Iranian Journal of Applied Animal Science

The Effects of Copper Nano Oxide and Prebiotic on Growth Performance, Blood Antioxidant Activity and Health Status of Holstein Suckling Calves

Research Article

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Received on: 5 Aug 2024
Revised on: 3 Jan 2025
Accepted on: 17 Jan 2025
Online Published on: Mar 2025

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Online version is available on: www.ijas.ir
https://doi.org/10.71798/ijas.2025.1202950

ABSTRACT

This study aimed to examine the effects of copper nano oxide (NCu) and Celmanax prebiotic (PBC) on growth performance, health status and blood parameters in Holstein suckling calves. For this purpose, 28 Holstein calves with an average weight of 36 ±19 kg with 4 treatments and 7 replications were selected. This study was conducted as a 2 × 2 factorial arrangements with two factors of copper nano oxide (0 and 15 mg/kg) and prebiotic (0 and 4 g) for 65 days. Blood samples were taken on day 30 and 60 of the experiment. Results indicated that PBC significantly affected final body weight (FBW) and average daily gain (ADG), with PBC-supplemented calves exhibiting the highest weight. However, NCu and the PBC × NCu interaction had no effect on FBW and ADG. Daily feed intake (DFI) and feed conversion ratio (FCR) were not affected by any treatment. PBC supplementation significantly reduced the time to diarrhea resolution and improved fecal consistency score, while NCu and the interaction had no effect. The incidence of diarrhea was influenced by PBC factor and the PBC × NCu interaction. The NCu factor increased blood copper concentration, urea, albumin, and total protein at 65 days, while the treatments had no effect on blood copper concentration at 30 days. Moreover, NCu factor significantly altered glutathione peroxidase activity at both 30 and 65 days. In general, the results of this study showed that the addition of prebiotics to the diet improved weight and daily weight gain. However, supplementing the diet with PBC and NCu increased blood NCu concentration and glutathione peroxidase concentration.

KEY WORDS blood parameters, Celmanax prebiotic, copper nano oxide, diarrhea, suckling calves.

INTRODUCTION

The future of any dairy farm relies significantly on the successful rearing of calves and replacement heifers. The most economical approach to supply the necessary heifers for the herd is through the rearing of dairy calves. These suckling calves represent the next generation of the herd and constitute a substantial investment for dairy cattle breeders. Respiratory diseases, diarrhea, and other gastrointestinal issues are the most prevalent ailments in suckling calves, and effective interventions to mitigate these problems can enhance calf efficiency (Dinardo *et al.* 2022). Nutritional manipulation is an important management strategy to reduce the incidence of disease and decrease calf mortality. Given the high nutritional needs of young calves and their limited nutrient reserves, the early feeding stage is critical. Nutritional requirements for minerals vary based on age, size, physiological state (pregnancy, lactation, growth), activity level, species, and genetics (Kertz *et al.* 2017).

Trace elements, particularly copper, play a crucial role in the proper functioning of the immune system (Pandey *et al.* 2022).

Copper exhibits antimicrobial, antifungal, and antiviral properties (Li *et al.* 2010), and serves as a cofactor for enzymes such as ceruloplasmin, cytochrome c oxidase, and superoxide dismutase (Underwood and Suttle, 1999).

This essential mineral is involved in cellular metabolism, development, and integrity, making it vital for both calves and adult cattle (Humann-Ziehank, 2016). The requirement for copper in calves is reported to be 12 mg per day according to the NRC (NRC, 2001) and between 8 to 14 mg per day in the ARC (ARC, 1980).

Suttle *et al.* (2010) noted that different forms of copper demonstrate varying absorption rates. Thus, introducing a suitable mineral form with high bioavailability can effectively reduce total mineral consumption (Creech *et al.* 2001). Research indicates that nano-copper has significantly higher bioavailability compared to conventional copper sulfate (Gonzales-Eguia *et al.* 2009). In addition to mineral forms, there is growing interest in enhancing mineral absorption through the consumption of oligosaccharides as prebiotics (Sarvestani *et al.* 2016).

Studies have shown that prebiotics can enhance the intestinal absorption of minerals such as calcium, iron, magnesium, and copper by reducing intestinal pH (Monchios *et al.* 1999). Prebiotics are defined as non-digestible substances that promote health and growth performance by modulating the composition of the gastrointestinal microbiota (Cangiano *et al.* 2020; Lopes *et al.* 2021). They contribute to the growth, nutrient absorption, and development of the ruminal epithelium in calves (Alves Costa *et al.* 2019; Lopes *et al.* 2021). However, literature on the combined use of nano-copper and prebiotics in calf nutrition is limited.

This study hypothesizes that the simultaneous use of copper and prebiotics in the diet of dairy calves can improve growth performance by enhancing the absorption efficiency of copper and other nutrients. Therefore, the aim of this research is to investigate the effects of NCu and PBC on growth, health status, and blood parameters in Holstein suckling calves.

MATERIALS AND METHODS

Animal ethics

This study was conducted on the animal husbandry dairy herd at Moghan Agro-Industrial Complex in Parsabad, Ardabil Province, Iran. All animal operations were performed in compliance with protocols approved by the Research Ethics Committees of the University of Mohaghegh Ardabili (Ethics Approval Code: IR.UMA.REC.1401.100). Animal procedures were carried out according to protocol no. 19293, approved by the Iranian Council of Animal Care.IR.UMA.RE.

Animals and diet

Twenty-eight male Holstein calves with an average weight of 36 ± 19 kg were selected for this study. A 2 × 2 factorial design was employed with two factors: copper nano-oxide (0 and 15 mg/kg) and prebiotic (0 and 4 g). The experimental treatments consisted of: 1) control group receiving a basal diet (CON), 2) the basal diet supplemented with 4 g/day of prebiotic (PBC), 3) the basal diet supplemented with 15 mg/kg of copper nano-oxide (NCu), and 4) the basal diet supplemented with both 4 g/day of prebiotic and 15 mg/kg of copper nano-oxide (PBC×NCu). The prebiotic used in this study was Celmanax®, manufactured by the American company Arm & Hammer Animal Nutrition (VICOR). Celmanax® contains hydrolyzed yeast culture products and yeast extract, which are natural sources of β-glucan, mannan-oligosaccharides, proteins, amino acids, minerals, and B vitamins. The copper nano-oxide (99.99% purity and 54.79 molecular weight) used in this study was sourced from US Research. Calves received NCu and PBC mixed with their liquid feed (colostrum and whole milk). In the first 24 hours after birth, calves were separated from their mothers and randomly allocated to the experimental groups. Calves received 5 liters of colostrum with a pacifier twice within the first 8 h post-birth. From the second feeding until day 3 of life, all calves were fed colostrum and transition milk via bottles with nipples twice daily. The quality of colostrum was measured using a digital Brix refractometer, and it was discarded if the measurement was below 22 on the Brix scale (Bielmann *et al.* 2010).

Whole milk was offered twice daily at 8:30 a.m. and 6:00 p.m., with careful monitoring to ensure complete consumption. During the breeding period, 10 milk samples were sent to a laboratory to analyze dry matter, fat, protein, and lactose content.

The milk consumed by the calves contained an average of 11.50% dry matter, 3.1% crude protein, 3.5% ether extract, and 4.35% lactose. On day 4, water was made available *ad libitum*. From days 4 to 19, the calves were given starter feed, and from days 20 to 65, 10% dried alfalfa (1-3 cm) was mixed with the starter. The alfalfa forage contained 15% crude protein, 1.9% ether extract, 50% ADF, and 37% NDF. The experimental diets and their chemical composition are detailed in Table 1.

Sampling and analyses procedures

Dry matter (DM), Crude protein (CP), crude fat (CF), and ash of feed samples, were determined by the Association of Official Agricultural Chemists (AOAC, 1990). Also, the neutral detergent fiber (NDF) and acid detergent fiber (ADF) of the samples were determined by the method of Van Soest *et al.* (1991). Daily feed intake (DFI) was measured by measuring daily feed offered and refusal.

Table 1 Basal diet and chemical composition of diet

Feed ingredients	% of dry matter	Chemical composition	Amounts
Corn	40.5	Dry matter (%DM)	90.71
Barley	14.0	Crude protein (%DM)	20.80
Wheat bran	4.0	ADF (%DM)	15.71
Soybean meal	38.6	NDF (%DM)	29.41
Salt	0.4	EE (%DM)	2.21
Shelf powder	1.0	Calcium (%DM)	0.94
Mineral premix	0.5	Phosphor (%DM)	0.78
Vitamin premix[1]	0.5	Copper (mg/kg)	12.10
Sodium bicarbonate	0.5	Iron (mg/kg)	398.01

[1] Vitamin Supplement: vitamin A: 500000 IU/kg; vitamin E: 100 mg/kg; vitamin D3: 100000 IU/kg; Calcium: 195000 mg; Phosphorus: 90000 mg; Magnesium: 90000 mg; Sodium: 55000 mg; Zinc: 3000 mg; Iron: 300 mg; Manganese: 2000 mg; Cobalt: 100 mg; Selenium: 1 mg and Antioxidant: 400 mg.

Body weight change was determined on day 30, 60 and 65 of experimental period before the morning feeding. Feed conversion ratio for each calf is the ratio of daily DM intake to daily weight gain.

Blood samples were collected from all calves from jugular veins puncture on day 30 and 65 of trial, 4 h after morning feeding. Then centrifuged at 3500 rpm for 15 min at 4 °C to obtain the plasma and kept at −20 °C till the analyses. Serum concentrations of glucose, cholesterol, triglyceride, albumin, total protein and blood urea nitrogen (BUN) were measured by using the commercial kits (Pars Azmoon co, Tehran, Iran). In the blood samples, the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured by Ransod and Ransel kits, respectively (Randox Laboratories, UK). Plasma copper was determined using an atomic absorption spectrophotometer (Varian SpectrAA220, Australia). Measurement of total antioxidant capacity (TAC) was done in serum samples by a commercially available kit according to the manufacturer's protocol (Total Antioxidant Status, Randox Laboratories, Co. An-trim, UK).

Diarrhea frequency, time until resolution of diarrhea, incidence of diarrhea, faecal consistency score, number of calves affected with pneumonia, number of calves affected with navel ill were used to access the health status of experimental calves. The incidence of diarrhea in each group was calculated using the following formula.

Insidence of diarrhea (%)= (diarrhoeal calves in each group×darrhoea days/total calves in each group×experimental days) × 100

Faecal score was established as 1= normal, thick inconsistency; 2= normal, but less thick; 3= abnormallythin, but not watery; 4= watery; 5= watery withabnormal colouring (Kertz and Chester-Jones, 2004).

Statistical analyses

Data were analyzed using mixed procedure of statistical analyses software (SAS, 2004) with factorial arrangement according to the following model:

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

Where:
Y_{ijk}: observations.
μ: overall average.
A_i: effect of primary factor (PBC).
B_j: effect of second factor (NCu).
AB_{ij}: interaction of the factors (PBC×NCu).
e_{ijk}: effect of trial error.

Dietary treatment means were compared for significant differences according to LSMeans test and $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The effects of dietary prebiotics and copper nano-oxide on final body weight (FBW), average daily feed intake (DFI), average daily weight gain (ADG), and feed conversion ratio (FCR) are presented in Table 2. Results showed that FBW and ADG of suckling calves were affected by the prebiotic factor (P<0.05); however, the NCu factor and their interactions had no effect on FBW and ADG. Among the experimental treatments, calves receiving prebiotics had the highest weight compared to the control group. DFI and FCR of suckling calves were not affected by the PBC factor, NCu factor, and their interactions.

The effects of experimental treatments on health status are shown in Table 3. The prebiotic factor significantly influenced the time until resolution of diarrhea and faecal consistency score in suckling calves, such that calves receiving prebiotics had a shorter time until resolution of diarrhea and better fecal consistency score. However, the NCu factor and the interactions PBC × NCu had no effect. The Incidence of diarrhea was affected by the PBC factor and the interaction PBC × NCu ($P<0.05$); however, the NCu factor did not have any effect on the diarrhea index of suckling calves. One calf in both the control and NCu groups was affected by navel ill. No cases of pneumonia were reported in any of the four groups.

The effects of dietary prebiotics and copper nano-oxide on blood parameters are shown in Table 4. At 30 days of age, the interaction between PBC and NCu significantly affected glucose concentration ($P<0.05$). Specifically, glucose concentration decreased in calves receiving PBC + NCu. However, by 65 days of age, neither factor had a significant effect on glucose concentration. Cholesterol and triglyceride concentrations were not affected by the PBC, NCu, and their interaction at both 30 and 65 days of age. At 30 days of age, there was no significant difference in blood urea concentration among the calves. However, at 65 days of age, NCu significantly altered urea concentration ($P<0.05$), with calves fed diets containing NCu exhibiting higher urea concentrations compared to the control group. Albumin and total blood protein concentrations were not affected by the experimental treatments at 30 days of age. However, at 65 days of age, copper nanoparticles significantly altered albumin and total protein concentrations ($P<0.05$), with experimental groups receiving NCu having higher concentrations than the control group.

The effects of experimental treatments on plasma copper concentration and antioxidant activity are presented in Table 5. At 30 days of age, PBC, NCu, and their interaction did not significantly affect blood copper concentration. However, at 65 days of age, NCu significantly increased blood copper concentration ($P<0.05$); the PBC and the interaction factor had no effect on blood copper concentration. Blood total antioxidant capacity and superoxide dismutase (SOD) activity were not affected by PBC, NCu, or their interaction. At 30 days of age, glutathione peroxidase (GPx) activity was affected by NCu and the interaction of PBC + NCu ($P<0.05$); calves receiving NCu and PBC + NCu had higher GPx activity than the control group. At 65 days of age, the NCu also significantly increased GPx activity ($P<0.05$); however, the PBC and the PBC+ NCu interaction had no effect on GPx activity.

The results of the present study indicated that calves fed the PBC exhibited higher FBW and ADG compared to the control group, while no significant effect was observed with NCu. Additionally, no significant differences were observed among treatments regarding DFI and FCR (Table 2).

The beneficial effects of mannooligosaccharides as prebiotics on the growth performance of suckling calves are well-supported by previous studies. Ghosh and Mehla (2012) reported that the inclusion of mannooligosaccharides (4 g/day) in the diets of suckling calves improved feed intake. This observation is consistent with earlier research by Heinrichs *et al.* (2003) and Morrison *et al.* (2010), which demonstrated that calves receiving prebiotics tended to show higher feed intake compared to un-supplemented calves. However, the findings from Roodposhti and Dabiri (2012) did not align with this, as their study showed that while mannooligosaccharides increased feed intake, there was no corresponding improvement in final body weight (FBW). In our study, the inclusion of prebiotics (PBC) resulted in significantly higher FBW and ADG compared to the control group, which supports the results of Chang *et al.* (2022), who found that dietary prebiotics not only improved final weight but also increased weight gain in suckling calves.

Additionally, Lesmeister *et al.* (2004) showed that calves fed mannooligosaccharides exhibited higher average daily gain (ADG) compared to the control group, further validating the positive effects of prebiotics on growth performance. The mechanisms underlying these benefits likely involve the fermentation of prebiotics in the colon and large intestine by beneficial bacteria. This process can alter the composition of the gut microbiota, fostering a more favorable environment for nutrient absorption and energy utilization.

The fermentation products, such as volatile fatty acids (VFAs), could contribute to improved energy efficiency and enhanced growth performance. Overduin *et al.* (2013) suggested that prebiotics could influence fat metabolism directly and indirectly, thereby improving body weight and growth, a finding that is in agreement with the results from our study, where significant improvements in ADG and FBW were observed. Furthermore, it is well-established that prebiotics enhance the growth performance of animals (Abecia *et al.* 2013; Richards *et al.* 2020). The observed improvements in growth parameters, particularly FBW and ADG, could be attributed to the beneficial effects on gut health and nutrient digestion. These changes in intestinal microbiology and histology might explain the improved weight gain observed in later stages of growth (Roodposhti and Dabiri, 2012).

On the other hand, the use of nano copper (NCu) did not yield significant effects on the growth performance of calves in our study. This is in line with the findings of Dezfoulian *et al.* (2012), who reported that copper supplementation, whether organic or inorganic, had no significant impact on the growth performance of lambs.

Table 2 Effects of prebiotics and copper nano oxide on performance of Holstein calves

Traits	Experimental treatments				SEM	P-value		
	CON	PBC	NCu	PBC × NCu		PBC	NCu	PBC × NCu
Birth weight (kg)	35.16	36.13	36.66	36.83	1.361	0.471	0.631	0.714
Final weight (kg)	63.66[b]	78.50[a]	77.66[ab]	76.98[ab]	1.302	0.050	0.482	0.071
Feed intake (kg/d)	1.11	1.11	1.09	1.17	0.594	0.641	0.436	0.450
Average daily gain (g/d)	593[b]	636[a]	606[ab]	617[ab]	25.124	0.041	0.841	0.184
Feed conversion ratio	1.87	1.73	1.73	1.90	0.091	0.967	0.731	0.104

Con: control; NCu: copper nano oxide and PBC: Celmanax prebiotic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of prebiotics and copper nano oxide on health status of Holstein calves

Traits	Experimental treatments				SEM	P-value		
	CON	PBC	NCu	PBC × NCu		PBC	NCu	PBC × NCu
No. of calves in trial	7	7	7	7		-	-	-
No. of calves diagnosed at least once for diarrhea	3.00	1.00	2.00	1.00		-	-	-
Proportion (%) of examined calves identified with diarrhea	42.85	14.28	28.57	14.28		-	-	-
Time until resolution of diarrhea (day)	6.00[b]	3.00[a]	5.00[ab]	4.00[a]	0.281	0.031	0.141	0.091
Incidence of diarrhea	3.95[b]	0.65[a]	2.19[ab]	0.87[a]	0.110	0.011	0.090	0.032
Faecal consistency score	3.21[b]	1.78[a]	2.98[b]	2.78[b]	0.181	0.040	0.080	0.111
No. calves affected with pneumonia	NS	NS	NS	NS		-	-	-
No. calves affected with navel ill	1.00	NS	1.00	NS		-	-	-

Con: control; NCu: copper nano oxide and PBC: Celmanax prebiotic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of prebiotics and copper nano oxide on blood parameters of Holstein calves

	Experimental treatments				SEM	P-value		
	CON	PBC	NCu	PBC × NCu		PBC	NCu	PBC × NCu
Glucose (mg/dL)								
30 days	94.1[a]	97.8[a]	97.0[a]	81.50[b]	11.941	0.147	0.205	0.041
65 days	76.6	77.0	88.0	75.2	10.101	0.134	0.262	0.147
Cholesterol (mg/dL)								
30 days	117.5	106.5	116.5	102.5	9.112	0.081	0.720	0.88
65 days	107.4	104.4	104.2	111.0	17.614	0.980	0.380	0.915
Triglyceride (mg/dL)								
30 days	14.2	14.3	14.5	12.8	3.760	0.630	0.700	0.578
65 days	13.7	14.2	14.4	16.4	2.324	0.222	0.926	0.28
BUN (mg/dL)								
30 days	18.6	19.00	19.3	18.3	3.281	0.797	0.996	0.597
65 days	16.4[b]	15.8[b]	18.9[a]	18.2[ab]	2.162	0.344	0.014	0.077
Total protein (g/dL)								
30 days	5.41	5.46	5.70	5.96	0.373	0.514	0.111	0.657
65 days	5.54[b]	5.96[ab]	6.68[a]	6.04[ab]	0.574	0.461	0.001	0.324
Albumin (g/dL)								
30 days	2.95	2.85	3.05	3.06	0.184	0.560	0.261	0.771
65 days	2.94[b]	2.86[b]	3.84[a]	2.96[ab]	0.171	0.294	0.001	0.401

Con: control; NCu: copper nano oxide and PBC: Celmanax prebiotic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Similarly, Pandey *et al.* (2022) found that 10 mg/kg nano copper supplementation did not significantly affect the daily weight gain of calves, suggesting that copper supplementation may not always be effective in improving growth parameters in certain animal species or under specific conditions. Kushwaha *et al.* (2021) also reported no effects of various copper sources (inorganic Cu, nano Cu) on the growth performance of Sahiwal heifers, further supporting the idea that copper supplementation might not universally enhance growth.

Table 5 Effects of prebiotics and copper nano oxide on plasma Cu concentration and antioxidant activity of plasma in Holstein calves

Metabolites	Experimental treatments				SEM	P-value		
	CON	PBC	NCu	PBC × NCu		PBC	NCu	PBC × NCu
Copper (μg/dL)								
30 days	93.88	97.50	96.16	99.16	5.031	0.391	0.551	0.792
65 days	109.18[b]	121.12[ab]	138.14[a]	112.20[ab]	7.242	0.060	0.050	0.090
Total Antioxidant activity (μmol/L)								
30 days	0.360	0.392	0.370	0.388	0.021	0.354	0.911	0.794
65 days	0.414	0.442	0.418	0.424	0.020	0.511	0.830	0.694
Superoxide dismutase (U/g)								
30 days	50.11	50.71	55.50	54.83	4.020	0.960	0.265	0.851
65 days	69.80	75.51	64.04	71.44	3.621	0.115	0.230	0.921
Glutathione peroxidase (U/g)								
30 days	583.21[b]	602.30[ab]	694.44[a]	674.01[a]	26.940	0.874	0.010	0.040
65 days	716.00[b]	728.30[ab]	788.01[a]	763.61[ab]	46.270	0.381	0.023	0.091

Con: control; NCu: copper nano oxide and PBC: Celmanax prebiotic.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Vaswani *et al.* (2018) found no effect of different copper sources (Cu-proteinate, Cu-propionate, Cu sulfate) on ADG in growing heifers, which aligns with our results. However, not all studies have found similar results. For example, Zhang *et al.* (2023) observed a positive effect on the ADG of goats when 20 mg/kg of copper sulfate was added to their diet. This suggests that the effects of copper supplementation may depend on factors such as the animal species, copper form, and dosage. Iqbal *et al.* (2012) also reported that 500 mg of copper sulfate increased feed intake in broiler chicks, though no significant effect on feed conversion ratio (FCR) or ADG was observed. This finding is somewhat similar to our study, where no significant changes in DFI or FCR were observed, further emphasizing that copper supplementation might not consistently influence growth performance, especially in terms of feed efficiency. The lack of significant interactions between prebiotics and NCu in the present study suggests that the combination of these two interventions may not provide the expected synergistic effects on growth performance in calves. This lack of interaction is consistent with previous research indicating that the effects of copper supplementation on growth performance are highly variable and may not be enhanced by the inclusion of prebiotics.

In the current study, it was observed that calves receiving PBC and the combination of PBC and NCu exhibited a lower incidence of diarrhea compared to the control group. Additionally, the fecal consistency score was notably reduced in calves fed with PBC. These findings align with previous research, such as the study by Pandey *et al.* (2022), which reported no significant incidence of diarrhea in calves receiving a lower dose of 10 mg/kg of nano copper. The anti-diarrheal effects associated with copper may be linked to its role in enhancing immune function, as suggested by Pei *et al.* (2019).

Supporting this, Suttle and Angus (1976) noted a correlation between copper deficiency and the onset of diarrhea and anemia in calves, indicating the importance of adequate copper levels in maintaining calf health. While information on the effects of NCu on diarrhea occurrence is limited, other studies have demonstrated the efficacy of prebiotics. In study of Bagheri Varzaneh (2022), adding prebiotics to the diet of mid-lactation dairy cows under heat stress improved fecal consistency scores, suggesting better nutrient absorption and digestive health. Heinrichs *et al.* (2003) emphasized that dietary supplementation with prebiotics significantly reduces diarrhea and enhances normal fecal consistency in calves. However, not all studies have found consistent results; for instance, Didarkhah and Bashtani (2018) reported no significant effect of prebiotics on the duration of diarrhea days in nursing calves. Conversely, Ghosh and Mehla (2012) showed that prebiotic supplementation effectively reduced fecal consistency scores. Morrison *et al.* (2010) and Nargeskhani *et al.* (2010) further confirmed these benefits, reporting that the use of mannan oligosaccharides significantly improved fecal consistency in suckling calves compared to the control group.

The results of the present study showed that the inclusion of PBC and NCu had no significant effect on most blood microflora. These findings are consistent with the study of Shams *et al.* (2022), which similarly indicated that the application of probiotics in Holstein calves failed to produce significant changes in plasma concentrations of glucose, cholesterol, blood urea nitrogen (BUN), and albumin. However, it is noteworthy that our investigation revealed a significant impact of NCu on BUN, total protein, and albumin levels by day 65. This finding could imply a positive influence of NCu on protein metabolism, although the broader implications for overall metabolic health. In contrast, Heinrichs *et al.* (2003) reported that feeding calves diets containing prebiotics did not significantly alter plasma levels of albumin, urea, or protein. This outcome corroborates our findings regarding the lack of effect on plasma albumin concentrations, suggesting that prebiotics may not substantially influence protein metabolism or immune responses in the contexts studied. Such results collectively imply that the effects of prebiotics may be limited and context-dependent, potentially influenced by species, production conditions, and the specific parameters measured. The study by Bagheri Varzaneh (2022) provides additional context, indicating that the incorporation of prebiotics in the diets of dairy cows under heat stress did not significantly affect glucose concentrations.

Copper (Cu) is an essential trace element in living organisms, significantly influencing biochemistry, enzyme activities, cellular respiration, free radical defense, neurotransmitter function, and tissue biosynthesis (Hefnawy and Khaiat, 2015). High dietary levels of copper are associated with increased superoxide dismutase (SOD) activity, highlighting the importance of its supplementation in enhancing antioxidant mechanisms in animals. SOD is responsible for converting two superoxide radicals into oxygen and hydrogen peroxide, which are then utilized as substrates by catalase (CAT) and glutathione peroxidase (GPx) enzymes to eliminate harmful reactive oxygen species (ROS) from the cellular environment (Sevcikova *et al.* 2016; Saffari *et al.* 2017). Previous studies have reported similar findings. Zhao *et al.* (2020) observed that copper supplementation improved the antioxidant status in Sahiwal calves. Shen *et al.* (2021) emphasized that the addition of nano copper and CuSO4 to the diet of goats increased serum concentrations of SOD and GPx compared to the control group (copper-deprived). However, Dezfoulian *et al.* (2012) showed that the use of copper in lambs increased total antioxidant capacity (TAC) from day 0 to 65, but the source and level of copper did not significantly affect SOD activity. Additionally, the study by Sansinanea *et al.* (1993) indicated that the use of copper in sheep reduced SOD levels. Alterations in antioxidant enzyme activities and the loss of some nonenzymatic antioxidants are the most common signs of oxidative stress (Alkhudhayri *et al.* 2020). Glutathione peroxidase (GPx) is a key antioxidant enzyme that uses the cofactor glutathione to convert hydrogen peroxide to water. Glutathione peroxidase is largely found in the cytoplasm and has great specialization for the detoxification of both lipid and organic hydroperoxides (Bun *et al.* 2011). However, in the present study, the experimental treatments did not have a significant effect on the antioxidant capacity of suckling calves.

Plasma Cu concentration was increased by NCu factor on day 65 of this experiment. Organic and inorganic copper supplements did not have a significant effect on copper plasma concentrations. Some researchers stated that plasma copper concentrations were higher in Simmental steers that were fed with basic diet (9.8 mg Cu/kg DM) supplemented with 10 or 40 mg copper per kg/DM until day 56 of the growing period. Also, the amount of plasma copper remained higher when it is supplemented with 10 or 40 mg of copper per kg/DM in the basal diet (5.1 mg Cu/kg DM) during the whole 28 days of the final phase of the sampling period (Engle and Spears, 2001).

The observed differences between these studies may be due to genetic, environmental, and dietary factors, including the concentration of copper in the basal diet, level, and duration of copper supplementation (Engle and Spears, 2001). Chang *et al.* (2022) reported that the use of mannan oligosaccharide in diet of calves had no significant effect on blood concentration of copper. There are limited studies regarding the effects of prebiotics on plasma copper concentration, so it was not possible to compare with the results of other studies.

CONCLUSION

The results of this study indicated that the addition of PBC to the diet of suckling calves improved growth performance and reduced the incidence of diarrhea. Meanwhile, NCu contributed to increased copper concentration and glutathione peroxidase activity. Overall, the positive effects of both supplements were observed in enhancing health status and blood parameters. However, there was no significant evidence of an interactive effect between NCu and PBC on the performance of the calves.

ACKNOWLEDGEMENT

This project was completed with support from the authors organisations and, in particular, the University of Mohaghegh Ardabili. The authors also thank the dairy cow station personnel of the Moghan Agro-Industrial Complex for their assistance.

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