approved by a research ethics committee at the institution or practice at which the studies were conducted. The approval for this study was granted by the Committee on Ethics in the Use of Animals of the Faculty of Veterinary Medicine and Animal Science (FMVZ) of the University of São Paulo (USP), protocol #3886141020.

Conflict of Interest Statement

This work was supported by Virbac Animal Health. Authors B.S.L. and L.D. are currently employed by Virbac Animal Health. This study was conducted using a product manufactured by Virbac, which may be perceived as a financial interest related to the publication of this manuscript. R.S.M., F.A.P., C.S.M., S.S.-R and R.A. declare they have no financial interests.

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (Finance Code 88887.619707/2021-00) and Virbac Animal Health. Authors B.S.L. and V.G. have received research support from Virbac Animal Health.

Author Contributions

Conceptualization, B.S.L., L.D. and V.G.; methodology, R.S.M., C.S.M., R.A., B.S.L., L.D. and V.G.; software, R.S.M., F.A.P. and V.G.; validation, R.S.M. and V.G.; formal analysis, R.S.M., F.A.P. and R.A.; investigation, R.S.M. and F.A.P.; resources, R.S.M., F.A.P. and V.G.; data curation, R.S.M., F.A.P. and V.G.; writing—original draft preparation, R.S.M., S.S.-R., L.D. and V.G.; writing—review and editing, S.S.-R., L.D. and R.A.; visualization, R.S.M., S.S.-R, L.D., R.A. and V.G.; supervision, V.G.; project administration, V.G.; funding acquisition, B.S.L, L.D. and V.G. All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available in the Zenodo repository, https://doi.org/10.5281/zenodo.14530068.

Ethics approval

The approval for this study was granted by the Committee on Ethics in the Use of Animals of the Faculty of Veterinary Medicine and Animal Science (FMVZ) of the University of São Paulo (USP), protocol #3886141020.

References

1. Abuelo A, Hernández J, Benedito JL, Castillo C (2013) Oxidative stress index (OSi) as a new tool to assess redox status in dairy cattle during the transition period. Animal 7:1374–1378. https://doi.org/10.1017/S1751731113000396
2. Abuelo A, Hernández J, Benedito JL, Castillo C (2015) The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. J Anim Physiol Anim Nutr 99:1003–1016. https://doi.org/10.1111/jpn.12273
3. Bertoni G, Trevisi E, Han X, Bionaz M (2008) Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. J Dairy Sci 91:3300–3310. https://doi.org/10.3168/jds.2008-0995
4. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61:882–888.
5. Bittar JHJ, Hoyos-Jaramillo A, Hurley DJ, Woolums AR, Havenga LJ, Lourenço JM, Barnett G, Gomes V, Saliki JT, Harmon DD, Palomares RA (2018) Effects of injectable trace minerals administered concurrently with a modified live virus vaccine on long-term protection against bovine viral diarrhea virus acute infection in dairy calves. Res Vet Sci 119:250–258. https://doi.org/10.1016/j.rvsc.2018.07.003
6. Bittar JHJ, Palomares RA, Hurley DJ, Hoyos-Jaramillo A, Rodriguez A, Stoskute A, Hamrick B, Norton N, Adkins M, Saliki JT, Sanchez S, Lauber K (2020) Immune response and onset of protection from Bovine viral diarrhea virus 2 infection induced by modified-live virus vaccination concurrent with

injectable trace minerals administration in newly received beef calves. Vet Immunol Immunopathol 225:110055. https://doi.org/10.1016/j.vetimm.2020.110055

7. Clinical and Laboratory Standards Institute (2005) Performance Standards for Antimicrobial Susceptibility Testing, Fifteenth Informational Supplement, M100-S15. Wayne, USA.
8. Eckersall PD, Bell R (2010) Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet J 185:23–27. https://doi.org/10.1016/j.tvjl.2010.04.009
9. Enjalbert F, Lebreton P, Salat O (2006) Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: Retrospective study. J Anim Physiol Anim Nutr 90:459–466. https://doi.org/10.1111/j.1439-0396.2006.00627.x
10. Gomes V, Barros BP, Castro-Tardón DI, Martin CC, Santos FCR, Knöbl T, Santarosa BP, Padilha LM, Hurley DJ (2023) The role of anti-E. coli antibody from maternal colostrum on the colonization of newborn dairy calves gut with Escherichia coli and the development of clinical diarrhea. Animal - Open Space 2:100037. https://doi.org/10.1016/j.anopes.2023.100037
11. Grünberg W, Constable PD (2009) Chapter 6 - Function and Dysfunction of the Ruminant Forestomach. In Food Animal Practice, 5th ed. Netherlands, pp. 12–19. ISBN 978-141-603-591-6.
12. Huang Z, Rose AH, Hoffmann PR (2012) The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal 16:705–743. https://doi.org/10.1089/ars.2011.4145
13. Ingvartsen KL, Dewhurst RJ, Friggens NC (2003) On the relationship between lactational performance and health: Is it yield or metabolic imbalance that cause production diseases in dairy cattle? A position paper. Livest Prod Sci 83:277–308. https://doi.org/10.1016/S0301-6226(03)00110-6
14. Kaneko JJ, Harvey JW, Bruss ML (2008) Appendix VIII blood analyte reference values in large animals. In Clinical Bichemistry of Domestic Animals, 6th ed. San Diego, USA.
15. Koller LD, South PJ, Exon JH, Whitbeck GA, Maas J (1984) Comparison of selenium levels and glutathione peroxidase activity in bovine whole blood. Can J Comp Med 48:431–433.
16. Kreipe L, Vernay MCMB, Oppliger A, Wellnitz O, Bruckmaier RM, Van Dorland HA (2011) Induced hypoglycemia for 48 hours indicates differential glucose and insulin effects on liver metabolism in dairy cows. J Dairy Sci 94:5435–5448. https://doi.org/10.3168/jds.2011-4208
17. LeBlanc SJ, Lissemore KD, Kelton DF, Duffield TF, Leslie KE (2006) Major advances in disease prevention in dairy cattle. J Dairy Sci 89:1267–1279. https://doi.org/10.3168/jds.S0022-0302(06)72195-6
18. Machado VS, Bicalho ML, Pereira RV, Caixeta LS, Knauer WA, Oikonomou G, Gilbert RO, Bicalho RC (2013) Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. Vet J 197:451–456. https://doi.org/10.1016/j.tvjl.2013.02.022
19. Machado VS, Oikonomou G, Lima SF, Bicalho ML, Kacar C, Foditsch C, Felippe MJ, Gilbert RO, Bicalho RC (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. https://doi.org/10.1016/j.tvjl.2014.02.026

20. Mann S, McArt J, Abuelo A (2019) Production-related metabolic disorders of cattle: Ketosis, milk fever and grass staggers. In Practice 41:205–219. https://doi.org/10.1136/inp.l3041
21. Mattioli GA, Rosa DE, Turic E, Picco SJ, Raggio SJ, Minervino AHH, Fazzio LE (2020) Effects of parenteral supplementation with minerals and vitamins on oxidative stress and humoral immune response of weaning calves. Animals (Basel) 10:1298. https://doi.org/10.3390/ani10081298
22. McArt JAA, Neves RC (2020) Association of transient, persistent, or delayed subclinical hypocalcemia with early lactation disease, removal, and milk yield in Holstein cows. J Dairy Sci 103:690–701. https://doi.org/10.3168/jds.2019-17191
23. McArt JAA, Nydam DV, Oetzel GR (2012) Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci 95:5056–5066. https://doi.org/10.3168/jds.2012-5443
24. Moyes KM, Larsen T, Friggens NC, Drackley JK, Ingvartsen KL (2009) Identification of potential markers in blood for the development of subclinical and clinical mastitis in dairy cattle at parturition and during early lactation. J Dairy Sci 92:5419–5428. https://doi.org/10.3168/jds.2009-2088
25. National Academies of Sciences, Engineering, and Medicine (2021) Nutrient Requirements of Dairy Cattle, 8th Rev. ed. Washington, USA. https://doi.org/10.17226/25806.
26. National Mastitis Council (1999) Laboratory handbook on bovine mastitis, Rev. ed. Madison, USA. ISBN 978-093-214-703-5
27. Nemec A, Drobnič-Košorok M, Skitek M, Pavlica Z, Galac S, Butinar J (2000) Total Antioxidant Capacity (TAC) values and their correlation with individual antioxidants in healthy beagles. Acta Vet Brno 69:297–303. https://doi.org/10.2754/avb200069040297
28. Omur A, Kirbas A, Aksu E, Kandemir F, Dorman E, Kaynar O, Ucar O (2016) Effects of antioxidant vitamins (A, D, E) and trace elements (Cu, Mn, Se, Zn) on some metabolic and reproductive profiles in dairy cows during transition period. Pol J Vet Sci 19:697–706. https://doi.org/10.1515/pjvs-2016-0088
29. Ospina PA, Nydam DV, Stokol T, Overton TR (2010) Associations of elevated nonesterified fatty acids and beta-hydroxybutyrate concentrations with early lactation reproductive performance and milk production in transition dairy cattle in the northeastern United States. J Dairy Sci 93:1596–1603. https://doi.org/10.3168/jds.2009-2852
30. Pogge DJ, Richter EL, Drewnoski ME, Hansen SL (2012) Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. J Anim Sci 90:2692–2698. https://doi.org/10.2527/jas.2012-4482
31. Ramos JS, Madureira KM, Da Silva KN, Bosco KA, Morita LM, Guimarães JE, Gomes V (2021) Haptoglobin and its association with naturally occurring diseases in Holstein heifer calves. Arq Bras Med Vet Zootec 73:551–559. https://doi.org/10.1590/1678-4162-12159
32. Reinhardt TA, Lippolis JD, McCluskey BJ, Goff JP, Horst RL (2011) Prevalence of subclinical hypocalcemia in dairy herds. Vet J 188:122–124. https://doi.org/10.1016/j.tvjl.2010.03.025

33. Rodrigues CM, Parmeggiani EB, Leal KW, Schneider M, Collet SG, Cibin FWS, Gomes V, Blagitz MG, Rocha JBTD, Leal MLDR (2023) Effect of prepartum maternal supplementation with diphenyl diselenide on biochemical, immunological, and oxidative parameters of the offspring. Animals (Basel) 14:10. https://doi.org/10.3390/ani14010010
34. Silva TH, Guimaraes I, Menta PR, Fernandes L, Paiva D, Ribeiro TL, Celestino ML, Netto AS, Ballou MA, Machado VS (2022) Effect of injectable trace mineral supplementation on peripheral polymorphonuclear leukocyte function, antioxidant enzymes, health, and performance in dairy cows in semi-arid conditions. J Dairy Sci 105:1649–1660. https://doi.org/10.3168/jds.2021-20624
35. Smith BP (2008) Large Animal Internal Medicine, 4th ed. Missouri, USA.
36. Soldá NM, Glombowsky P, Campigotto G, Bottari NB, Schetinger MRC, Morsch VM, Favero JF, Baldissera MD, Schogor ALB, Barreta D, Machado G, Da Silva AS (2017) Injectable mineral supplementation to transition period dairy cows and its effects on animal health. Comp Clin Path 26:335–342. https://doi.org/10.1007/s00580-016-2378-y
37. Sordillo LM, Aitken SL (2009) Impact of oxidative stress on the health and immune function of dairy cattle. Vet Immunol Immunopathol 128:104–109. https://doi.org/10.1016/j.vetimm.2008.10.305
38. Sordillo LM, Mavangira V (2014) The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim Prod Sci 54:1204–1214. https://doi.org/10.1071/AN14503
39. Spears JW (2003) Trace mineral bioavailability in ruminants. J Nutr 133:1506S–1509S. https://doi.org/10.1093/jn/133.5.1506S
40. Stefanache A, Lungu II, Butnariu IA, Calin G, Gutu C, Marcu C, Grierosu C, Bogdan Goroftei ER, Duceac LD, Dabija MG, Popa F, Damir D (2023) Understanding how minerals contribute to optimal immune function. J Immunol Res 3355733. https://doi.org/10.1155/2023/3355733
41. Trevisi E, Amadori M, Cogrossi S, Razzuoli E, Bertoni G (2012) Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. Res Vet Sci 93:695–704. https://doi.org/10.1016/j.rvsc.2011.11.008
42. Yazlık MO, Çolakoğlu HE, Pekcan M, Kaya U, Küplülü Ş, Kaçar C, Polat M, Vural MR (2021) Effects of injectable trace element and vitamin supplementation during the gestational, peri-parturient, or early lactational periods on neutrophil functions and pregnancy rate in dairy cows. Anim Reprod Sci 225:1–11. https://doi.org/10.1016/j.anireprosci.2021.106686

Figures

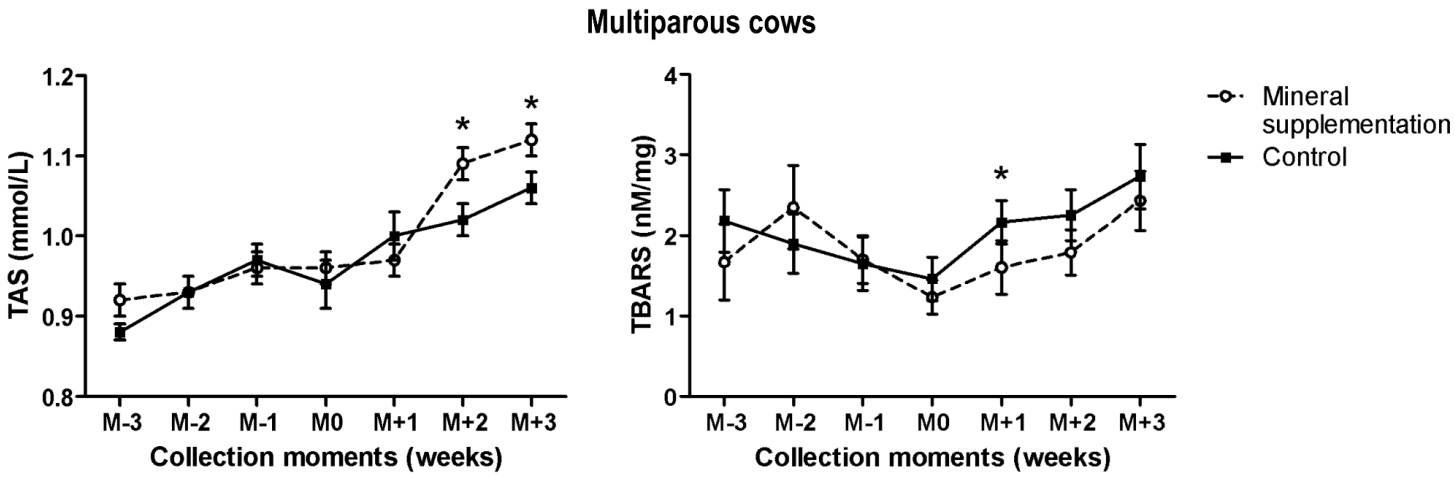

Figure 1

Mean values, standard error (SE), and differences for TAS (a) and TBARS (b) between control and mineral supplementation groups in Holstein dairy cows during the transition period

Abbreviations: NIMS = Control group, animals did not receive injectable mineral supplementation; IMS = Injectable mineral supplementation group, animals were injected 3 times, between D-30 to D-20 prepartum, on calving day and D+15 postpartum; TBARS = Thiobarbituric acid; TAS = Total antioxidant status; M-3 = Three weeks prepartum; M-2 = Two weeks prepartum; M-1 = One week prepartum; M0 = Peripartum; M+1 = One week postpartum; M+2 = Two weeks postpartum; M+3 = Three weeks postpartum.

* Differences were considered significant when $P < 0.05$ (Student's *t*-test).

Total animals per group: NIMS, $n = 17$; IMS $n = 24$.

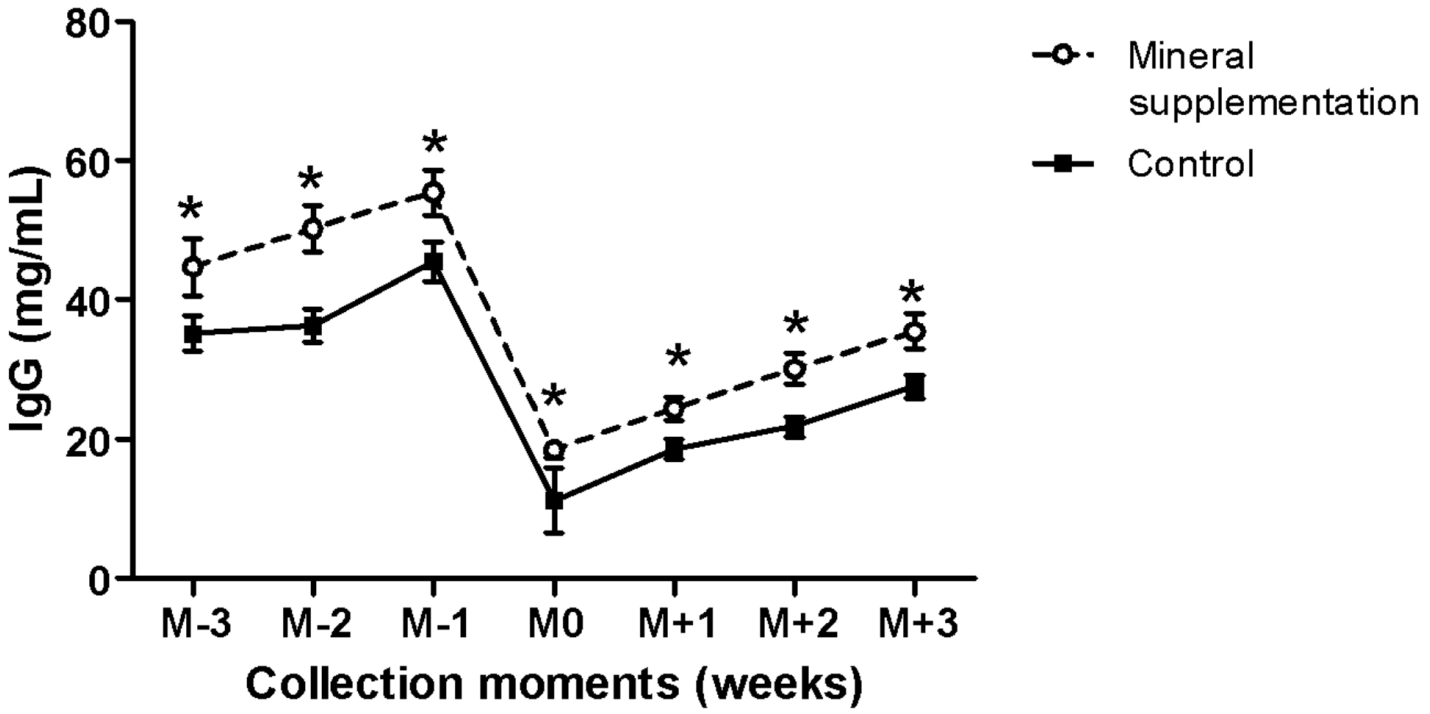

Figure 2

Mean values, significant *P*-value, and differences for IgG between control and mineral supplementation for the total of animals

Abbreviations: NIMS = Control group, animals did not receive injectable mineral supplementation; IMS = Injectable mineral supplementation group, animals were injected 3 times, between D-30 to D-20 prepartum, on calving day and D+15 postpartum; HAP = Haptoglobin; IgG = Immunoglobulin G; M-3 = Three weeks prepartum; M-2 = Two weeks prepartum; M-1 = One-week prepartum; M0 = Peripartum; M+1 = One week postpartum; M+2 = Two weeks postpartum; M+3 = Three weeks postpartum.

* Differences between groups are considered significant when $p < 0.05$ (Student's *t*-test).

Total animals per group: NIMS, $n = 32$; IMS, $n = 34$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile.docx