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The effect of milk enrichment with vitamin C and copper on performance, skeletal growth index, digestibility, blood biochemical attributes and liver enzyme activity in suckling calves

Abdolhakim Toghdory[1,*]*, Taghi Ghoorchi*[1]*, Mohammad Asadi*[1]*, Ayda Teymouri*[1]*, Mohammad Eteraf*[1]*, Kerim Yaziyev*[2]

[1]*Department of Animal and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran*
[2]*Faculty of Veterinary, Turkmen Agricultural University named after S.A.Niyazov*

*Corresponding author,
E-mail address:
toghdory@yahoo.com

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ORCID
Abdolhakim Toghdory
0000-0002-5198-1940
Taghi Ghoorchi
0000-0002-6869-2932
Mohammad Asadi
0000-0001-8199-3953
Ayda Teymouri
0009-0004-9508-0695
Mohammad Eteraf
0009-0007-2335-4636
Kerim Yaziyev
0009-0002-8363-7594

Abstract To investigate the effect of milk enrichment with vitamin C and copper on dairy calves, 20 newborn calves were used in a completely randomized factorial design with 4 treatments of 5 replication each. Experimental treatments included: 1) control group (without copper and vitamin C supplementation), 2) copper added to milk, 3) vitamin C added to milk and 4) supplementation of milk with copper and vitamin C. Vitamin C and/or copper supplements for each treatment were completely dissolved in milk before feeding. The group receiving vitamin C and copper simultaneously (P<0.05) recorded the best performance in weight on day 63, daily weight gain on days 1-21 and 1-63, and feed intake on days 22-42, 43-63, and 1-63. The highest levels of glucose, triglycerides, LDL, and VLDL, and the lowest level of HDL were observed in the group receiving vitamin C and copper simultaneously (P<0.05). The lowest cortisol and highest insulin levels were observed in the group receiving vitamin C and copper simultaneously (P<0.05). The enrichment of milk with vitamin C, copper, and the combination of vitamin C and copper had no effect on the withers height, hip height, hip width, body length, heart girth and body barrel of the calves. Also, no significant differences were observed between the treatments containing copper and vitamin C and the control group regarding the digestibility of dry matter, organic matter, ether extract, crude protein, neutral detergent fiber, and acid detergent fiber. In general, it is concluded that the simultaneous enrichment of milk with vitamin C and copper during the first two months of calves' lives enhanced growth performance, reduced steress and bolsterd their immune system.

Keywords: antioxidant, immune response, newborn calve, trace element, vitamin

Introduction

Successful rearing of calf and heifer is crucial for the sustainability of dairy cattle breeding (Moran, 2002). Proper nutrition during the neonatal and early developmental stages of calves is crucial for their subsequent growth, development, and overall performance (Drackley, 2008). The initial liquid nourishment for a calf is milk, which serves as a source of essential proteins but lacks sufficient minerals (Sahinduran and Albay, 2004). Furthermore, a newborn calf possesses an underdeveloped immune system and experiences stress due to the transition from the intrauterine to the external environment (Cortese, 2009). Calves that are fed only with milk may be susceptible to disease and oxidative stress, as minerals and vitamins are crucial for the optimal functioning of the immune system (Suttle, 2010). Among the therapeutic minerals and vitamins, vitamin C and copper are particularly noteworthy.

Ascorbic acid, is a criticial antioxidant for the proper functioning of cells; This vitamin accumulates significantly in endocrine tissues and cells, such as leukocytes, and acts as a free radical scavenger (Berean et al., 2024). Vitamin C indirectly enhances the immune response by maintaining vitamin E levels and protects cytosolic substances from oxidative damage (Comb, 1992). Furthermore, vitamin C contributes to adrenal hormone synthesis and attenuates stress responses by modulating corticosteroid release (Calsamiglia et al., 2024). Vitamin C deficiency in suckling calves can lead to diarrhea and pneumonia. Additionally, calves that receive vitamin C supplements exhibit enhanced immune resistance to infections (Dogan, 2023). Copper is a trace element that is essential for various biological processes, including the production of hemoglobin (Tapiero et al., 2003). Copper serves as a essential component of the enzymes cytochrome oxidase and superoxide dismutase (Suttle, 2010). Deficiency of copper in neutrophils leads to an increase in the activity of superoxide and hydrogen peroxide radicals, thereby exposing the animal to oxidative stress (Muehlenbein et al., 2001). Furthermore, copper's role in hematopoiesis is mediated through ceruloplasmin, which exhibits anti-inflammatory properties and mitigates the effects of free radicals (Suttle, 2010).

Calves cannot synthesize vitamin C until 3 months of age, and milk provides only 0.15 mg/kg of copper, far below the 2–4 mg/kg dry matter required for suckling calves (Comb, 1992). To address these deficiencies and explore the potential antioxidant synergy of vitamin C and copper, this study was conducted to investigate the effect of enriching milk with vitamin C and copper on performance, skeletal growth index, digestibility, blood biochemical attributes and liver enzyme activity in suckling calves.

Material and methods

Experimental treatments

This experiment was conducted in 2025 at Gorgan Agricultural Sciences and Natural Resources University, Gorgan, Golestan province, Iran in accordance with the guidelines for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010). Twenty newborn calves were randomly divided into 4 groups with 5 replications. Experimental treatments included: 1) control group (without copper and vitamin C supplementation), 2) copper added to milk, 3) vitamin C added to milk and 4) milk supplemented with both copper and vitamin C. The duration of the experiment was 63 days. Calves were separated from their dam at 24 h after birth, the navels were disinfected with tincture solution, weighed, and transferred to individual boxes. Then, 4 liters of colostrum were fed on two occasions during the first 8 hours of life. Colostrum feeding was continued for another 2 days based on 10% of body weight (BW). The calves were fed milk twice a day (8:00 A.M. and 4:00 P.M.). Before feeding milk, vitamin C and copper supplements for each treatment were completely dissolved in milk and fed to the calves. The starter diet was adjusted based on NRC (2001; Table 1) recommendations.

Table 1. Ingredients and chemical composition of the starter diet (in DM)

Ingredients	%	Chemical composition	Amount
Alfalfa	10.00	Dry matter (%)	87.31
Soybean meal	30.60	Metabolizable energy (kcal)	2.62
Corn grain	32.90	Crude protein (%)	21.69
Barley grain	18.00	Neutral detergent fiber (%)	27.15
Full-fat soybean	2.70	Acid detergent fiber (%)	20.09
Mineral-vitamin premix[1]	2.20	Ether extract (%)	0.94
Sodium bicarbonate	1.30	Ash (%)	6.55
Magnesium oxide	0.40	Calcium (%)	0.81
Calcium carbonate	1.40	Phosphorus (%)	51.0
Toxin binder	0.50		

[1]Vitamin-trace mineral pre-mix provides per kg of mixed ration: vitamin A: 1000000 IU; vitamin D3: 75000 IU; Antioxidants: 3000 (mg); Ca: 150000 (mg); P: 60000 (mg); Mg: 300009 (mg); Mn: 2000 (mg); Fe: 3000 (mg); Cu: 500 (mg); Zn: 2500 (mg); Co: 10 (mg) and I: 20 (mg).

Performance

To investigate the performance factors and assess daily and cumulative weight changes, calves were weighed at 21-day intervals. Starter feed was fed on daily basis, and the leftover feed was weighed daily. Individual feed consumption was calculated by determining the difference between the amount of feed provided and the orts. Additionally, any increase in the amount of feed given to the animals was based on the leftover feed observed on the subsequent day. Specifically, if an animal left less than 10% of its feed for three consecutive days, the amount of feed provided to that animal was increased. For weight change calculations, animals were weighed weekly after a fasting period of 16 hours using a digital scale. The average daily weight gain (DWG) was computed by dividing the total weight gain during the experimental period by the number of days within that same period. Furthermore, the feed conversion ratio (FCR) was calculated by dividing the average DM intake for each treatment by the average DWG corresponding to that treatment throughout the experimental duration.

Skeletal indices, including the withers height, hip height, hip width, body length, heart girth and body barrel, were measured every 21 days using a measuring tape and caliper, following the methodology outlined by Khan et al. (2007).

Digestibility

Throughout the experiment, samples of the feed provided for each group were collected weekly. The remaining feed for each animal within each treatment group was stored daily and also sampled weekly. From the accumulated samples of both the provided and remaining feed over the entire duration, a 100-gram sample for each treatment was stored in a sealed container. To evaluate the apparent digestibility of DM, organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent insoluble fiber (NDF), and acid detergent fiber (ADF), the acid-insoluble ash (AIA) method was utilized as an internal marker. For this purpose, fecal samples were collected from the rectum of the calves during the final six days of the experiment using artificial insemination gloves. These samples were then combined, and a 100-gram sample from each calf was taken and dried in an oven at 64°C for 48 hours. The dried samples were ground using a mill equipped with a 1-mm sieve. The chemical composition of both the feed and feces was analyzed. The apparent digestibility of DM, OM, CP, and EE was determined according to AOAC (2000) methods, while NDF and ADF were assessed as described by Van Soest (1994).

Blood parameters

Blood samples were collected from the jugular vein on the final day of the experiment using vacuum tubes without anticoagulant. Serum was separated by centrifugation at 1000 × g for 15 minutes at 4°C and stored at -80°C for subsequent analysis. Concentrations of albumin, total protein, glucose, triglycerides, total cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL), and blood urea nitrogen (BUN) were measured using commercial kits from Pars Azmoun Company and analyzed with an automated microplate reader (EON-BIOTEK, America). The activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase were also determined using commercial kits from Pars Azmoun Company and an automated microplate reader (EON-BIOTEK, America). Tumor necrosis factor (TNF-α), insulin, insulin-like growth factor (IGF-1), thyroid hormones including triiodothyronine (T3), and tetraiodothyronine (T4), as well as cortisol, were measured using [Pars Azmon Company kit by ELISA (ELX808; TEX-Bio)].

Statistical analyses

The data, collected in a completely randomized design with factorial arrangement, were analyzed using the PROC GLM in SAS (9/1) using the following model:

$$Y_{ij} = \mu + A_i + B_j + AB_{ij} + e_{ij}$$

where, Y_{ij}: observed value, μ: mean, A_i: vitamin C, B_j: copper, AB_{ij}: interaction of vitamin C and copper, e_{ij}: experimental error.
Comparison of means was carried out by the Tukey Kramer's test at 5% probability level.

Results

Summarized data on growth performance are reported in Table 2. Significant differences were observed in calf weights on days 21, 42, and 63, DWG on days 1-21, 22-42, 43-63, and 1-63, feed intake (FI) on days 22-42, 43-63, and 1-63 and FCR on days 1-21 and 43-63 between calves receiving vitamin C and copper and the control group. The best performance in BW on day 63, DWG on days 1-21 and 1-63, and FI on days 22-42, 43-63, and 1-63 was for the group receiving vitamin C and copper simultaneously. The experimental treatments did not significantly affect FI on days 1-21 and FCR on days 22-42 and 1-63.

The results of the effect of milk enrichment with vitamin C and copper on skeletal indices of suckling calves are reported in Table 3. The fortification of milk with vitamin C, copper, and the combination of vitamin C and copper had no effect on the withers height, hip height, hip width, Body length, heart girth and body barrel of suckling calves.

The effect of milk enrichment with vitamin C and copper on digestibility in suckling calves is reported in Table 4. No significant differences were observed between the treatments receiving copper and vitamin C and the control group regarding the digestibility of DM, OM, EE, CP, NDF, and ADF.

The effect of milk enrichment with vitamin C and copper on blood biochemical parameters is shown in Table 5. In treatments receiving vitamin C and copper, there was an increase in glucose, triglycerides and HDL, but LDL and VLDL decreased compared to the control group. The highest levels of glucose, triglycerides, HDL, and the lowest levels of LDL and VLDL were observed in the treatment receiving vitamin C and copper simultaneously. Vitamin C and copper had no significant effect on cholesterol, BUN, total protein, albumin, globulin, and the albumin:globulin ratio.

The data on hormone and liver enzyme activity in calves are reported in Table 6. A significant increase in insulin and a decrease in cortisol were recorded in calves consuming milk containing vitamin C and copper. The lowest cortisol and highest insulin levels were observed in the group receiving vitamin C and copper simultaneously. Vitamin C and copper had no effect on insulin -like growth factor 1, tumor necrosis factor-α, triiodothyronine, tetraiodothyronine, lactate dehydrogenase, creatine phosphokinase, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase.

Table 2. The effect of milk enrichment with vitamin C and copper on growth performance of calves

Parameter	-Vit C		+ Vit C		SEM	P-value		
	-Cu	+Cu	-Cu	+Cu		Vit C	Cu	Vit C * Cu
Body weight (kg)								
Weight on day 1	37.48	38.65	37.94	38.27	0.974	0.7482	0.8182	0.7694
Weight on day 21	44.62[b]	47.01[a]	47.66[a]	48.52[a]	1.211	0.0120	0.0488	0.0297
Weight on day 42	55.67[b]	59.89[a]	58.99[a]	60.44[a]	1.686	0.0411	0.0228	0.0001
Weight on day 63	67.06[c]	71.99[b]	71.25[b]	73.71[a]	0.813	0.0164	0.0211	0.0081
Daily weight gain (g/day)								
Day 1-21	340.00[c]	398.09[b]	462.85[a]	488.09[a]	24.869	0.0014	0.0171	0.0001
Day 22-42	526.19[b]	613.33[a]	539.52[b]	567.61[a]	31.554	0.0406	0.0279	0.0121
Day 43-63	542.38[b]	576.19[b]	583.81[b]	631.90[a]	35.812	0.0296	0.0417	0.0111
Day 1-63	469.52[c]	529.24[b]	528.78[b]	562.55[a]	19.412	0.0056	0.0001	0.0012
Feed intake (g/day)								
Day 1-21	512.44	524.18	563.09	579.74	6.782	0.0899	0.1207	0.0769
Day 22-42	812.98[c]	902.99[b]	938.27[a]	944.10[a]	38.480	0.0011	0.0005	0.0001
Day 43-63	994.96[c]	1042.22[b]	1098.29[a]	1188.97[a]	40.529	0.0001	0.0021	0.0001
Day 1-63	602.72[c]	647.17[b]	660.26[b]	714.95[a]	21.706	0.0121	0.0077	0.0001
Feed conversion ratio								
Day 1-21	1.50[a]	1.32[ab]	1.22[b]	1.19[b]	0.0148	0.0248	0.0112	0.0020
Day 22-42	1.54	1.47	1.72	1.66	0.0129	0.0647	0.0567	0.0656
Day 43-63	2.11[a]	1.81[b]	1.88[b]	1.88[b]	0.0279	0.0018	0.0055	0.0007
Day 1-63	1.28	1.22	1.24	1.27	0.0087	0.2418	0.4155	0.2977

[a,b] Within row, means with common superscript (s) do not differ significantly (*P*>0.05).

Table 3. The effect of milk enrichment with vitamin C and copper on skeletal indices of calves

Structural growth (cm)	-Vit C		+ Vit C		SEM	P-value		
	-Cu	+Cu	-Cu	+Cu		Vit C	Cu	Vit C * Cu
Withers height	89.20	91.27	92.01	91.49	1.762	0.1429	0.2244	0.1917
Hip height	92.11	94.46	94.65	94.29	1.828	0.4196	0.2971	0.5564
Hip width	22.14	22.76	22.85	22.79	0.414	0.7944	0.6902	0.6711
Body length	47.56	49.06	49.45	49.29	0.976	0.6242	0.4197	0.5519
Heart girth	98.99	99.87	100.04	100.20	1.264	0.6995	0.7171	0.5856
Body barrel	114.14	116.84	117.19	116.88	1.776	0.7401	0.7980	0.5682

Table 4. The effect of milk enrichment with vitamin C and copper on nutrient digestibility (g/kg) in calves

Parameter	-Vit C		+ Vit C		SEM	P-value		
	-Cu	+Cu	-Cu	+Cu		Vit C	Cu	Vit C * Cu
Dry matter	682.11	674.49	677.01	680.44	19.521	0.8401	0.6868	0.7402
Organic matter	700.82	697.65	704.29	699.86	22.332	0.6941	0.8419	0.7270
Ether extract	579.02	577.89	581.96	582.52	17.540	0.4218	0.6024	0.5521
Crude protein	616.56	614.62	620.74	619.78	21.764	0.6842	0.8102	0.7971
Neutral detergent fiber	576.22	568.99	567.42	570.86	20.496	0.6980	0.8487	0.7605
Acid detergent fiber	512.08	518.57	517.96	515.75	18.774	0.5675	0.6849	0.6006

Table 5. The effect of milk enrichment with vitamin C and copper on blood biochemical parameters in calves

Parameter	-Vit C		+ Vit C		SEM	P-value		
	-Cu	+Cu	-Cu	+Cu		Vit C	Cu	Vit C *Cu
Glucose (mg/dL)	84.08[c]	89.12[b]	88.55[b]	94.97[a]	2.191	0.0001	0.0001	0.0001
Cholesterol (mg/dL)	75.22	74.89	72.98	73.61	1.849	0.6478	0.5798	0.5249
BUN (mg/dL)	22.19	20.24	20.49	19.47	0.697	0.1495	0.2411	0.2986
Triglyceride (g/dL)	17.27[c]	21.65[b]	20.92[b]	25.09[a]	1.001	0.0001	0.0001	0.0001
HDL (mg/dL)	24.97[c]	28.54[b]	29.06[b]	33.47[a]	1.314	0.0001	0.0041	0.0001
LDL (mg/dL)	9.88[a]	8.83[b]	8.74[b]	7.67[c]	0.671	0.0121	0.0011	0.0056
VLDL (mg/dL)	5.98[a]	5.18[b]	5.09[b]	4.19[c]	0.418	0.0289	0.0127	0.0166
Total protein (g/dL)	8.68	8.79	8.66	8.70	0.246	0.7911	0.8014	0.7479
Albumin (g/dL)	5.14	5.21	5.19	5.26	0.343	0.6940	0.5562	0.3449
Globulin (g/dL)	3.54	3.58	3.47	3.44	0.298	0.7112	0.5602	0.6109
Albumin : Globulin	1.45	1.45	1.66	1.52	0.088	0.5068	0.8984	0.6690

[a,b] Within row, means with common superscript (s) do not differ significantly (*P*>0.05).
BUN: Blood nitrogen urea; HDL: High-density lipoprotein; LDL: Low-density lipoprotein;
VLDL: Very-low-density lipoprotein.

Table 6. The effect of milk enrichment with vitamin C and copper on hormones and liver enzyme activity in calves

Parameter	-Vit C		+ Vit C		SEM	P-value		
	-Cu	+Cu	-Cu	+Cu		Vit C	Cu	Vit C * Cu
Insulin (ng/mL)	0.47[c]	0.54[b]	0.55[b]	0.60[a]	0.019	0.0001	0.0001	0.0001
Insulin -like growth factor 1 (ng/mL)	54.18	52.89	55.27	53.01	2.742	0.7812	0.4411	0.5627
Tumor necrosis factor-α (pg/mL)	94.61	97.54	95.11	96.27	4.792	0.2418	0.5029	0.4197
Triiodothyronine (nmol/L)	9.42	10.16	10.29	10.40	0.461	0.7482	0.6911	0.4242
Tetraiodothyronine (nmol/L)	107.29	111.92	112.61	106.99	4.521	0.5560	0.6721	0.4201
Lactate dehydrogenase (u/L)	242.18	229.84	240.21	233.62	17.546	0.4892	0.6984	0.5976
Creatine phosphokinase (u/L)	64.28	62.24	65.18	66.02	2.749	0.6984	0.4892	0.2477
Alkaline phosphatase (u/L)	14.27	16.01	15.66	15.47	0.898	0.6842	0.2697	0.1247
Alanine aminotransferase (u/L)	204.47	218.21	211.69	209.55	16.162	0.5562	0.7821	0.2948
Aspartate aminotransferase (u/L)	72.14	68.97	70.21	69.52	2.979	0.7498	0.6811	0.5624
Cortisol (ng/mL)	6.19[a]	5.52[b]	5.28[b]	4.67[c]	0.7942	0.0001	0.0001	0.0001

[a,b] Within row, means with common superscript (s) do not differ significantly (P>0.05).

Discussion

Minerals and vitamins play significant roles at early developmental stages in calves (Osorio et al., 2012). Copper and vitamin C are known to enhance growth performance due to their antioxidant properties (Spears and Weiss, 2008). The kidneys are integral to the synthesis of vitamin C; however, this process is not highly active during the initial months of life. Therefore, administration of vitamin C supplements and its incorporation into milk can improve animal performance (Sahinduran and Albay, 2004). The beneficial effects of vitamin C may be attributed to its role in collagen synthesis, influence on vitamin D_3 metabolism, involvement in carnitine-mediated fatty acid oxidation, impact on amino acid oxidation, participation in cellular electron transport, and capacity to neutralize free radicals (Comb, 1992). The increase in feed intake may be associated with vitamin C's role in the synthesis and secretion of steroids, which can lower body temperature, enhance FI, and reduce the FCR (Matsui, 2012). Furthermore, the combined effects of copper and vitamin C can elevate thyroid hormone levels, thereby promoting calf growth by increasing feed intake and enhancing the digestion and absorption of nutrients (Comb, 1992; Suttle, 2010). It is likely that the antioxidant properties of vitamin C and copper positively influence the immune system, enabling calves to better manage the metabolic challenges in early life (Naeem et al., 2022). Consequently, an increase in dry matter intake and weight gain was observed, along with a reduction in stress and disease incidence (Spears and Weiss, 2008).

Vitamin C supplementation has been shown to significantly enhance the final BW and average DWG of suckling calves (Seifzadeh et al., 2022). Additionally, inclusion of organic copper at 10 mg/kg of DM in the diet of Zandi lambs improved their DWG compared to the control group (Hoseinpour et al., 2014). In alignment with the findings of the present study, Seifzadeh et al. (2021) reported that the inclusion of vitamin C in the calves' diet significantly enhanced DWG and FI throughout the experimental period. Hozhabri et al. (2018) found that various forms of copper reduced the DWG of Sanjabi lambs without affecting their final BW, FI, or FCR. In contrast, the glycinate form of copper, did not affect BW, average DWG, average FI, or FCR in suckling calves (Moazeni Zadeh et al., 2024). Also, Vahedi et al. (2022) observed that nano copper oxide did not influence FI, DWG, FCR and final BW of suckling calves.

Trace elements such as zinc, copper, iron, and manganese, which are present in bone, are integral to enzyme systems that facilitate the normal function of bone cells and cartilage; these trace elements are associated with organic or inorganic components of the matrix and impact the physical properties of bone (Suttle, 2010). Collagen, the primary structural protein of the extracellular matrix and connective tissues, including cartilage and bone, is influenced by the copper-dependent enzyme lysyl oxidase, which is involved in the formation and maintenance of bone collagen and other connective tissues (Suttle, 2010). Skeletal abnormalities have been observed in copper-deficient lambs (Bao et al., 2007). It is plausible that the basal diet's trace mineral and vitamin levels were adequate to support skeletal growth in suckling calves, as most parameters related to skeletal growth in this experiment did not significantly differ between the groups. Mousavi Haghshenas et al. (2022) reported that organic supplementation of trace elements in suckling calves increased hip bone width. Nano copper oxide supplementation did not affect skeletal growth indices, including chest circumference, withers height, and body length (Vahedi et al., 2022). Moazeni Zadeh et al. (2024) reported that the glycinate form of mineral elements, such as copper, did not influence body length, chest circumference, back height, withers height, tibia length, hip bone width and height in suckling calves, although it did increase carpus bone length.

The absence of treatment effects on digestibility could be due to the same diet and equal environmental conditions. The enhancement in digestibility may be attributed to the role of micronutrients in mitigating oxidative stress (Dogan, 2023). In alignment with the findings of the present study, Akinmoladun et al. (2021) found that nutrient digestibility was not significantly affected in the group receiving vitamin C compared to the control group. Contrary to the findings of the present study, incorporating vitamin C into the diets of suckling calves enhanced the digestibility of DM, OM, CP, EE

(Hidiroglou, 1999). Furthermore, adding a compound containing copper in organic and inorganic forms to the diets of fattening lambs improved their DM, OM and NDF digestibility (El Ashry et al., 2012). In a study examining the use of various forms of mineral elements in inorganic forms, Genther and Hansen (2015) observed that hydroxy manganese, copper, and iron reduced the digestibility of DM, whereas sulfated manganese, copper, and iron did not affect DM digestibility in calves.

Micronutrients, particularly copper and vitamin C, are essential for the metabolism of lipids, proteins, and carbohydrates (Spears and Weiss, 2008). Deficiencies in these micronutrients can limit glucose metabolism, insulin secretion, and insulin-induced glucose uptake (Asadi et al., 2024). Therefore, the elevated glucose levels observed in suckling calves treated with vitamin C and copper in this study may be linked to their roles in gluconeogenesis and insulin secretion (Comb, 1992; Suttle, 2010). The relationship between inflammation and lipid metabolism is well established. High-density lipoproteins (HDL) play a crucial role in innate immunity, and their circulating levels typically decrease during inflammatory responses (Yamamoto et al., 2000). In the present study, calves supplemented with vitamin C and copper exhibited significantly higher serum HDL levels compared to the control group. Given the recognized inverse association between systemic inflammation and HDL concentration, this improvement in HDL levels suggests that the supplemented calves likely experienced a reduced state of inflammation and oxidative stress, which is consistent with the known antioxidant and anti-inflammatory functions of vitamin C and copper (White et al., 2017). Similarly, the lipid profile improvement was further evidenced by a significant reduction in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) levels in the supplemented groups, which are often associated with pro-inflammatory states and elevated blood triglyceride concentrations (Nazir et al., 2020; Abolghasemi et al., 2023).

Moazeni Zadeh et al. (2024) reported that calves receiving glycinate supplements of trace elements containing copper exhibited higher serum albumin, glucose, and HDL concentrations, alongside lower LDL concentrations. In contrast, Khatami Khalkhoran et al. (2024) found that copper supplements in nano and sulfate forms increased BUN in fattening lambs, without affecting other blood biochemical parameters or liver enzyme activities. Also, Eftekhari et al. (2020) observed that copper supplementation, through the diet of Mahabadi goat kids with copper sulfate, did not significantly influence blood parameters such as glucose, triglycerides, and plasma cholesterol. Conversely, Datta et al. (2007) noted that while plasma glucose, total protein, and triglyceride concentrations remained unaffected by experimental treatments, although cholesterol concentrations were reduced by copper supplementation. Khodamoradi et al. (2020) reported that maternal vitamin C and copper injection did not impact glucose, protein, cholesterol, triglycerides, and HDL levels in newborn calves. Similarly, Jafari et al. (2024) reported no significant effect of vitamin C and copper injection on blood glucose, total protein, triglycerides, and cholesterol in late-pregnant cows. The differences may be due to species and breed.

Hormonal fluctuations and liver enzyme activity serve as indicators for assessing animal welfare. Cortisol is recognized as an immunosuppressive agent (Aguiar et al., 2023), with elevated cortisol levels inhibiting the antibody function and production, alongside diminishing the number and activity of lymphocytes. Micronutrients have the potential to lower cortisol levels and enhance immune responses during periods of stress and inflammation; the response of insulin concentrations to minerals and vitamins can be partially attributed to the reduction in blood cortisol levels (Adam et al., 2010). Given that cortisol and insulin exert opposing effects on metabolism, it is plausible that micronutrients may elevate insulin levels (Adam et al., 2010). This antagonism, coupled with alterations in overall metabolic processes due to minerals and vitamins (Asadi et al., 2025), can enhance the production, proliferation, and activation of immune cells, thereby improving the body's resistance to diseases (Zhao et al., 2023). When the calves experience stress, cortisol levels rise to facilitate appropriate responses to the stressor. This elevation in cortisol can lead to increased oxidative stress (Chandimali et al., 2025). Vitamin C and copper are recognized as potent antioxidants that may mitigate oxidative stress and safeguard the body from the detrimental effects of free radicals (Suttle, 2010). Furthermore, vitamin C and copper may indirectly influence the synthesis or degradation of cortisol during periods of stress. Specifically, vitamin C has been shown to inhibit the secretion of corticosteroid hormone (Kegley et al., 2016).

In line with the present study, administration of vitamin C to stressed ewes resulted in a reduction of blood cortisol levels (Kafilzadeh et al., 2012). Moreover, copper supplementation in both nano and sulfate forms did not influence hormone synthesis or liver enzyme activity in fattening lambs (Khatame Khalkhoran et al., 2024). On the other hand, Datta et al. (2007) noted that the addition of copper sulfate and copper proteinate to the diets of goats did not affect ALT and AST enzyme levels. In a study conducted by Seifzadeh et al (2022), vitamin C supplementation in the diets of pre-partum cows led to a decrease in aspartate aminotransferase activity; additionally, they reported that vitamin C supplementation in suckling calves resulted in a reduction of alanine aminotransferase activity on days 7 and 21, as well as a decrease in aspartate aminotransferase activity on day 7.

Conclusion

The results of this study showed milk enrichment with vitamin C and copper improved growth performance (final BW, total weight gain, average DWG and FI), and several blood biochemical parameters (glucose, triglyceride, LDL, VLDL), but reduced HDL levels. On the other hand, a significant increase in insulin and a decrease in cortisol were reported in calves consuming milk containing vitamin C and copper. The most favorable outcomes were noted in the cohort receiving both vitamin C and copper concurrently. Therefore, based on the results obtained, simultaneous enrichment of milk with vitamin C and copper during the first two months of calves' lives may enhance growth performance and bolster their immune system.

Author Contributions

Conceptualization, Mohammad Asadi and Ayda Teymouri; methodology, Mohammad Asadi; software, Mohammad Asadi; validation, Mohammad Asadi and Taghi Ghoorchi; formal analysis, Mohammad Asadi; investigation, Mohammad Asadi and Ayda Teymouri; resources, Ayda Teymouri; data curation, Abdolhakim Toghdory and Mohammad Eteraf; writing—original draft preparation, Mohammad Asadi and Ayda Teymouri; writing—review and editing, Abdolhakim Toghdory; supervision, Taghi Ghoorchi and Mohammad Eteraf; project administration, Abdolhakim Toghdory; All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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