

Journal of the Hellenic Veterinary Medical Society

Vol 77, No 1 (2026)

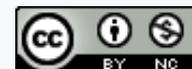


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doi: [10.12681/jhvms.41607](https://doi.org/10.12681/jhvms.41607)

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To cite this article:

Ahmadikhatir, A., Ghoorchi, T., Toghdory, A., & Asadi, M. (2026). Effect of Chromium on Performance, Antioxidant Status, and Hormonal Parameters of Holstein Calves under Heat Stress. *Journal of the Hellenic Veterinary Medical Society*, 77(1), 10197–10210. <https://doi.org/10.12681/jhvms.41607>

Effect of Chromium on Performance, Antioxidant Status, and Hormonal Parameters of *Holstein* Calves under Heat Stress

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ABSTRACT: The mineral status of newborn calves, including chromium (Cr), is critical for early growth, stress mitigation, and overall health. Consequently, it is essential to supply minerals at sufficient levels to suckling calves. In order to evaluate of the effect of feeding milk enriched of inorganic, organic and chromium nanoparticles supplements on performance, blood minerals, biochemical, antioxidant and hormonal of Holstein suckling calves under heat stress, 32 calves with an average weight of 37 ± 3 kg in a complete design were randomly selected with eight replications and four treatments. Experimental treatments included milk without chromium supplement (control), milk containing 3 mg of chromium in mineral form per day, milk containing 3 mg of chromium in the form of chromium-methionine per day, and milk containing 3 mg of chromium in the form of chromium nanoparticles per day. The results showed that milk enrichment with chromium nanoparticles increased the final weight, daily weight gain, and dry matter intake. Treatments receiving all three forms of chromium showed higher chromium, insulin, glucose, SOD, triiodothyronine, tetraiodothyronine, glutathione peroxidase and catalase levels in blood than control group. However, cortisol, total antioxidant capacity and malondialdehyde level of blood in calves consuming different forms of chromium decreased compared to the control group. In general, the use of chromium, especially in the form of chromium-methionine and chromium nanoparticles, is recommended for calves affected by heat stress.

Keyword: Antioxidant; Blood Parameters; Chromium nanoparticles; Heat Stress; Suckling calves

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Date of submission: 28-5-2025

Date of acceptance: 7-11-2025

INTRODUCTION

The successful development of a profitable dairy herd relies heavily on the proper management and care of calves during early life stages (Kumar et al., 2013). Key factors such as optimal nutrition and effective disease control play a vital role in minimizing calf mortality and associated economic losses (Ghavidel et al., 2024). During the suckling period, calves experience considerable metabolic changes and require adequate nutrient intake in both quality and quantity to support healthy development (Ryan et al., 2015). On the other hand, heat stress is one of the most important stress factors which has a devastating effect on farm animals' growth and productivity (Sejian et al., 2018). High temperatures may reduce food intake, growth and welfare of the animals and disrupt the antioxidant defense system (Asadi et al., 2023). Because of the insufficient growth and development of the rumen during the initial weeks of a calf's life, milk serves as the primary source of energy, protein, vitamins, and minerals for growth (Asadi et al., 2018). Milk is often low in certain vitamins and minerals; minerals are necessary nutrients required by livestock and have an important role in the animals' health, growth, and welfare (Toghory et al., 2023). Use of metabolic improver, such as minerals, may promote animal growth and immune system strength (Kaki Soumar et al., 2018). Minerals, with their varied actions, have the ability to improve livestock health and performance by acting as enzyme cofactors or contributing to antioxidant activity (Domínguez-Vara et al., 2023). The use of chromium as a metabolic Improver is one of the options to optimizing production conditions and animal health by enhancing nutrition metabolism and eliminating or reducing stress circumstances (Moreira et al., 2020). Trivalent Chromium's participation in the structure of the glucose tolerance factor improves insulin function and, as a result, the metabolism of carbohydrates, lipids, and proteins (Pechova and Pavlata, 2007). Additionally, chromium improves insulin action by enhancing its binding to cellular receptors, which leads to increased glucose uptake in the cell and decreased negative stress effects (Asadi et al., 2024). Chromium is used in livestock feed in three different forms: inorganic, organic, and chromium nanoparticles. Organic forms of chromium, such as chromium-methionine and chromium nanoparticles, absorb more than inorganic forms (Asadi et al., 2023). Organic chromium exhibits a bioavailability of 25%, whereas the bioavailability of inorganic chromium

is 0.5%. Due to this low bioavailability and potential toxicity, the utilization of inorganic chromium is not recommended (Ohh and Lee, 2007). In contrast, numerous researchers argue that the bioavailability of the inorganic form of elements is generally lower than that of the nano-forms and organic complexes of elements (Phan et al., 2020; Hill et al., 2017). Because of their advantageous bioavailability, organic nanoparticles are therefore likely to be used to improve the nutritional value of food systems (Kargar et al., 2018). Furthermore, nano-minerals have demonstrated significant beneficial effects even at lower doses compared to conventional mineral sources (An-Qiang et al., 2009). The majority of investigators concluded that the immunological activity of chromium supplementation mitigated the adverse effects of heat stress in ruminants and the positive effects of chromium on growth performance are more pronounced under stress conditions (Besong et al., 2009). Research has shown that supplementing heat-stressed calves' diet with chromium increases their weight gain and improves their feed conversion ratio during stressful weaning conditions (Uyanik, 2001). According to scientific literature, newborn calves need 3 to 5 mg of chromium per day (Lashkari et al., 2018). Given the effects of chromium on essential metabolism processes and immune function in livestock, and considering the minimal chromium content in milk, the present study was conducted with the aim of the effect of enriching milk with inorganic, organic and chromium nanoparticles supplements on performance, blood minerals, biochemical, antioxidant and hormonal of Holstein suckling calves under the influence of heat stress.

MATERIALS AND METHODS

All experimental procedures involving animals were approved by the Animal Welfare and Ethics Committee of Gorgan University Agricultural Science and Natural resources, Gorgan, Iran (approval number: N.T. 24/1147). This study was conducted at Mahdasht Milk and Meat Company in Sari, Iran, in July to September of 2024.

Experimental design, animals, and treatments

In this study, thirty-two Holstein calves, with a mean weight of 37 ± 3 kg, were randomly assigned to one of four treatment groups in a completely randomized design beginning two days after birth. The duration of this study was 63 days. Calves were given special health care and colostrum for two days after birth and then entered the experimental design. Treatments

included: 1) control group (no chromium supplement was added to the calves' milk), 2) addition of 3 mg of inorganic chromium supplement to the milk consumed, 3) addition of 3 mg of chromium supplement in the form of chromium-methionine to the milk consumed, and 4) addition of 3 mg of chromium supplement in the form of chromium nanoparticles to the milk consumed. Calves received milk daily at a rate of 10% of their body weight, in two doses. before feeding the milk, the chromium supplements for each treatment were completely dissolved in the milk and ingested by the calves. Calves had free access to water and feed throughout the experiment. The composition and nutrients of the starter diet are listed in Table 1.

Performance and dry matter intake

Calves were given starter feed every day, and the amount of feed left for each animal was weighed and recorded every day in order to analyze performance parameters and weight changes. Calves were weighed every 21 days. The difference between each animal's residual feed and the provided ration was used to compute each animal's feed consumption. Additionally, each animal's residual feed the following day was used to calculate the increase in the amount of feed supplied to the animals. Therefore, the animal's feed was raised if it left less than 10% of the remaining feed for three days in a row (Asadi et al., 2024). After 16 hours of hunger, the animals were weighed once a week using a digital scale to determine weight changes.

Blood samples, and laboratory analyses

On the last day of the experiment, blood was collected into K₂EDTA tubes approximately 4 hours after morning feeding from every (Asadi et al., 2024). Blood samples were centrifuged at 3000g for 10 minutes at room temperature in order to produce the plasma. Ultimately, until the analysis, plasma samples were frozen at -20°C.

Concentrations of minerals (chromium, calcium, phosphorus, zinc, copper, iron) and activities of aminotransferase, alkaline phosphatase, alanine aminotransferase, superoxide dismutase, cholesterol, triglyceride, urea, glucose, LDL, HDL, VLDL, total protein (TP), albumin, and globulin were measured using kits manufactured by Pars Azmoun Company (Pars Azmoun, Iran). The photometric spectrometer (UV-Vis model 365 LAMBDA, Perkinelmer, NY, USA) with the emission wavelength specific to each element was used. The level of insulin was determined in plasma by the Pars Azmoun Company kit (Pars Azmoun, Iran) by ELISA (ELX808. TEXBio, BioTek Instruments, Frankfurt, Germany). Enzyme activities of glutathione peroxidase (GPX), Cortisol, malondialdehyde (MDA), total antioxidant capacity (TAC) and catalase were also measured using a kit (Rancel, product of Randox Company) according to the manufacturer's instructions and by a spectrophotometer. The concentration of tetraiodothyronine (T4) and triiodothyronine (T3) hormones was measured using the kit of Antigen Gostar Company by the Elizarider device (Togh dory et al., 2023).

Table 1. Ingredient composition and nutrient content of the starter diet (% of DM unless otherwise stated)

Ingredients	Percent	Dietary nutrients	Amount
Alfalfa	10.00	Dry matter (%)	87.31
Soybean meal	30.60	Metabolic energy (kcal/kg)	3.27
Corn grain	32.90	Crude protein (%)	21.69
Barley grain	18.00	Neutral detergent fiber (%)	27.15
Full fat soy	2.70	Acid detergent fiber (%)	20.09
Mineral-vitamin supplement*	1.70	Ether extract (%)	0.94
Sodium bicarbonate	1.30	Ash (%)	6.55
Magnesium oxide	0.40	Calcium (%)	0.81
Calcium carbonate	1.40	Phosphorus (%)	0.51
Toxin binder	0.50	Chromium (mg/kg)	0.67
Salt	0.50		

*Mineral-vitamin supplement: 195 g calcium, 90 g phosphorus, 20 g, magnesium, 3 g Zinc, 3 g iron, 280 mg copper, 100 mg cobalt, 100 mg, iodine, 400 mg antioxidants, 10 mg sodium selenite per kg supplement.

Statistical analysis

The present study was conducted with four treatments and eight repetitions in the form of a completely randomized design. GLM software (SAS, 2003) was used for data analysis and Duncan's multiple tests was used to determine significant differences between treatments. The significance level of the results was considered statistically $P < 0.05$. The statistical model of the basic design was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Y_{ij} = dependent variable (each observation), μ = mean of all observations, T_i = treatment fixed effect, e_{ij} = experimental error.

The statistical model of the data repeated example weight and DMI was as follows:

$$Y_{ijk} = \mu + A_i + E_{aik} + B_j + AB_{ij} + E_{bijk}$$

Y_{ijk} = observation related to treatment i and measurement time j in replication k , μ = mean of all observations, A_i = treatment fixed effect, E_{aik} = experimental error, B_j = effect of measurement time j , AB_{ij} = interaction of treatment i and measurement time j , E_{bijk} = sub-error.

Temperature-Humidity Index (THI)

To calculate the temperature-humidity index, the data of the Meteorological Department of Sari city were used. This information was obtained from the station inside the educational and research complex, which was adjacent to the cow farming unit. Environmental data, including ambient temperature and relative humidity, were used to evaluate the

degree of heat stress via the temperature-humidity index (THI). But the THI index is obtained from the combination of dry air temperature and relative humidity. In terms of degrees Fahrenheit, 72 or less means a cool area, 73-77 is mild heat stress, 78-89 is moderate heat stress and above 90 is known as heat stress severe. The current research was conducted between July and September (the range of moderate heat stress, $THI > 80$), as shown in Figure 1. The obtained data included the maximum, minimum and average temperature and daily relative humidity percentage, which was calculated based on the Alfano (Alfano et al., 2011). formula:

$$THI = 0.8 \times \text{maximum temperature} + (\text{minimum relative humidity} / 100) \times (\text{maximum temperature} - 14.4) + 46.4$$

RESULTS

Performance

The results of the effect of milk fortification with chromium supplementation on the performance of suckling calves are shown in Figure 2 and Figure 3. According to the present study, total weight gain, average daily weight gain, total dry matter intake, and final weight increased in the treatment receiving milk enriched with chromium nanoparticles compared to other treatments ($P < 0.05$).

Minerals

The information related to the effect of milk fortification with chromium supplementation on blood minerals in suckling calves is presented in Table

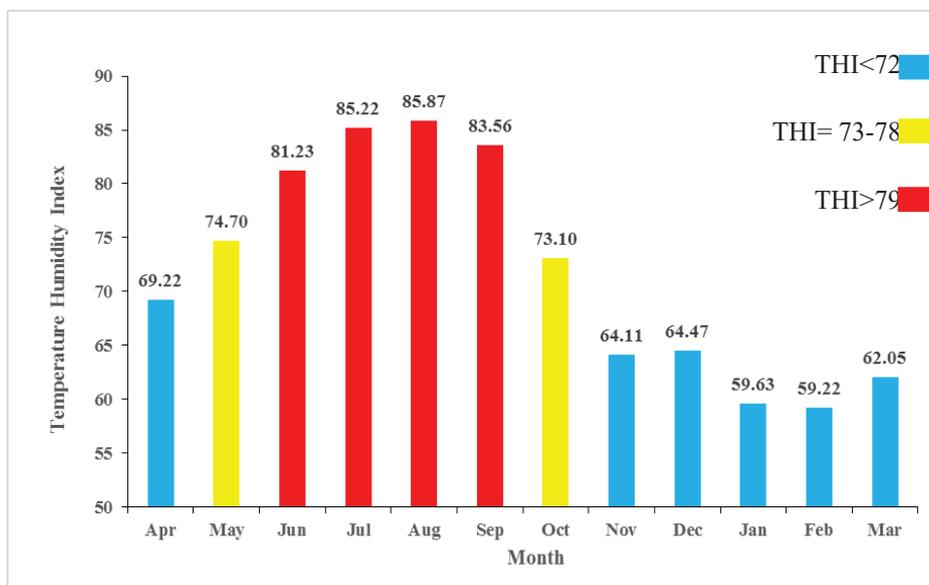


Figure 1. Temperature-humidity index of cow farming.

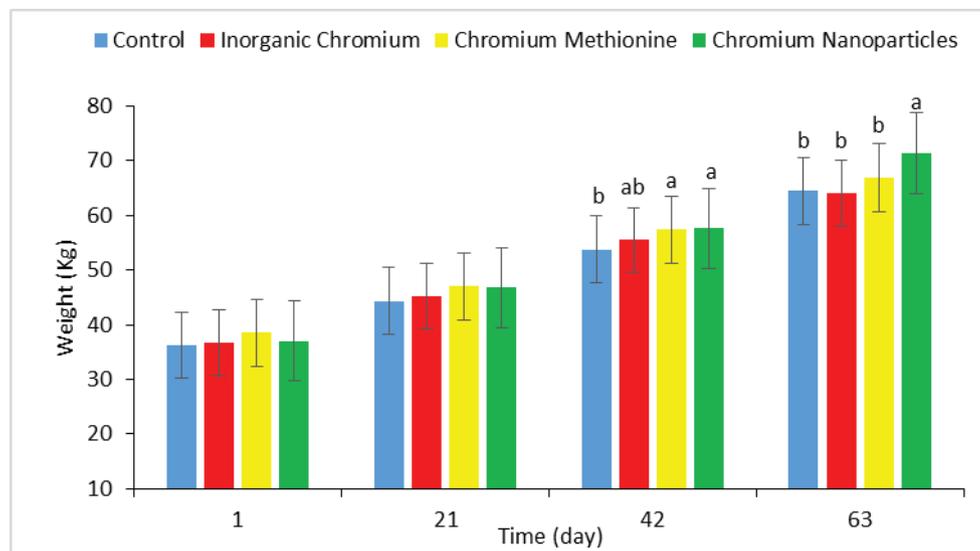


Figure 2. The effect of milk fortification with chromium supplementation on weight of suckling calves.

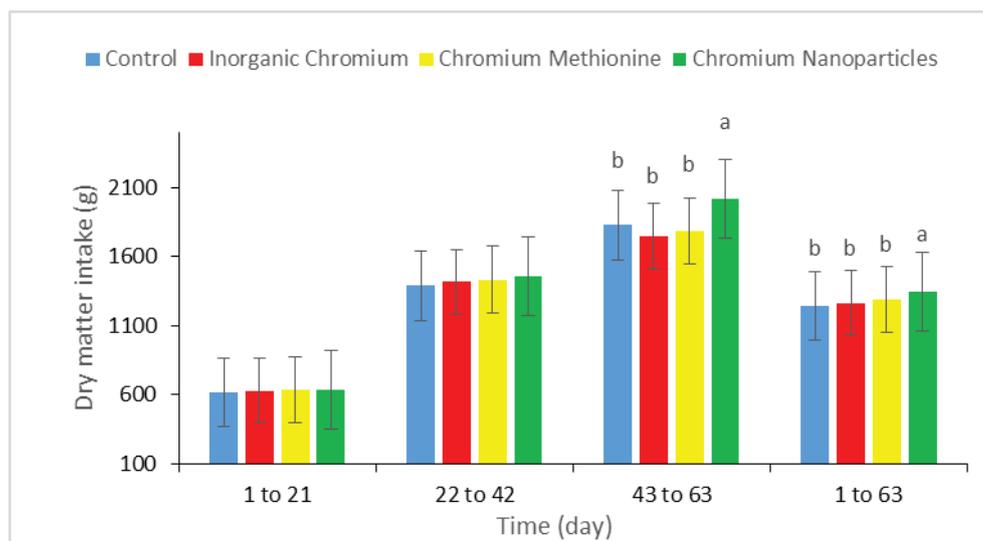


Figure 3. The effect of milk fortification with chromium supplementation on dry matter intake of suckling calves.

2. According to the results obtained in the present experiment, the blood chromium concentration increased in the treatments receiving all three forms of chromium compared to the control group. The blood chromium concentration was significantly higher in the treatments receiving chromium in the form of chromium-methionine and chromium nanoparticles than in the treatments receiving chromium in the form of inorganic chromium and the control treatment ($P < 0.05$).

Biochemical parameters

The information related to the effect of milk fortification with chromium supplementation on blood biochemical parameters in suckling calve is presented in Table 3. According to the obtained results, Biochemical parameters, including cholesterol, triglycerides, and others, showed no significant differences across treatments, except for glucose ($P > 0.05$). The blood glucose increased in treatments receiving chromium in different forms compared to the control group ($P < 0.05$).

Table 2. The effect of milk fortification with chromium supplementation on blood minerals in suckling calves

Blood Minerals	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Phosphorus (mmol/L)	1.74	1.78	1.69	1.70	0.102	0.6762
Calcium (mmol/L)	3.75	3.58	3.70	3.62	0.081	0.2419
Chromium (ng/L)	69.25 ^c	82.33 ^b	93.79 ^a	99.68 ^a	7.414	0.0001
Iron (mg/L)	1.86	1.79	1.82	1.85	0.246	0.4112
Zinc (mg/L)	1.69	1.67	1.62	1.59	0.189	0.5605
Copper (mg/L)	0.80	0.81	0.79	0.78	0.024	0.5644

SEM= standard error of means;

^{a,b}Means with different superscripts in the same row differ significantly (P< 0.05).**Table 3.** The effect of milk fortification with chromium supplementation on blood biochemical parameters in suckling calves

Blood Biochemistry	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Cholesterol (mg/L)	63.62	68.80	66.04	69.44	3.405	0.5533
Triglyceride (mg/L)	34.46	32.69	33.04	35.84	2.412	0.4778
Glucose (mmol/L)	84.89 ^b	92.82 ^a	94.34 ^a	95.35 ^a	4.011	0.0001
Urea (mg/L)	21.71	21.03	22.24	22.10	0.962	0.7841
Total protein (g/dl)	8.04	8.43	8.79	8.81	1.024	0.3442
Albumin (g/dl)	4.98	5.03	5.19	5.45	0.628	0.4181
Globulin (g/dl)	3.05	3.40	3.61	3.36	0.742	0.2986
Albumin: Globulin	1.64	1.48	1.44	1.63	0.149	0.5541
LDL (mg/dl)	8.20	7.89	8.02	7.97	0.482	0.2145
HDL (mg/dl)	32.09	30.54	30.74	31.68	4.029	0.6799
VLDL (mg/dl)	3.80	3.94	3.76	3.96	0.066	0.2144

SEM= standard error of means;

^{a,b}Means with different superscripts in the same row differ significantly (P< 0.05).

Antioxidants and hormones

The information related to the effect of milk fortification with chromium supplementation on blood antioxidants and hormones in suckling calves is presented in Table 4. According to the obtained results, chromium-supplemented treatments showed significantly higher levels of insulin, SOD, T3, T4, glutathione peroxidase, and catalase compared to the control group (P < 0.05). But cortisol, total antioxidant capacity and malondialdehyde of blood in calves consuming different types of chromium decreased compared to the control group (P<0.05).

Liver enzymes

The information related to the effect of milk fortification with chromium supplementation on blood

liver enzymes in suckling calves is presented in Table 5. According to the obtained results, there were no significant differences in liver enzyme concentrations among the treatment groups (P > 0.05).

DISCUSSION

Due to variations in the rate at which chromium is absorbed from the digestive tract, different forms of chromium can have varying effects on livestock growth performance at different concentrations; Additionally, weather and ambient temperature can have an impact on livestock growth performance and how it reacts to chromium consumption (Soltan, 2010). The beneficial impact of chromium supplementation on calves' performance may be linked to chromium's function in protein and carbohydrate

Table 4. The effect of milk fortification with chromium supplementation on blood antioxidants and hormones in suckling calves

Antioxidant and Hormonal Blood	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Insulin (ng/mL)	0.42 ^b	0.50 ^a	0.53 ^a	0.53 ^a	0.089	0.0011
SOD (U/mg of proteins)	15.74 ^b	22.13 ^a	24.34 ^a	25.44 ^a	1.545	0.0001
Cortisol (ng/ml)	5.29 ^a	4.59 ^b	3.88 ^c	3.79 ^c	0.885	0.0001
Triiodothyronine (ng/dl)	9.12 ^b	10.07 ^a	10.34 ^a	11.35 ^a	1.133	0.0084
Tetraiodothyronine (µg/dl)	95.41 ^b	101.26 ^a	108.86 ^a	114.38 ^a	11.074	0.0001
T4: T3	10.46	10.05	10.52	10.07	0.556	0.7469
Glutathione peroxidase (nmol NADPHox/mg)	6.69 ^b	7.18 ^{ab}	7.69 ^{ab}	8.16 ^a	1.044	0.0001
Total Antioxidant Capacity (mmol/l)	1.38 ^a	1.26 ^b	1.18 ^c	1.12 ^c	0.074	0.0005
Malondialdehyde (nmol/ml)	1.35 ^a	1.23 ^b	1.21 ^b	1.19 ^b	0.331	0.0001
Catalase (U/mg of proteins)	0.29 ^b	0.37 ^a	0.39 ^a	0.38 ^a	0.045	0.0001

SEM= standard error of means;

^{a,c} Means with different superscripts in the same row differ significantly (P< 0.05).**Table 5.** The effect of milk fortification with chromium supplementation on blood liver enzymes in suckling calves

Blood Liver Enzymes	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Alanine Phosphatase (U/L)	223.02	224.73	229.71	223.51	18.525	0.7194
Alanine Aminotransferase (U/L)	16.16	15.98	15.65	15.57	1.408	0.6276
Aspartate Aminotransferase (U/L)	58.91	59.33	59.46	59.68	4.130	0.8016

SEM= standard error of means;

metabolism. Insulin's ability to attach to its cell receptor is improved by chromium. Protein synthesis, amino acid transfer efficiency, protein breakdown rate, and lipid and carbohydrate utilization are all increased when insulin signaling is improved (Yari et al., 2010). The beneficial benefits of chromium on calves' performance may be due to the energy generated from these activities (Asadi et al., 2024). then it can be concluded that the use of chromium in calf nutrition, especially chromium nanoparticles that have better accessibility, digestibility and absorption, has probably increased feed intake and subsequently improved calf performance (Asadi et al., 2021). The different results can be due to the type of species, form and level of chromium supplementation, the age of the animal and the development of the animal's digestive system. In a study by Kargar et al. (2018), it was stated that adding organic chromium supplementation to milk improves feed intake in suckling calves. Also, Seifalinasab et al. (2019),

reported that adding 3 mg of organic chromium to the lamb diet resulted in a higher final weight compared to 1.5 mg of organic chromium. In the study of Mousaie et al. (2014), it was stated that adding organic chromium supplement to the diet of sheep increased feed intake and average daily weight gain. Also, Bell et al. (2005) showed in their study that adding organic form supplement to the basal diet linearly increased weight gain of fattening calves. In contrast to the present study, Ghorbani et al. (2012) showed that the use of chromium-methionine supplement in the milk consumed by calves did not affect the intake of starter feed and dry matter consumed throughout the period. The lack of significance of weight gain and feed intake in calves consuming chromium was also reported in the reports of Kraidees et al. (2009) and Bunting et al. (1994). In a study, it was reported that feeding chromium mineral supplement to calves did not have a significant effect on final weight, daily weight gain and dry matter intake (Ryan et al., 2015).

The current study's observations indicate that the treatments that received chromium in the form of chromium-methionine and chromium nanoparticles had a noticeably increased blood chromium content. These findings can be explained by the fact that organic chromium has more than 25% intestinal absorption (Moreira et al., 2020), but inorganic chromium has a bioavailability of around 0.5% (Asadi et al., 2024; Ohh and Lee, 2013). According to several researches, organic complexes and nanoforms often have higher bioavailability than mineral sources of elements (Phan et al., 2020; Hill et al., 2017; Maximino et al., 2018). In one study, supplementation of milk with chromium supplementation increased blood chromium concentrations in calves (Glombowsky et al., 2024). However, Sousa et al. (2020) reported that dietary supplementation with different levels of organic chromium had no significant effect on blood chromium concentrations in stressed calves. Application of different levels of chromium chloride in winter increased blood chromium concentrations but did not affect the concentrations of other minerals in the blood of buffalo calves Moreira (Kumar et al., 2013). Interactions between chromium, calcium and magnesium have been reported by Moonsie-Shageer and Mowat (1993) that chromium supplementation increased calcium and magnesium concentrations. Similarly, Stahlhut et al. (2006) reported that chromium had no effect on plasma copper concentrations in cows and increased plasma copper concentrations in calves. Anderson et al. (1997) found that iron concentrations decreased as chromium concentrations increased. There is relatively little literature on the effects of chromium supplementation on the metabolism of other minerals. Because iron and chromium are both transported linked to transferrin, the interaction between the two minerals has been further investigated (Asadi et al., 2022). Iron and chromium preferentially bind to distinct binding sites at low iron saturation. The two minerals, however, vie for the same binding sites at increasing iron concentrations (Pechova and Pavlata, 2007).

In order to increase the efficiency of glucose absorption by cells, chromium contributes to the stimulation of insulin receptors, an increase in membrane permeability, and eventually insulin uptake by the animal cell (Mayorga et al., 2019). Insulin is good for controlling energy production, boosting volume, protein and fat metabolism, and enhancing immune system function. It also plays a significant role in glucose metabolism and the uptake of amino acids

by muscle cells (Nonaka et al., 2008; Soriani et al., 2013). Reduced glucose burning due to low blood insulin levels causes the glucose to be converted and stored as fat within the cell. Additionally, low insulin causes muscle cells to absorb less amino acids, which slows muscular development. Chromium enhances and boosts the activity of the insulin receptor tyrosine kinase in the inner portion of the cell membrane by binding to the chromomodulin protein. It also activates the transporter, which makes it easier for glucose to enter the cell and, eventually, lowers blood sugar levels (Vincent, 2015).

As the most abundant plasma protein, albumin makes up 35-50% of plasma protein and provides amino acids for the normal recycling of protein in body tissues (Mousavi et al., 2019). Total blood protein concentration is a suitable index for evaluating the long-term effects of diet on nutrient absorption, especially amino acids (Yari et al., 2010). Additionally, albumin is a regulatory and inhibitory factor for lipoprotein production in the liver, and by reducing this inhibitory factor, more lipoprotein is released into the plasma (Zhao et al., 2019). During stress (e.g., early lactation or high ambient temperatures), the immune system is challenged, and the need for the synthesis of essential proteins like albumin and globulins increases (Asadi et al., 2024).

In a study by Kargar et al. (2018), it was shown that adding 0.05 mg of chromium to the diet of calves increased glucose concentrations and insulin-to-glucose ratios. Also, adding chromium (4 mg/day) to the diet of dairy cows had no effect on serum cholesterol, triglyceride, urea, albumin, and blood glucose levels, but reduced total blood protein concentration (Soltan et al., 2010). Recently, in calves under heat stress, chromium-methionine supplementation had no significant effect on blood glucose, albumin, cholesterol, triglycerides, and blood urea nitrogen concentrations. However, serum globulin concentrations increased in calves receiving chromium during the experimental (Mousavi et al., 2019). Similar results were also reported in studies by Ghorbani et al. (2012) and Yari et al. (2010). Also, Jin et al. (2017) reported that adding 20 and 40 mg/kg dry matter of chromium picolinate to the diet of lactating cows under heat stress conditions did not show any changes in glucose, urea nitrogen, cholesterol and creatinine. It was found that adding chromium to the diet increased the albumin to globulin ratio and also the cholesterol level of dairy cows (Stewart et al., 2012). In a study by Pechova et al. (2002),

increased serum albumin levels were reported in cows receiving chromium supplementation. In line with the present study, studies have shown that the use of chromium supplementation increased the total blood protein concentration of goats (Haldar et al., 2009); however, in studies conducted on fattening lambs, it did not affect the total blood protein concentration (Kaki Soumar et al., 2018; Mousaie et al., 2014). The results of the present study regarding the effect of chromium on total protein concentration are consistent with the results of studies conducted on lambs (Domínguez et al., 2009).

Similar to the present results, it has been reported that chromium supplementation did not have a significant effect on plasma cholesterol concentration in lambs (Domínguez et al., 2009). It also did not have a significant effect on plasma cholesterol and urea concentration in lambs (Domínguez et al., 2009). In another study, adding 370 µg/kg of chromium tripicolinate supplement to the lamb diet did not have a significant effect on blood cholesterol and triglyceride concentration (Forbes et al., 1998). The results regarding the effect of chromium supplementation on blood triglyceride concentrations have been mixed in several studies. A decrease in blood triglyceride concentrations has been reported in response to chromium intake in lambs (Uyanik et al., 2001) and calves (Besong et al., 2001). Also, in another study conducted on goats, it was found that the use of chromium supplementation in the short term had no effect on blood triglyceride concentrations, but in the long term, increasing the dietary chromium concentration reduced blood triglyceride concentrations (Haldar et al., 2009). However, some other studies on lambs reported no effect of chromium supplementation on blood triglyceride concentrations (Soumar et al., 2018; Domínguez et al., 2009). The reasons for the disagreement include differences in diet, especially its fat content and the production stage of the animal, along with the chromium concentration in the diet (Haldar et al., 2009).

Animal welfare may be evaluated using blood parameters. The immune system is known to be suppressed by stress and cortisol. According to research, the decrease in blood cortisol levels may be partly responsible for the response of insulin and glucose concentrations to chromium supplementation (Khan-sari et al., 1998; Lashkari et al., 2018). Studies have indicated that chromium lowers blood cortisol levels, while it is yet unknown exactly how chromium strengthens the immune system (Besong et al., 2001;

Kafilzadeh et al., 2012). It has been demonstrated that high cortisol suppresses the immune system by lowering lymphocyte counts and activity as well as blocking the synthesis and function of antibodies (Sordillo and Aitken, 2009). Also, insulin and cortisol act oppositely on metabolism, and it is well established that chromium supplementation has the potential to increase insulin levels. Therefore, this antagonist and alteration of the general metabolism of the body by chromium is converted into the processes of production, proliferation and activation of immune cells as well as the body's resistance to diseases (Lashkari et al., 2018).

On the other hand, increasing temperature causes heat stress, which changes the physiological performance of animals and affects the sustainability of livestock production (Das et al., 2016; Bagath et al., 2019; Keshri et al., 2019). Under normal conditions, free radicals produced by the body are neutralized by the antioxidant system (Sun et al., 2019). Heat stress increases free radicals, increases the production of lipid peroxides in the cell membrane and weakens the antioxidant defense function, thereby causing oxidative stress (Keshri et al., 2021). Studies show that raising cattle in a hot environment leads to oxidative stress (Bernabucci et al., 2002). When oxidative stress occurs, reactive oxygen species (ROS) are overproduced and accumulated, which affects the capacity of the antioxidant system and predisposes animals to metabolic diseases (Sun et al., 2001). The body has enzymatic antioxidants such as superoxide dismutase and glutathione peroxidase that can protect against the negative effects of ROS (Bernabucci et al., 2002). Superoxide dismutase is one of the important markers that indicate the antioxidant status of animals (Gong and Xiao, 2016). It is a precursor for scavenging free radicals and can resist and block the damage caused by oxygen free radicals and repair damaged cells in time (Sun et al., 2017). Glutathione peroxidase has the ability to scavenge free radicals (Gong et al., 2016). In addition, total antioxidant capacity is a comprehensive indicator of the functional status of the normal antioxidant system (Cao et al., 2014). In contrast, malondialdehyde is a marker of lipid peroxidation, which mainly changes depending on the availability of polyunsaturated fatty acids and antioxidant defense (Kargar et al., 2018).

In the present study, superoxide dismutase and glutathione peroxidase activities and total antioxidant capacity in serum increased, but malondialdehyde concentration decreased, indicating that chro-

mium supplementation improved the antioxidant capacity of heat-stressed calves. It has been reported that serum total antioxidant capacity in treatments receiving black seed and chromium methionine showed a significant improvement in malondialdehyde concentration and increased serum antioxidant activity (Kaki Soumar et al., 2018). In another study, when lambs under transport stress were fed chromium-methionine, malondialdehyde concentration decreased and serum total antioxidant capacity increased, which was consistent with the results of the present study (Mousaie et al., 2014). The chromium supplementation to summer-weaned dairy calves effectively improved antioxidant capacity and prevented lipid (Mousavi et al., 2019). The results also show that adding 0.8 mg of chromium supplement in the form of mineral, organic, and nano-chromium cream reduced the level of malondialdehyde enzyme and increased the activity of glutathione peroxidase in fattening lambs of the Mehraban breed (Ghasemi Kasmaei et al., 2022).

Heat shock protein 72 (Hsp72) has been proposed to inhibit the release of inflammatory mediators under stress conditions (Gehrig et al., 2012). Oxidative stress may be the primary regulator of Hsp72, and elevated expression of Hsp72 may be a sign of enhanced reactive oxygen species generation or oxidation of mitochondrial proteins (Zhang et al., 2014). Consequently, elevated Hsp72 serum levels could result in enhanced antioxidant capacity. To fully understand how chromium affects ruminant antioxidant levels, more research is necessary. In dairy cows bred under heat stress, chromium supplementation has been shown to elevate serum levels of Hsp72 and interleukin-10, an anti-inflammatory mediator, but to have no effect on serum levels of tumor necrosis factor-alpha (Yuan et al., 2014).

Thyroid hormones play an important role in growth, cell division, and regulation of basal metabolism. Similarly, chromium supplementation (0.05 mg/kg body weight) was reported to have no effect on serum T3, T4, and cortisol levels when measured before and after weaning in calves (Kargar et al., 2018). Calves fed 0.04 mg/kg body weight of chromium showed no difference in blood T3 and T4 concentrations. Similar results were observed in buffalo calves (Kumar et al., 2009). However, chromium supplementation at 0.03 mg/kg body weight increased blood T4 concentrations and decreased the T3:T4 ratio in dairy calves (Ghorbani et al., 2012). On the other hand, it has been reported that increas-

ing chromium levels (from 0 to 0.02 and 0.04 mg/kg body weight) resulted in a decrease in serum T4, while blood T3 was only reduced with higher chromium intake in dairy calves (Yari et al., 2010). Decrease in total antioxidant capacity reduces free radicals (Mousaie et al., 2014; Regoli et al., 1995; Sahin et al., 2003; Kumar et al., 2009) a similar result was obtained in this study. High ambient temperatures and heat stress increase serum levels of liver enzymes in ruminants, and increased activity of these enzymes causes liver cell damage in these animals (Giri et al., 2017). During stress, especially in the warm season, the concentrations of the enzymes aspartate aminotransferase (ALT) and alanine aminotransferase (AST) increase. The reduction in serum concentrations of liver enzymes in calves may be due to its potential role in promoting liver health and function. Chromium supports glucose and lipid metabolism, which in turn can positively affect liver function and enzyme activity (Asadi et al., 2024). A healthier liver may lead to reduced levels of liver enzymes in the bloodstream. Chromium increases the catalytic activity of aspartate aminotransferase in sensitive livestock conditions (Melendez et al., 2004).

In an experiment conducted by Hayirli et al. (2001) chromium in the form of chromium-methionine was added to the diet of dairy cows at four levels of 0, 0.03, 0.06, and 0.12 mg/kg metabolic weight from 28 days before calving to 28 days after calving. These researchers concluded that Chromium supplementation had no effect on liver enzyme concentrations. In a study, chromium-methionine supplementation had no effect on alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities in dairy cows (Wu et al., 2021). Chromium supplementation had no effect on blood aspartate aminotransferase and alanine aminotransferase activities in cows during the transition period (Ghandehari et al., 2018). Chromium nanoparticle and chromium picolinate supplementation had no effect on blood alanine aminotransferase in calves during the pre-weaning period (1-90 days) and post-weaning period (90-180 days), but decreased aspartate aminotransferase in both periods and alkaline phosphatase on day 45 and the entire post-weaning period (Bharadwaj et al., 2023). Various studies have reported that chromium supplementation had no effect on blood ALT activity in Holstein cows and sheep (An-Qiang et al., 2009; Uyanik et al., 2001). Similarly, Bagath et al. (2019) reported that chromium supplementation significantly reduced

AST activity in crossbred calves during the summer. However, in one study, chromium supplementation increased aspartate aminotransferase activity in the postpartum period (Pechova et al., 2003). Several studies have reported that chromium supplementation had no effect on blood alkaline phosphatase activity (Sultana et al., 2022; Hala et al., 2014; Patil et al., 2017).

CONCLUSION

The results showed that milk enrichment with chromium nanoparticles increased final weight, daily

weight gain and dry matter intake. Treatments receiving all three form of chromium showed higher chromium, insulin, glucose, SOD, triiodothyronine, tetraiodothyronine, glutathione peroxidase and catalase levels in blood than control group. But cortisol, total antioxidant capacity and malondialdehyde of blood in calves consuming different forms of chromium decreased compared to the control group. In general, the use of chromium, especially in the forms of chromium-methionine and chromium nanoparticles, is recommended in calves affected by heat stress.

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