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Effects of different copper supplement sources on growth performance, nutrient digestibility, and blood parameters in Holstein suckling calves

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Abstract To evaluate the effects of copper (Cu) supplement source on Holstein suckling calves, 40 newborn male calves (mean birth weight: 39.6±2.4 kg) were randomly assigned to a completely randomized design for 70 days. The four experimental treatments consisted of the basal diet (7.89 mg Cu/kg dry matter) supplemented with 10 mg Cu/kg dry matter from different copper sources: Cu-acetate (27% purity), Cu-chloride (36% purity), Cu-sulfate (23% purity), and Cu-tribasic (58% purity). Calf body weight (BW) was measured weekly, while starter intake was recorded daily. Apparent digestibility was assessed using acid-insoluble ash as an internal marker. Blood samples were drawn from the jugular vein at the end of the trial. Final BW, total weight gain, and average daily gain were significantly greater in calves fed Cu-acetate and Cu-sulfate compared to those receiving Cu-chloride and Cu-tribasic ($P<0.01$). Dry matter digestibility was significantly enhanced in calves fed Cu-acetate compared to the other experimental treatments ($P<0.01$). Apparent digestibility of ether extract ($P<0.01$) and acid detergent fiber ($P<0.05$) was greater in calves fed Cu-acetate than in those fed Cu-chloride or Cu-tribasic. Plasma globulin concentration was significantly greater in calves fed Cu-tribasic compared to those receiving Cu-acetate and Cu-chloride ($P<0.05$). Plasma cholesterol levels were significantly higher in the Cu-sulfate group than in the other treatments ($P<0.05$). The concentration of low-density lipoprotein was significantly lower in the Cu-acetate group compared to other treatments ($P<0.05$). Plasma zinc levels were significantly increased in calves receiving Cu-chloride compared to other groups ($P<0.01$). Plasma aspartate aminotransferase activity was significantly greater in calves fed Cu-acetate compared to those receiving Cu-chloride or Cu-tribasic ($P<0.01$). It was concluded that adding 10 mg Cu/kg dry matter to the basal diet through Cu-acetate and Cu-sulfate supplements had a more favorable effect on the growth performance in Holstein suckling calves compared to Cu-chloride and Cu-tribasic salts.

Keywords: bioavailability, calf, copper acetate, liver enzymes, mineral antagonism

Introduction

Optimal nutritional management of suckling calves is essential for successful dairy cattle production, with trace mineral supplementation, particularly copper (Cu), playing a vital role in calf growth and health (Fulton et al., 2023). Cu is a key component of numerous metalloenzymes involved in metabolic pathways, hematopoiesis, immune response, and oxidative defense mechanisms

(Yang et al., 2011). There is a significant positive correlation between superoxide dismutase activity and blood Cu concentration in calves (Safonov et al., 2021). Supplementation with Cu has been shown to enhance antioxidant status and immune function, which can lead to improved health and increased average daily gain (Mattioli et al., 2019; Pandey et al., 2023). However, Cu absorption and bioavailability are influenced by dietary antagonists such as molybdenum, sulfur, and iron, which may necessitate higher Cu supplementation depending on the diet composition (Spears et al., 2022). Thus, the chemical form of Cu used in supplementation plays a pivotal role in optimizing its absorption, promoting growth, and minimizing oxidative stress (Kegley et al., 2016). In commercial livestock diets, Cu sulfate is commonly used as the primary source of Cu; however, its bioavailability is relatively low (Kushwaha et al., 2021). In contrast, tribasic Cu chloride, an inorganic Cu source with low water solubility, is more stable in the rumen environment and potentially more bioavailable due to improved solubility in the acidic abomasum (Cheng et al., 2011). Organic sources like Cu acetate may offer superior ruminal bypass and enhanced intestinal absorption due to reduced reactivity with dietary antagonists (Byrne and Murphy, 2022). When the diet contains a normal concentration of Cu, the Cu content of milk is approximately 0.04 mg/kg (Faulkner et al., 2017). As noted by Suttle (2010), a dietary Cu level of 12–36 mg/kg dry matter (DM) may lead to chronic Cu poisoning. Therefore, in the present study, we selected a safe and physiologically relevant supplementation level of 10 mg Cu/kg DM from different Cu sources. Studies on ruminant livestock have reported varying effects of different Cu supplement sources on growth performance, ranging from no impact (Vaswani et al., 2018; Khatami Khalkhoran et al., 2024) to significant improvements (Vahedi et al., 2022; Hoseinpour et al., 2014). Additionally, inconsistent results have been observed regarding the influence of various Cu sources on nutrient digestibility (Kushwaha et al., 2021; Hoseinpour et al., 2014), blood metabolite profiles, Cu concentrations, and interactions with other minerals (Khatami Khalkhoran et al., 2024; Garrine et al., 2019; Hozhabri et al., 2018). Therefore, this study aimed to compare the effects of different Cu supplement sources on growth performance, feed nutrient digestibility, and certain blood parameters in Holstein suckling calves.

Materials and methods

Animals and experimental design

The experiment was conducted from February to April 2024 at Mahdasht Dairy Farm Company in Sari, Mazandaran, Iran. This research involved 40 newborn male Holstein calves (mean birth weight: 39.6±2.4 kg) born to multiparous cows, evaluated from 4 days of age until weaning (70 days), using a completely randomized design. The four experimental treatments consisted of the basal diet (7.89 mg Cu/kg dry matter) supplemented with 10 mg Cu/kg dry matter from different copper sources: Cu-acetate (27% purity), Cu-chloride (36% purity), Cu-sulfate (23% purity), and Cu-tribasic (58% purity). The Cu supplements used in this study were obtained from Merka Company (Sari, Iran) and possessed to specified purity levels. The starter diets containing the Cu supplements were prepared daily, ensuring precise and homogeneous incorporation of the Cu sources into the feed. Before housing the calves, individual pens were thoroughly washed, disinfected, and flame-sterilized. The starter diets were formulated to the NRC (2001) requirements. The chemical composition of feed samples, including DM, crude protein (CP), ether extract (EE), ash, and organic matter (OM), was determined using the AOAC (2005) method. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed according to Van Soest et al. (1991). The Cu concentration in the basal diet was measured using an atomic absorption spectrophotometer (Model FX210, Rayleigh, China). The ingredients and chemical composition of the diets are presented in Table 1.

Table 1. Ingredients and chemical compounds of experimental diet (% DM)

Ingredients and chemical composition	Amount[1]
Alfalfa hay, 15 CP	5.57
Wheat bran	1.00
Barley grain	13.22
Corn grain, ground	28.36
Soybean meal 44%	31.57
Soybean whole, roast	9.22
Canola meal solvent	4.76
Sodium bicarbonate	1.53
Magnesium oxide	0.51
Mineral and vitamin permix[2]	2.00
Calcium carbonate	0.76
Di-calcium phosphate	0.50
Salt, white	0.50
Mycotoxin binder	0.50
Dry matter	90.69
Organic matter	93.45
Crude protein	21.69
Ether extract	3.88
Ash	6.55
Neutral detergent fiber	17.15
Acid detergent fiber	10.09
Non-fiber carbohydrate[3]	47.90
Methionine	1.62
Lysine	6.06
Calcium	0.50
Phosphorus	0.47
Metabolizable energy (Mcal/kg)	2.62
Copper (mg/kg DM)	7.89

[1]Percent %), unless otherwise indicated.
[2]Mineral supplements: Calcium 195,000 mg; Phosphorus 90,000 mg; Magnesium 90,000 mg ؛Sodium 55000 mg; Zinc 3000 mg Iron 300 mg; Manganese 2000 mg; Cobalt 100 mg; Selenium 1 mg; Antioxidant 400 mg. Vitamin supplement: Vitamin A, 500,000 IU/ kg; Vitamin E, 100 mg/ kg; Vitamin D3 100,000 IU/ kg.
[3]NFC = 100 − (%NDF + %Ash + %Fat + %CP) (NRC, 2001).

Measurements and sample collection

After birth, the calves were separated from their mothers and individually weighed before being moved to separate concrete pens (2×1 m) within the calf-rearing

facility. Each calf received four liters of high-quality colostrum, administered in two feedings within the first six hours after birth. During the experimental period, calves were fed a milk replacer equivalent to approximately 10% of their body weight (BW), divided into two equal meals at 08:00 and 17:00 daily. Calves had *ad libitum* access to starter feed and clean drinking water. To determine the daily feed intake, the amount of refused starter feed was collected and recorded every morning before feeding. Body weights were recorded weekly after a 12- to 14-hour period of feed and water deprivation to minimize the effects of gut fill on weight measurements. A digital scale (Model SV7000, Iran) was used for all weight measurements.

Total mixed ration (TMR) samples were collected weekly and stored at –20°C until further analysis. Dry matter content was determined by drying samples in a forced-air oven at 60°C for 48 h. Weekly dried diet samples were ground using a 1-mm screen and analyzed for composition. Fecal grab samples were collected biweekly after morning and afternoon feedings and stored at –20°C until analysis. These samples were dried in a forced-air oven at 60°C for 72 h, ground to pass through a 1-mm screen, and analyzed for ash, ADF, NDF, CP, and EE, following the same procedures used for feed and orts. Apparent total-tract digestibility of nutrients was determined using the acid-insoluble ash (AIA) internal marker method, as described by Van Keulen and Young (1977).

Blood samples were collected from the jugular vein into evacuated tubes containing the anticoagulant EDTA at the end of the experiment. The samples were centrifuged at 2,000 × g for 20 minutes at 4°C to separate the plasma. Each sample was then divided into three aliquots and frozen at –20°C until further analysis. Blood metabolite concentrations were determined using commercially available kits (Pars Azmoon Company, Tehran) according to the manufacturer's instructions. Plasma concentrations of glucose, total cholesterol, total triglycerides, low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), total protein, albumin, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine aminotransferase (ALT), creatine phosphokinase (CPK), and alkaline phosphatase (ALP) were determined automatically using standard test kits (Pars Azmoon Co., Tehran, Iran) on an ALCYON 300i automatic analyzer (Cobas Integra 400 plus, Roche, Germany). The analyzer was calibrated with control sera (N and P; TrueLab N® and TrueLab P®, respectively; Pars Azmoon Co., Tehran, Iran) and a calibrator solution (TrueCal U®, Pars Azmoon Co., Tehran, Iran) to ensure optimal assay performance. The globulin concentration was calculated by subtracting albumin from total protein. Plasma concentrations of Cu, iron (Fe), and zinc (Zn) were measured using commercial kits from Dialab (Austria) and a photometric method with an atomic absorption spectrophotometer (SpectrAA220 FS, Varian, Australia).

Statistical analyses

The experiment was conducted using 40 suckling calves, assigned to four treatment groups with 10 replicates each, in a completely randomized design. The effect of the treatment was analyzed using analysis of variance (ANOVA) with the general linear model (GLM) procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The means were compared using the Duncan's multiple range test. The significance level was set at 95% probability.

Results and discussion

Growth performance

The effects of treatments on growth performance in Holstein suckling calves are summarized in Table 2. Calves supplemented with Cu-acetate and Cu-sulfate exhibited significantly higher final BW compared to those receiving Cu-chloride and Cu-tribasic (P=0.0006). Total weight gain also differed significantly among the treatment groups (P=0.0002), with the Cu-acetate (38.20 kg) and Cu-sulfate (37.32 kg) groups demonstrating greater gains than the Cu-chloride (33.97 kg) and Cu-tribasic (34.57 kg) groups. Accordingly, average daily gain (ADG) was significantly improved in calves receiving Cu-acetate and Cu-sulfate (P=0.0002), with the highest ADG observed in the Cu-acetate group (636 g/d) and the lowest in the Cu-chloride group (566 g/d). The superior performance observed in the Cu-acetate and Cu-sulfate groups is likely attributable to their higher solubility and greater intestinal absorption. Organic and sulfate forms of Cu are known to possess greater bioavailability than oxide or chloride forms, a finding consistent with previous research in ruminants (Spears, 2003; López-Alonso, 2012). The enhanced ADG, particularly in the Cu-acetate group, may reflect more efficient Cu transport and cellular utilization, potentially influencing the key metabolic pathways related to energy metabolism and protein synthesis (Byrne and Murphy, 2022). In contrast, DM intake and feed conversion ratio (FCR) were not significantly affected by the Cu sources (P>0.05). Nevertheless, numerically lower (i.e., improved) FCR values were observed in the Cu-acetate and Cu-sulfate groups compared to those receiving Cu-chloride and Cu-tribasic. Previous studies have also indicated that more bioavailable Cu sources can indirectly improve FCR by enhancing rumen microbial activity, increasing fiber digestibility, and promoting more efficient nutrient utilization (Solaiman et al., 2007; Zhang et al., 2009). Furthermore, Pandey et al. (2023) emphasized Cu's essential role in regulating intestinal transit, preserving mucosal integrity, enhancing immune responses, and modulating oxidative stress, all of which contribute to improved growth performance in calves. Consistent with the results of this study, Hoseinpour et al. (2014) demonstrated that Cu supplementation from both Cu sulfate and Cu proteinate enhanced ADG and feed conversion efficiency (FCE) in fattening Zandi lambs, without significantly affecting daily feed intake (DFI). Similarly, Fagari-Nobijari et al. (2013) reported that dietary supplementation with 30 mg of Cu sulfate

($CuSO_4$) improved ADG and reduced the incidence of lameness in fattening calves. In contrast, Garrine et al. (2019) and Khatami Khalkhoran et al. (2024) found that neither the organic nor inorganic Cu supplementation significantly influenced the growth performance in male lambs. Likewise, Vaswani et al. (2018) observed no significant differences in ADG among growing heifers supplemented with 8.0 mg Cu/kg DM, regardless of the Cu source (Cu proteinate, Cu propionate, or Cu sulfate). Kushwaha et al. (2021) also reported no significant improvement in growth performance in growing Sahiwal heifers supplemented with 10 ppm Cu sulfate or with 5.0 and 10.0 ppm nano-Cu. Variations in growth performance observed across different studies are likely influenced by differences in diet composition, the source and concentration of Cu in the basal diet, and the presence of Cu antagonists.

Table 2. Effects of dietray Cu supplements on growth performance in calves

Item	Experimental treatments				SEM[1]	*P*-value
	Cu-acetate	Cu-chloride	Cu-sulfate	Cu-tribasic		
Initial body weight (kg)	39.37	39.41	39.95	40.03	0.661	0.8408
Final body weight (kg)	77.57[a]	73.38[b]	77.27[a]	74.60[b]	0.685	0.0006
Total weight gain (kg)	38.20[a]	33.97[b]	37.32[a]	34.57[b]	0.627	0.0002
Average daily gain (g/d)	636[a]	566[b]	622[a]	576[b]	0.010	0.0002
Dry matter intake (kg/d)	1.405	1.309	1.364	1.317	0.030	0.1156
Feed conversion ratio	2.20	2.31	2.19	2.28	0.063	0.4737

a, b: Within row, means with common superscript (s) are not different (P>0.05).
[1]SEM: Standard error of the mean.

Apparent digestibility

The effects of Cu source on nutrient digestibility are summarized in Table 3. Dry matter digestibility was significantly higher in calves receiving Cu-acetate compared to those in the other treatment groups (P=0.0054). Among the treatments, the Cu-acetate group exhibited the highest DM digestibility (85.25%), while the Cu-sulfate group showed the lowest (82.48%). Previous studies suggested that the bioavailability and solubility of Cu salts play a critical role in modulating the ruminal fermentation and nutrient absorption (Spears and Kegley, 2002). The observed improvements in DM digestibility in the Cu-acetate group may be attributed to Cu enhancing the ruminal fermentation (Engle and Spears, 2000) or stimulating the growth of ruminal microorganisms (Zhang et al., 2008). Ether extract digestibility was also significantly enhanced in the Cu-acetate group compared to calves supplemented with Cu-chloride and Cu-tribasic (P=0.0079). Furthermore, ADF digestibility was significantly greater in calves fed Cu-acetate than in those receiving Cu-chloride or Cu-tribasic (P=0.0427). Notably, ADF digestibility in the Cu-sulfate group was significantly higher than in the Cu-tribasic group. The Cu-acetate group recorded the highest ADF digestibility value (44.85%), whereas the lowest value was observed in the Cu-tribasic group (40.92%). The enhanced fiber digestion in the Cu-acetate group indicates more favorable ruminal conditions for cellulolytic activity. Previous research demonstrated that Cu supplementation, particularly from more bioavailable sources, can stimulate the growth of fiber-degrading microbial communities, such as *Ruminococcus spp.* and *Fibrobacter succinogenes* (Zhang et al., 2007). In contrast, no significant differences were detected among treatment groups in terms of CP, NDF, or non-fiber carbohydrate (NFC) digestibility (P>0.05). Similar to our results, Hoseinpour et al. (2014) reported improved digestibility of DM, OM, CP, and NDF in Zandi fattening lambs supplemented with organic Cu compared to those receiving inorganic sources. Similarly, Dezfoulian et al. (2012) observed that supplementation with Cu proteinate and Cu sulfate at 10 and 20 mg/kg DM enhanced DM and CP digestibility in fattening lambs. Mondal and Biswas (2007) also reported improved EE and crude fiber digestibility in Black Bengal kids supplemented with Cu proteinate. In contrast, Vaswani et al. (2018) found no significant changes in nutrient digestibility in Hariana heifers supplemented with Cu sulfate or Cu propionate. Likewise, Kushwaha et al. (2021) and Waghmare et al. (2014) found no improvement in the digestibility of DM, OM, EE, and crude fiber in Sahiwal heifers and goat kids supplemented with inorganic or organic sources. Additionally, Zhang et al. (2008) emphasized that interactions with other dietary minerals and baseline Cu intake could alter the physiological response to supplementation.

Blood parameters

The results pertaining to the effects of dietary Cu sources on blood parameters are presented in Table 4. Plasma globulin concentration was significantly higher (P=0.0353) in calves supplemented with Cu-tribasic compared to those receiving Cu-acetate and Cu-chloride. Consequently, the albumin-to-globulin ratio was significantly lower in the Cu-tribasic group than in the other treatment groups (P=0.0435). The elevated plasma globulin concentration observed in calves supplemented with Cu-tribasic suggests an enhancement in humoral immune responses or a shift in hepatic protein synthesis. Globulins (particularly gamma globulins) are closely associated with immune function, and elevated levels may indicate increased immunocompetence or inflammatory activity (López-Alonso, 2020). Plasma cholesterol concentration differed significantly among treatments (P=0.0398), with the highest value observed in calves supplemented with Cu-sulfate (140.58 mg/dL), and the lowest in those receiving

Cu-acetate (128.83 mg/dL). Plasma LDL levels also showed significant variation across treatments (P=0.0122), with calves in the Cu-acetate group exhibiting significantly lower LDL concentrations (75.25 mg/dL) compared to the other groups, while the highest LDL concentration was recorded in the Cu-sulfate group (87.89 mg/dL). Cu sulfate, known for its high solubility and bioavailability, may elicit a more pronounced hepatic response, thereby influencing lipoprotein synthesis and secretion (Hurlbert, 2023). Conversely, the significantly LDL concentration observed in the Cu-acetate group may have important implications for oxidative stress and cardiovascular risk in animals, as LDL serves as a primary cholesterol carrier and is particularly susceptible to oxidative modification (Correa et al., 2012). However, the experimental treatments had no significant effect on plasma concentrations of glucose, total protein, albumin, urea nitrogen, triglycerides, HDL, or VLDL (P>0.05). Collectively, these findings underscore that the bioavailability and physiological effects of Cu are strongly influenced by its chemical form. Similar to our results, Asli et al. (2020) highlighted the Cu's central role in glucose metabolism by facilitating enzyme activity in glycolysis and glucose uptake, aligning with the observed glucose stability across treatments in our study. Hoseinpour et al. (2014) also found that organic Cu supplementation reduced BUN and affected lipid profiles, consistent with our observations of form-dependent variations in cholesterol and LDL levels. In contrast, Garrine et al. (2019) reported that supplementing the fattening lamb diet with 10 and 30 mg Cu/kg DM as Cu-sulfate or Cu-methionine had no significant effect on cholesterol, HDL, LDL, or triglyceride levels. Khatami Khalkhoran et al. (2024) found a significant increase in BUN after Cu-sulfate and Cu-nano-oxide supplementation without a significant effect on glucose, cholesterol, triglycerides, partially contradicting the lipid-related outcomes in our study. Cheraghi Mashoof et al. (2018) reported no significant effects of Cu and Zn sulfate supplementation on blood parameters in ewes and lambs throughout the experiment.

Table 3. Effects of dietary Cu supplements on nutrient digestibility (%)

Digestibility	Experimental treatments				SEM[1]	*P*-value
	Cu-acetate	Cu-chloride	Cu-sulfate	Cu-tribasic		
Dry matter	85.25[a]	82.75[b]	82.48[b]	83.40[b]	0.408	0.0054
Ether extract	74.80[a]	71.03[c]	73.10[ab]	71.68[bc]	0.582	0.0079
Crude protein	75.20	73.64	74.13	73.48	0.978	0.6167
Neutral detergent fiber	65.91	63.11	65.56	64.02	1.115	0.3142
Acid detergent fiber	44.85[a]	41.48[bc]	44.29[ab]	40.92[c]	0.944	0.0427
Nonfiber carbohydrate	97.84	96.69	97.67	98.08	0.502	0.2938

a, b: Within row, means with common superscript (s) are not different (P>0.05).
[1]SEM: Standard error of the mean.

Table 4. Effects of dietary Cu supplements on blood parameters in calves

Item	Experimental treatments				SEM[1]	*P*-value
	Cu-acetate	Cu-chloride	Cu-sulfate	Cu-tribasic		
Glucose (mg/dL)	82.50	85.50	86.16	83.83	2.681	0.9605
Urea nitrogen (mg/dL)	24.16	21.50	26.83	21.91	0.615	0.0530
Total protein (g/dL)	5.93	5.80	6.03	5.76	0.091	0.2211
Albumin (g/dL)	3.66	3.53	3.66	3.30	0.095	0.0804
Globulin (g/dL)	2.26[b]	2.26[b]	2.36[ab]	2.46[a]	0.044	0.0353
Albumin/globulin	1.61[a]	1.56[a]	1.54[a]	1.33[b]	0.058	0.0435
Cholesterol (mg/dL)	128.83[b]	131.75[b]	140.58[a]	132.50[b]	1.188	0.0398
Triglyceride (mg/dL)	29.50	23.58	25.83	26.75	1.043	0.3183
HDL (mg/dL)	47.68	45.05	47.52	42.58	1.109	0.3782
LDL (mg/dL)	75.25[b]	81.97[a]	87.89[a]	84.56[a]	1.007	0.0122
VLDL (mg/dL)	5.90	4.71	5.16	5.35	0.417	0.3183

a, b: Within row, means with common superscript (s) are not different (P>0.05).
[1]SEM: Standard error of the mean.

Plasma minerals and liver enzymes

The effects of treatments on plasma mineral concentrations and hepatic enzyme activities in Holstein suckling calves are summarized in Table 5. Plasma Zn concentration was significantly higher (P=0.0055) in calves supplemented with Cu-chloride compared to other treatment groups. The highest plasma Zn level was observed in the Cu-chloride group (99.50 µg/dL), while the lowest was recorded in the Cu-acetate group (86.50 µg/dL). This finding is consistent with previous reports indicating the potential antagonistic interactions between Cu and Zn at the level of intestinal absorption (Spears, 2003; Hozhabri et al., 2018). However, the elevated Zn concentration observed in the Cu-chloride group may suggest that this source exerts a lower degree of competitive inhibition on Zn absorption, or possibly enhances Zn transporter expression. Similar results were reported by Zhang et al. (2020), who demonstrated that Cu source can affect the trace mineral status by modulating the expression of transporter genes and metallothionein activity. Plasma concentrations of Cu and Fe were not significantly affected by the treatments (P>0.05); however, numerically higher plasma Cu levels were noted in calves receiving Cu-acetate and Cu-sulfate compared to those supplemented with Cu-

chloride and Cu-tribasic. A significant difference was observed in plasma AST activity among the treatment groups (P=0.0064). Calves fed with Cu-acetate exhibited higher AST activity (92.16 U/L) than those receiving Cu-chloride (76.50 U/L) and Cu-tribasic (84.25 U/L). The increased AST levels observed in calves receiving Cu-acetate may indicate a mild hepatocellular response or oxidative stress, likely induced by the high solubility and rapid absorption of this Cu source (Lin et al., 2020). Plasma activities of ALT, CPK, and ALP were not significantly influenced by the dietary treatments (P>0.05). AST and ALT are commonly used as biochemical markers of hepatic injury. Cu contributes to the prevention of excessive lipid peroxidation in cell membranes by enhancing the antioxidant defense system and immune response, which may in turn reduce the release of AST and ALT into the bloodstream (Zhang et al., 2000). In agreement with our findings, Vaswani et al. (2018) reported that Cu supplementation at a level of 8 mg/kg DM, in the form of sulfate or propionate, significantly increased plasma Cu concentrations without affecting plasma AST, ALT, Fe, or Zn levels. Similarly, Dezfoulian et al. (2012) observed that Cu-proteinate and Cu-sulfate supplementation at 10 and 20 mg Cu/kg DM elevated plasma Cu concentrations and demonstrated a significant inverse relationship between plasma Cu and Fe levels. Hozhabri et al. (2018) found that supplementing the male Sanjabi lambs with 15 mg Cu/kg DM as Cu-lysine significantly increased plasma Cu concentrations compared to nano-Cu and control groups. Garrine et al. (2019) reported that Cu supplementation at levels ranging from 10 to 30 mg/kg DM, either as Cu sulfate or Cu methionine, did not significantly affect serum or hepatic Cu concentrations in lambs.

Table 5. Effects of dietary Cu supplements on plasma mineral and liver enzymes in calves

Item	Experimental treatments				SEM[1]	*P*-value
	Cu-acetate	Cu-chloride	Cu-sulfate	Cu-tribasic		
Minerals (µg/dL)						
Copper	136.50	129.92	134.42	130.83	1.158	0.2311
Iron	150.58	161.08	152.17	152.50	1.674	0.1921
Zinc	86.50^{b}	99.50^{a}	87.83^{b}	89.91^{b}	0.963	0.0055
Liver enzymes (U/L)						
Aspartate aminotransferase	92.16^{a}	76.50^{c}	86.83^{ab}	84.25^{b}	1.095	0.0064
Alanine aminotransferase	22.62	21.93	23.12	21.87	1.098	0.9595
Creatine phosphokinase	116.25	113.25	117.08	119.50	1.916	0.7223
Alkaline phosphatase	317.25	325.83	335.50	335.00	7.616	0.8089

a, b: Within row, means with common superscript (s) are not different (P>0.05).
[1]SEM: Standard error of the mean.

Conclusions

Supplementing the basal diet (containing 7.89 mg Cu/kg DM) with an additional 10 mg Cu/kg DM from Cu-acetate or Cu-sulfate significantly improved the ADG and nutrient digestibility in Holstein suckling calves, offering practical benefits for dairy production systems. Notably, Cu-acetate supplementation enhanced DM digestibility, as well as the apparent digestibility of EE and ADF, reflecting improved feed utilization and digestive efficiency. Moreover, the reduced concentration of LDL observed in calves receiving Cu-acetate suggested a favorable impact on lipid metabolism and overall metabolic health. These findings highlighted the critical role of Cu source selection in optimizing the calf growth performance and health, with Cu-acetate emerging as the most effective option in this context. Further research is warranted to evaluate the long-term outcomes and to elucidate the underlying mechanisms by which different Cu sources affect the physiological and metabolic functions in calves.

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Ethical approval

The animals were used solely for zootechnical evaluation purposes. Blood analyses were conducted as part of routine clinical examinations by the staff of the university veterinary hospital.

Disclosure statement

The authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence or bias the content of this manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [R], upon reasonable request.

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