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The Effect of a Pre-Mix of Essential Organic Minerals on Growth, Antioxidant Indices, and the Diarrhea Incidence in Dairy Calves Breed in Arid Climates

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Simple Summary: Diarrhea is a major health challenge for dairy calves, particularly in arid climates, where stress and poor nutrition can weaken their immune defenses. This study investigated whether supplementing calf diets with organic forms of copper, zinc, and manganese could enhance antioxidant defenses and reduce diarrhea incidence. Twenty-five male Holstein calves were fed different mineral supplements for 21 days. While mineral supplementation did not affect body weight or daily gain, it significantly increased blood mineral concentrations and improved antioxidant enzyme activity. Moreover, calves receiving zinc or copper showed a lower incidence of diarrhea compared with the control group. These findings suggest that organic trace minerals may support calf health and resilience under stressful environmental conditions.

Abstract: This study evaluated the effects of organic trace mineral supplementation on growth performance, antioxidant indices, mineral status, and diarrhea incidence in dairy calves raised in arid climates. Twenty-five male Holstein calves were randomly assigned to five dietary treatments for 21 days, as follows: (1) control group (no organic mineral supplementation), (2) copper-methionine (Cu-Met) supplemented diet, (3) zinc-methionine (Zn-Met) supplemented diet, (4) manganese-methionine (Mn-Met) supplemented diet, and (5) Zn-Met + Cu-Met + and Mn-Met in a premix supplemented diet. Mineral supplementation had no effect on final body weight or average daily gain. However, the concentrations of Zn, Cu, and Mn significantly increased ($p < 0.01$) in blood and feces of treated animals. The highest blood concentrations of Zn and Mn were observed in calves receiving Zn-Met, while Mn-Met supplementation significantly influenced blood Cu levels. The highest Zn excretion was recorded in calves receiving the organic mineral premix, whereas the highest Mn and Cu excretion was observed in the Cu-Met group. Additionally, mineral supplementation enhanced total antioxidant capacity and superoxide dismutase activity in plasma samples ($p < 0.01$). These findings suggest that organic mineral supplementation could be an effective strategy to improve mineral bioavailability and support the health of dairy calves during early life in arid climates.

Keywords: antioxidant indices; bioavailability; holstein calves; trace mineral

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1. Introduction

The dairy sector faces multiple challenges that affect both productivity and economic sustainability, especially the prevalence of neonatal diseases [1], the volatility of market conditions, and the need to adopt emerging technologies [2]. These challenges are further intensified by climate change, particularly in arid regions where heat stress impairs animal welfare and reduces milk production [3]. Within this complex scenario, calf health represents a critical point of vulnerability [4]. Neonatal diarrhea is one of the most common and economically significant disorders affecting dairy calves, leading to increased veterinary costs, impaired growth, delayed weaning, and higher mortality rates [5,6]. These early-life health setbacks often translate into long-term consequences such as reduced milk yield in the first lactation and increased replacement rates [7]. Managing and preventing diarrhea is therefore essential not only for animal welfare but also for the long-term profitability of dairy farms, especially in harsh environments where calves are already exposed to considerable physiological stress [8]. Multiple factors contribute to the onset of neonatal diarrhea, including inadequate maternal nutrition during gestation [9], difficult calving [10], poor colostrum intake [11], and suboptimal calf feeding [12,13]. Malnourished calves are particularly susceptible to infectious diseases like diarrhea and pneumonia, with higher mortality risk and reduced lifetime productivity [14]. Even when recovery is achieved, affected calves often experience long-lasting growth retardation, thus highlighting the importance of early nutritional and management strategies [7].

In this context, the role of trace minerals in supporting calf health has gained increasing attention. Essential elements such as zinc (Zn), copper (Cu), and manganese (Mn) are involved in a wide range of physiological functions including immune competence, skeletal development, cellular replication, and oxidative balance [15]. Deficiencies in these minerals can compromise growth, impair immune responses, and increase susceptibility to disease [16]. For instance, Zn is widely recognized for its anti-inflammatory and anti-diarrheal properties and has been used to prevent gastrointestinal infections in both human infants and neonatal animals [17,18]. Similarly, Cu and Mn are involved in numerous enzymatic systems and act as biomarkers of inflammation and tissue integrity [19].

One of the critical pathways through which trace minerals exert their protective effects is the antioxidant defense system [18,19]. During episodes of diarrhea, intestinal infections stimulate an overproduction of reactive oxygen species (ROS), which can damage epithelial cells, compromise barrier function, and impair nutrient absorption [20]. If not properly neutralized, oxidative stress exacerbates inflammation and delays recovery. Young calves are particularly vulnerable due to their immature antioxidant systems [4]. Enhancing antioxidant capacity through nutritional supplementation—particularly with trace minerals involved in antioxidant enzyme systems such as superoxide dismutase (SOD)—may mitigate intestinal damage, reduce the severity and duration of diarrhea, and support improved growth [18]. Moreover, the source and bioavailability of trace minerals significantly influence their effectiveness [21]. Organic forms, such as amino acid chelates (e.g., Cu-methionine [Cu-Met], Zn-methionine [Zn-Met], and Mn-methionine [Mn-Met]), are generally better absorbed than inorganic salts, leading to more stable blood mineral levels and improved physiological responses [22]. Previous studies have demonstrated that organic mineral supplementation can enhance growth performance and reduce disease incidence in calves [23–25]. However, there is little information on the beneficial effects of mineral supplementation on calves. Given these considerations, the present study aimed to investigate the effects of supplementing dairy calves with a premix of organic trace minerals—specifically Cu-Met, Zn-Met, and Mn-Met—at levels exceeding the National

Research Council (NRC) requirements [26]. The research focused on evaluating blood mineral concentrations, antioxidant status, growth performance, and the incidence of diarrhea in pre-weaning calves raised under arid climatic conditions.

2. Materials and Methods

This study was conducted following the ethical principles recommended by the animal care committee of the University of Mashhad, in accordance with the guidelines of the Iranian Council of Animal Care. The health status of each calf was recorded at birth and monitored throughout the trial period.

2.1. Animals and Experimental Diets

The experiment was carried out between August and September 2022 at the Dairy Cattle Research Center of Ferdowsi University of Mashhad, Iran. Twenty-five, one-month-old male Holstein calves with average body weight (BW) of 40.2 ± 1.6 kg were used in the study. These calves were sourced from the university's dairy farm, which specializes in breeding high-quality dairy cattle. Artificial insemination (AI) had been applied to synchronize calving, using hormonal treatments to induce ovulation and coordinate parturition, thereby ensuring uniformity in calf age at the start of the trial. Calves were selected based on similar age and health status to reduce variability and enhance the reliability of the experimental data. Upon arrival at the research center, all calves were weighed and evaluated for body dimensions, including chest circumference, body length, withers height, hip height, hip length, and pin length. Each calf received 2 L of colostrum twice a day (at 08:30 and 16:00 h) during the first three days of life. From day 4 onward, the experimental calves were fed raw cow milk at a rate of 10% of their BW until day 21. A starter concentrate was formulated according to NRC [26] recommendations, then provided to each calf from day 4 to day 21.

Each calf was then placed in an individual pen equipped with bedding made of dry manure and wheat straw, and provided with independent water troughs and feeders, within a covered housing system. To ensure acclimatization, calves were managed under uniform conditions for one week before the start of the experimental treatments. Following this acclimatization period, calves were randomly assigned into five treatment groups (n = 5 per group) using a random number generator. Randomization occurred immediately before the onset of the 21-day supplementation period. This approach ensured that all animals began the treatments under comparable conditions, minimizing potential confounding effects related to housing or handling stress.

From day 1 of the experiment, each calf received 4 L of whole milk daily (10% of BW), divided into two feedings (6:00 and 20:00 h). The mineral supplements used in the study were commercially available products that were mixed into the raw milk, and administered daily to calves in the morning feeding (6:00 h). The five experimental groups consisted of a control group, which received no additional mineral supplementation (CTR), and four treatment groups that received either 10 mg/day of Cu-Met (5% purity) (G1), 80 mg/day of Zn-Met (5% purity) (G2), 50 mg/day of Mn-Met (5% purity) (G3), or a premix containing all three organic minerals at the same respective doses (referred to as the Premix group). These supplementation levels were selected based on previously published studies [21,22,24] and were intended to reflect practical and physiologically relevant doses. The calves had ad libitum access to a starter feed composed of 10% alfalfa and 90% starter concentrate [26]. The total amount of the required starter feed was prepared in a single batch for uniformity, and the orts were weighed daily to estimate intake. The chemical composition and ingredients of the starter diet are presented in Table 1.

Table 1. The ingredients and chemical composition of the starter.

Ingredient (g/kg DM)	
Legume hay, mature	91.9
Barley grain, dry, ground	241.4
Corn grain dry, coarse grind	313.6
Soybean meal, solvent 48CP	233.6
Canola meal	27.6
Wheat bran	65.3
Calcium carbonate	10.2
Sodium bicarbonate	5.7
Sodium chloride (salt)	5.7
Mineral vitamin premix [1]	5
Chemical Composition	
Dry matter	885
Crude protein	183
Metabolizable energy, (Mcal/kg)	3.24
Neutral detergent fiber	179
Acid detergent fiber	95
Ash	65
Copper	0.0407
Zinc	0.1147
Manganese	0.1988

[1] Nutrient mix provided by the starter feed manufacturer per kilogram of diet: vitamin A, 500,000 IU; vitamin D, 120,000 IU; vitamin E, 1500 IU; vitamin C, 1000 mg/kg; Cu, 1000 mg; Zn, 9000 mg; Mn, 4000 mg; Se, 40 mg; I, 60 mg; Mg, 2500 mg.

2.2. Sample Collection

Three samples of the starter concentrate were collected and analyzed for crude protein (CP; AOAC, method 976.05), dry matter (DM; AOAC, method 930.15), and ether extract (EE; AOAC, method 4.5.05). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined following the methods described by Van Soest et al. [27] and previous studies [28].

The average daily gain (ADG) was determined by weighing each calf on days 1 and 21 of the experiment. Starter feed intake was recorded daily, and the average intake was calculated. Fecal samples were collected directly from the rectum of each calf on days 7, 14, and 21, then oven-dried to determine dry matter content. At the end of the experiment, fecal samples from each calf were pooled, ground, and analyzed for mineral content. Fecal consistency was assessed daily using a four-point scoring system, where a score of 1 corresponded to normal feces, 2 to pasty feces, 3 to semi-liquid feces, and 4 to liquid feces with an unusual color [15]. Diarrhea was defined as a fecal score of 3 or 4, according to Marcondes et al. [29]. The incidence of diarrhea in each treatment group was calculated using the formula described by Chang et al. [17].

$$\text{Incidence of diarrhea } (\%) = \frac{\text{Calves with diarrhea in each group } \times \text{ diarrhea days}}{\text{Total calves in each group } \times \text{ experimental days}} \times 100$$

Blood samples were collected from each calf on the morning of day 21. Serum was obtained by centrifuging the blood samples at 3000× g for 15 min at 4 °C, then stored at −20 °C until analysis. The oxidative status of the calves was evaluated by measuring total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx) concentrations in serum. These parameters were quantified using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. All analyses were performed by a certified veterinary diagnostic laboratory.

In addition, the concentrations of Zn, Cu, and Mn in the starter feed, serum, and feces were determined using inductively coupled plasma optical emission spectrometry (ICP-OES), following the protocols described by Enjalbert et al. [30] and later refined by subsequent studies [31].

2.3. Statistical Analysis

Data were analyzed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). A one-way ANOVA was performed using the MIXED procedure, and the least squares' means were reported. The statistical model used was the following:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

where, Y_{ij} is the observed response variable, μ is the overall mean, α_i is the fixed effect of the i-th treatment (five levels: CTR, Cu, Zn, Mn, and Pre-mix), β_j is the random effect of the j-th calf, and ε_{ij} is the residual error.

This mixed-effects model was used to evaluate treatment effects while accounting for calf-specific variability. The normality of residuals was verified to ensure the assumptions of ANOVA were met. Differences among treatment means were tested using Tukey's post hoc test, and statistical significance was set at $p < 0.05$.

3. Results

Starter intake, average daily gain (ADG), feed efficiency, and fecal scores are presented in Table 2. No significant differences ($p > 0.05$) were observed in these performance parameters among calves receiving mineral supplementation. The inclusion of Cu-Met, Zn-Met, Mn-Met, or their premix did not significantly affect initial body weight (BW), feed efficiency, or fecal scores compared to the control group (Table 2).

Table 2. Changes in body weight, average daily gain, and dry matter intake in Holstein calves supplemented with organic minerals [1].

Performance	CTR	G1	G2	G3	Pre-Mix	SEM	*p*-Value
Starter intake (g/day)	766.80	877.21	685.4	694.32	803.90	173.35	0.93
Initial body weight (kg)	41.32	41.96	40.68	40.56	41.04	2.44	0.99
Final body weight (kg)	51.08	56.14	55.48	55.00	52.78	3.28	0.80
Average daily gain (g/day)	0.69	0.67	0.7	0.69	0.60	0.08	0.99
Feed efficiency (%)	7.01	6.18	6.86	6.95	7.49	1.12	0.60
Average fecal score	2.05	2.05	2.07	2	2.04	0.03	0.52
Incidences of diarrhea (%)	8.57	8.57	3.80	3.80	3.80	1.27	0.94

[1] CTR: control group; G1: copper methionine (Cu-Met) group; G2: zinc methionine (Zn-Met) group; G3: manganese methionine (Mn-Met) group; pre-mix: Cu-Met + Zn-Met + Mn-Met.

The incidences of diarrhea in calves from control, G1 (Cu-Met), G2 (Zn-Met), G3 (Mn-Met), and Pre-mix groups were 8.57, 8.57, 3.80, 3.80, and 3.80%, respectively. The concentrations of Cu, Zn, and Mn in blood and feces are reported in Table 3. Mineral supplementation exceeding the requirements established by NRC [26] significantly affected the mineral concentrations in both blood and feces ($p < 0.01$). Zn-Met supplementation had a significant impact on blood concentrations of both Mn and Zn. The highest Zn excretion was observed in the group receiving the organic mineral mixture (Pre-mix), while the highest Mn and Cu excretion was recorded in calves supplemented with Cu-Met.

Table 3. Mean fecal and blood mineral concentrations (mg/L) in Holstein dairy calves supplemented with organic minerals [1].

Concentration	CTR	G1	G2	G3	Pre-Mix	SEM	*p*-Value
In plasma							
Manganese	0.00004^{c}	0.0001^{b}	0.0015^{a}	0.00038^{b}	0.0005^{b}	0.870	0.001
Zinc	0.042^{d}	0.054^{c}	0.083^{a}	0.039^{d}	0.075^{b}	0.001	0.0001
Copper	0.105^{b}	0.134^{b}	0.107^{b}	0.232^{a}	0.126^{b}	0.013	0.0001
In feces							
Manganese	14.11^{c}	27.10^{a}	23.55^{ab}	20.29^{b}	20.50^{b}	1.414	0.0001
Zinc	47.57^{b}	49.36^{b}	57.03^{ab}	53.89^{b}	70.33^{a}	4.651	0.0172
Copper	6.40^{b}	8.81^{a}	8.50^{ab}	8.64^{ab}	8.08^{ab}	0.702	0.0196

[a,b,c,d] Values within rows with different superscripts differ ($p \leq 0.01$). [1] CTR: control group; G1: copper methionine (Cu-Met) group; G2: zinc methionine (Zn-Met) group; G3: manganese methionine (Mn-Met) group; pre-mix: Cu-Met + Zn-Met + Mn-Met.

As shown in Table 4, dietary supplementation with organic minerals above the recommended levels significantly improved blood antioxidant indices, particularly SOD and TAC ($p < 0.01$). The highest blood SOD concentrations were observed in calves supplemented with Zn-Met and Cu-Met ($p < 0.01$), whereas GPx concentrations were highest in the control group ($p < 0.01$).

Table 4. Mean total antioxidant status in Holstein dairy calves supplemented with organic minerals [1].

Parameter [2]	CTR	G1	G2	G3	Pre-Mix	SEM	p-Value
SOD	0.00004 [c]	0.0001 [b]	0.0015 [a]	0.00038 [b]	0.0005 [b]	0.870	0.001
GPx	0. 042 [d]	0.054 [c]	0.083 [a]	0.039 [d]	0.075 [b]	0.001	0.0001
TAC	0.105 [b]	0.134 [b]	0.107 [b]	0.232 [a]	0.126 [b]	0.013	0.0001

[a,b,c,d] Values within rows with different superscripts differ ($p \leq 0.01$). [1] CTR: control group; G1: copper methionine (Cu-Met) group; G2: zinc methionine (Zn-Met) group; G3: manganese methionine (Mn-Met) group; pre-mix: Cu-Met + Zn-Met + Mn-Met. [2] SOD: superoxide dismutase; GPx: glutathione peroxidase; TAC: total antioxidant capacity.

4. Discussion

The present study demonstrated that supplementation with additional Cu-Met, Mn-Met, and Zn-Met had no significant effect on starter intake, feed efficiency, and initial body weight (BW) in dairy calves during the experiment. Similarly, Nair et al. [32] reported that trace mineral supplementation had no significant impact on BW, DMI, or ADG, whereas Chang et al. [17] found that feeding Zn-Met improved the feed-to-gain ratio, likely due to its higher bioavailability. Additionally, they suggested that Zn supplementation in the form of Zn-Met (rather than ZnO) enhanced ADG in dairy calves during the first two weeks of feeding. The growth-promoting effect of organic minerals was also found to be superior to that of inorganic minerals [33]. Several studies have indicated that additional trace mineral supplementation, regardless of source (inorganic or organic), does not significantly impact DMI or feed efficiency [34–37]. Similarly, Mudgal et al. [38] observed no effect of Cu and Zn supplementation (10–40 ppm) on BW in buffalo calves.

Although no statistically significant differences were observed, the reduced incidence of diarrhea in all mineral-supplemented groups compared to the control suggests a potential health-promoting effect of organic trace minerals. This trend may reflect an improvement in gut function and immune stability, possibly mediated by subtle, early-life physiological responses to enhanced mineral status. Given the short duration of the trial and the limited sample size, these results should be interpreted cautiously. However, the consistency of the reduction across all supplemented groups supports the hypothesis that organic forms of Zn, Cu, and Mn may contribute to improved gastrointestinal resilience in pre-weaning calves under environmental stress.

A positive effect of trace mineral supplementation was observed on total plasma mineral levels in dairy calves, highlighting a synergistic effect on Zn bioavailability. The highest mean blood Zn concentration was detected in calves supplemented with Zn-Met and the organic mineral premix. Consistently, Nair et al. [32] reported that supplementing Zn, Cu, Mn, and Cr increased serum concentrations of these minerals in male calves compared to the control group. Cazarotto et al. [39] found that selenium and Zn levels were higher in the serum of dairy lambs fed a supplemented diet. Xin et al. [40] suggested that plasma Cu concentrations could be influenced by disease status, dietary Cu levels, and gestation. In Cu-deficient diets, Cu supplementation significantly increased plasma Cu, whereas no significant increase was observed in lambs fed Cu-adequate diets. Previous studies also reported that moderate Zn supplementation (20–150 mg/kg diet) in Zn-adequate rations had little or no effect on serum or plasma Zn levels in dairy calves [41,42]. However, high Zn concentrations (300–500 mg/kg) significantly increased serum or plasma Zn levels. Similarly, increasing dietary Mn levels up to 240 mg/kg had no effect on plasma Mn concentrations in steers [43]. The addition of Mn from 13 to 45 mg/kg of DM increased plasma Mn concentrations on day 52 but had no effect on day 28 or day 84 in male lambs [44].

In this study, fecal mineral excretion significantly increased across treatment groups, suggesting that the level and source of Zn supplementation influenced its absorption in livestock. For instance, VanValin et al. [45] reported that lambs receiving Zn-sulfate supplementation at 40 mg/kg exhibited increased urinary and fecal Zn excretion compared to the control group. The study evaluated the effects of experimental treatments on the average stool consistency score and the incidence of diarrhea in calves. The results indicated that the experimental treatments did not significantly impact stool consistency in calves ($p > 0.05$). However, the addition of organic minerals was found to enhance stool consistency maintenance and lower the incidence of diarrhea throughout the experimental period. Supporting these findings, various researchers have indicated that supplementation with chelated forms of trace minerals in suckling calves can improve stool consistency and decrease diarrhea occurrences [46,47]. Proper inclusion of minerals in calf diets plays a vital role in promoting intestinal health, preventing infections, and enhancing growth performance. Zinc is particularly important for cell proliferation, maintaining cellular integrity, and supporting immune function. Immunoglobulins such as IgG, IgM, and IgA are crucial for defending against pathogenic invasions. These antibodies specifically target antigens and neutralize microorganisms, viruses, and other foreign agents, thus reducing diarrhea incidence and promoting calf health [48]. Zinc-methionine supplementation has been demonstrated to strengthen the intestinal mucosal barrier and increase villus height. Furthermore, organic zinc supplementation has been shown to decrease the incidence of diarrhea in newborn Holstein calves by reducing intestinal permeability, making it a promising strategy for managing diarrhea during the early calf-rearing period [49]. Additional studies examining the effects of different zinc sources on growth performance, immune function, and hematological parameters in suckling calves have confirmed that organic zinc supplementation can effectively decrease the incidence of diarrhea and enhance growth efficiency [17]. Mineral supplementation positively influenced plasma antioxidant indices in dairy calves and enhanced resistance to oxidative stress, likely due to the role of Zn and Cu in SOD activity. Minerals are essential for inflammatory and immune responses, and help prevent performance impairments in both humans and animals [50]. Nair et al. [32] reported that trace mineral supplementation improved antioxidant status and immunity in male calves. Several studies have demonstrated that supplementing dairy calf diets with trace minerals enhances antioxidant responses while reducing health issues such as diarrhea [46,47]. In the present study, significant differences in TAC were observed among experimental groups, with the highest values detected in Zn-supplemented groups (Table 2). Mn, Zn, and Cu are key components of mitochondrial SOD, functioning as antioxidants in the body [51]. These minerals help reduce free radical levels and mitigate oxidative stress [52]. ROS are commonly produced under various pathological conditions, leading to cellular damage through protein oxidation and lipid peroxidation [53,54]. Excessive extracellular ROS impair antioxidant defenses and weaken immune responses. Trace minerals such as Zn, Mn, and Cu play a crucial role in maintaining optimal immune function and antioxidant activity [55,56]. Thus, supplementing organic or inorganic minerals in calf diets may enhance antioxidant defenses during infections. Studies by Glombowsky et al. [46] and Soldá et al. [57] reported that SOD, GPx, and TAC levels were higher in mineral-supplemented dairy calves than in the control group. These findings suggest that mineral supplementation stimulates the antioxidant defense system, as demonstrated in dairy cows supplemented with minerals during the transition period [57]. In this study, the increase in antioxidant enzyme activity may be attributed to the higher bioavailability of Cu, Zn, and Mn, which serve as cofactors for these enzymes [58,59]. Recent research has highlighted the protective effects of Zn [60] and Cu [61] against oxidative stress through their role in enhancing antioxidant defenses, consistent with the results of this trial.

This study underscores the potential of organic mineral supplementation to improve the bioavailability of essential trace minerals, enhance antioxidant responses, and support the overall health of dairy calves during early development. Future research should investigate the long-term effects of these supplements on productivity, reproductive performance, and disease resilience, as well as their applicability across different livestock systems under varying management and environmental conditions. To maximize the impact of these findings, digital platforms could be leveraged to disseminate knowledge effectively [62]. Engaging formats such as visual content, educational materials, and collaborative discussions could help reach farmers, nutritionists, and other stakeholders, promoting evidence-based strategies for improved livestock health and management practices.

5. Conclusions

Supplementation with organic forms of Mn, Cu, and Zn (Mn-methionine, Cu-methionine, and Zn-methionine) increased plasma concentrations of these minerals in dairy calves over a short study period (21 days), likely due to their higher bioavailability and absorption. Additionally, Zn-Met supplementation significantly enhanced serum antioxidant indices (TAC, SOD), thereby improving the calves' resistance to oxidative stress. These findings suggest that Cu-Met supplementation, in particular, represents a valuable nutritional strategy to optimize mineral bioavailability and support the health and development of dairy calves during the early postnatal period.

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Abbreviations

The following abbreviations are used in this manuscript:

Cu-Met	Copper-methionine
Zn-Met	Zinc-methionine
Mn-Met	Manganese-methionine
ROS	Reactive oxygen species
SOD	Superoxide dismutase
AI	Artificial insemination
CTR	Control group
CP	Crude protein
DM	Dry matter
EE	Ether extract
ADF	Acid detergent fiber
NDF	Neutral detergent fiber
TAC	Total antioxidant capacity
GPx	Glutathione peroxidase

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