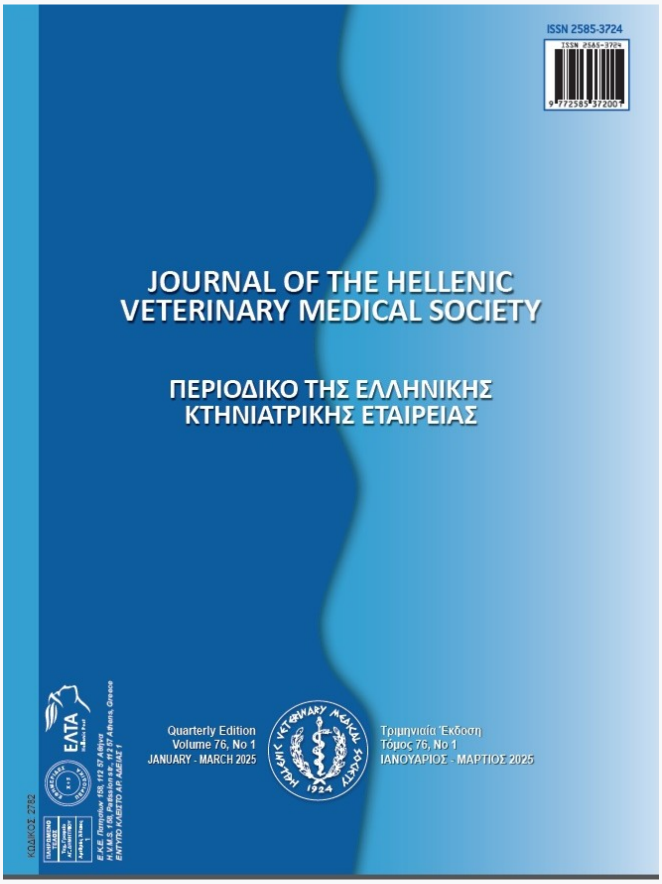
Journal of the Hellenic Veterinary Medical Society

Vol 76, No 1 (2025)



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doi: [10.12681/jhvms.35461](https://doi.org/10.12681/jhvms.35461)

J HELLENIC VET MED SOC 2025, 76 (1): 8575-8588
ΠΕΚΕ 2025, 76 (1): 8575-8588

Research article
Ερευνητικό άρθρο

Effect of dietary supplementation with different forms of chromium on feed intake, milk production and composition, blood parameters of *Afshari* ewes in transition period under the influence of heat stress

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ABSTRACT: The study evaluated the effect of using different forms of chromium in Transition period on feed intake, milk production and composition, blood parameters of *Afshari* ewes and their newborn lambs under the influence of heat stress. Forty pregnant ewes were assigned to four experimental treatments with ten replications from 42 ± 2 days before the expected birth in a completely randomized design. Experimental treatments were included basic diet without chromium supplement (control), basic diet containing 3 mg of chromium in mineral form per kg of dry matter, basic diet containing 3 mg of chromium in the form of chromium-methionine per kg of dry matter and diet the base contained 3 mg of chromium in the form of chromium nanoparticles per kilogram of dry matter. Basic ration was set based on the tables of National Research Council. The experiment started 42 days before lambing and lasted for 84 days. The results showed that there was no significant difference in the weight of the ewes before lambing. But from the beginning of ewes giving birth to 6 weeks after giving birth, the treatments receiving different forms of chromium performed better than the control group of ewes. Dry matter consumption of ewes also had an upward trend from the third to the sixth week after giving birth in the ewes receiving chromium-methionine and chromium nanoparticles ($P < 0.05$). Adding chromium to the diet of ewes under heat stress increases daily milk production and chromium concentration in milk ($P<0.05$). Blood malondialdehyde, cortisol concentrations, glucose, urea and cholesterol in the treatments receiving different forms of chromium also decreased compared to the control group ($P<0.05$). Also, by adding chromium supplement to the diet, the concentration of superoxide dismutase also increased ($P<0.05$). In general, it is recommended to receive chromium, especially in the form of chromium-methionine and chromium nanoparticles, during the transition period of ewes under the influence of heat stress.

Key words: Antioxidant Indices; Chromium; Dairy Ewes; Dry Matter Intake; Milk Composition.

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Date of initial submission: 22-09-2023
Date of acceptance: 20-01-2025

INTRODUCTION

In the perinatal period, ruminants make many metabolic adjustments to support the transition from pregnancy to lactation (Mullins et al., 2012). Before calving, cattle are subjected to many metabolic effects to prepare the physiological conditions of the body in the time after calving and during lactation (Vallimont et al., 2001). Dairy animals produce milk more than their ability to consume energy, as a result, they are in a negative energy balance at the beginning of lactation (Duffield et al., 2012; Moezzi et al., 2012). Heat stress in late pregnancy is aggravated by energy restriction. Environmental conditions and inappropriate nutrition along with physiological changes in late pregnancy and close to delivery, such as changes in the secretion of progesterone, lactoferrin and 17-beta estradiol, cause insulin resistance in the mother (Bell et al., 2005; Moezzi et al., 2012). Due to extensive metabolic changes, newborn animals have high flexibility in absorbing nutrients. These metabolic changes, such as decreased insulin production, cause the mobilization of adipose tissue and the use of non-esterified fatty acids and ketone bodies for milk production (Pechova et al., 2002). In such a situation, the use of management and nutritional methods will reduce the problems during the transfer period and heat stress (Kashfi et al., 2011). One of the solutions to optimize the productive and reproductive conditions of livestock, through improving the metabolism of nutrients and eliminating or reducing stress conditions, is the use of chromium as a metabolic improver (Kajori and Shirazi, 2007). Chromium supplementation may reduce lipolysis in cattle transitioning from pregnancy to lactation, thereby improving feed intake, health, and production (Pantelic et al., 2018; Kargar et al., 2018; Yari et al., 2010). Studies show that adding chromium to the diet of dairy cows improves reproduction and prevents post-partum weight loss (Stahlhut et al., 2006) and increases growth in heifers (Spears, 2019). The conducted studies show that the consumption of organic chromium in the feed of calves under heat stress increases their weight and improves the conversion factor under weaning stress conditions (Deka et al., 2015). In a research conducted on goats with a diet containing chromium, it has been stated that the consumption of chromium causes an increase in daily weight and feed consumption (Spears, 2019). Recent researches have shown that the chromium in feeds and other mineral sources containing chromium in the diet is not able to meet the chromium needs of farmed animals due to very low absorption and low bioavailability, and this situation increases the growth rate, especially in species and breeds that have a higher growth rate are intensified. Therefore, chromium should be provided as an essential and important element in stress conditions through food supplements (Mousavi et al., 2019; Lashkari et al., 2018).

The use of chromium in the ration of ruminants in the amount of 0.5 mg/kg of dry matter of the ration has been declared permissible, which is recommended in the conditions of thermal stress, dry animal feed, fresh animal feed and the occurrence of diseases (Hong et al., 2014). Scientific sources suggested the amount of chromium needed for sheep 3 to 5 mg per day and for dairy cows 15 to 50 mg per day (Mousaie et al., 2014; Kargar et al., 2018; Mousavi et al., 2019). Inorganic chromium bioavailability is around 0.5% (Oh and Lee, 2005), while organic chromium has more than 25% intestinal absorption (Moreira et al., 2020), Therefore, the use of inorganic chromium is not recommended due to its low bioavailability and toxic effects. Among the sources of organic chromium, chromium-methionine has been recognized by important global food and drug organizations as a compound with high bioavailability, impressive metabolic responses and no toxicity complications (Król et al., 2017). Many researchers believe that the bioavailability of mineral sources of elements is usually lower than nano forms and organic complexes (Mousavi et al., 2019; Lashkari et al., 2018; Phan et al., 2020). Due to bioavailability, organic nanoparticles will probably be used to increase the nutritional value of food systems (Hassan et al., 2017; Kargar et al., 2018; Travan et al., 2009). In addition, nano minerals have shown significant beneficial effects even at lower doses than conventional mineral sources (Choi et al., 2010, Yari et al., 2010).

The location of most of Iran's regions in arid and semi-arid regions and the lack of feedstuffs in animal nutrition require special attention to the use of metabolic enhancers. Sheep breeding in arid and semi-arid areas of Iran is facing many problems in terms of supplying the required food materials. Unsuitable weather conditions and the resulting stress have caused a decrease in production and reproduction performance (Kojouri and Shirazi, 2007). The purpose of this study is to investigate the effect of using different forms of chromium in Transition period on feed intake, milk production and composition, blood parameters of *Afshari* ewes under the influence of heat stress.

MATERIALS AND METHODS

Experimental locations, design and treatments

This research was conducted in an industrial sheep breeding unit located of educational and research farm in Bandargaz city in the summer of 2022. Forty pregnant Afshari ewes were assigned to four experimental treatments with ten replications from 42 ± 2 days before the expected birth in a completely randomized design. Experimental treatments were include basic diet without chromium supplement (control), basic diet containing 3 mg of chromium in mineral form per kg of dry matter, basic diet containing 3 mg of chromium in the form of chromium-methionine per kg of dry matter and basic diet containing 3 mg of chromium in the form of chromium nanoparticles per kilogram of dry matter. The experiment started 42 days before lambing and lasted for 84 days. The ewes were all balanced in terms of age, number of embryos, weight, expected lambing date and color. The accuracy of pregnancy and the possible time of delivery were confirmed through synchronization of estrus and ultrasound of the ewes before the experiment. The basic ration was set based on the tables of National Research Council (2007), which is shown in Table 1, and supplements were added to the basic ration daily (Kargar et al., 2018).

In addition to the beginning, the time of lambing and the end of the cycle, the ewes were weighed weekly. The remaining feed is weighed every day and the daily feed consumption of the ewes was calculated by deducting from the provided feed. Also, the birth weight of the born lambs and the time of placental discharge of the ewes after birth were also recorded.

To determine the composition of ewe's milk, all the ewe's milk was collected and mixed together, and 15-20 ml of ewe's milk was sent to the laboratory to measure its contents weekly. The concentrations of fat, protein, lactose and milk solids were measured with a Milkoscan device (MilcoscanTMS50-76510). After preparing the samples and preparing the calibration curve, the experiment was performed. An ef-

Table 1. The ingredients and chemical composition of dairy ewe diet

Ingredient (%) DM basis Pre-partum		**Ingredient (%) DM basis Post-partum**	
Wheat straw	5.7	Corn silage	34
Alfalfa	32	Alfalfa	30
Corn silage	30	Corn grain	19.75
Corn grain	18.5	Soybean meal	7.75
Soybean meal	7.2	Beet pulp sugar	2
Beet pulp sugar	1	Wheat bran	2.7
Wheat bran	2.9	Fat powder	2.8
Fat powder	1.5	Calcium carbonate	0.42
Calcium carbonate	0.7	Salt	0.33
Salt	0.3	Mineral-vitamin supplement	0.25
Mineral-vitamin supplement	0.2		
Chemical composition		**Chemical composition**	
Nutrients Diet	Amount	Nutrients Diet	Amount
Metabolic energy (Kcal/kg)	2.44	Metabolic energy (Kcal/kg)	2.54
Crude protein (%)	14.40	Crude protein (%)	14.40
Crude fat (%)	4.10	Crude fat (%)	5.20
Non-fibrous carbohydrates (%)	32.80	Non-fibrous carbohydrates (%)	32.10
NDF (%)	44.20	NDF (%)	40.90
Starch (%)	21.60	Starch (%)	25.00
Ash (%)	7.88	Ash (%)	8.40
Calcium (%)	1.42	Calcium (%)	0.89
Phosphorus (%)	0.71	Phosphorus (%)	0.52
Cr (mg/kg)	0.79	Cr (mg/kg)	0.82

*Each kilogram contained: 140 g of Ca, 20 g of P, 35 g of Mg, 40 g of S, 1200 mg Mn, 1000 mg of Zn, 800 mg of Cu, 8 mg of Co, 10 mg of I, 400 mg of Fe, 10 mg of Se, 20000 mg of Niacin (B3) and 350000, 60000 and 4000 IU of A, D and E respectively and 650 g of Anionic salts.

fective solution to eliminate the effects of the sample matrix is sample digestion, for which a microwave digestion device was used, with high productivity and excellent digestion capabilities. A flame atomic absorption spectrometer model (670-AA-Shimadzu) with a wavelength of 279.5 nm was used to measure the chromium concentration in milk. To calculate the milk production of the total ewe, the amount of production in different weeks was added (Asadi et al., 2023).

In order to measure blood glucose, urea and cholesterol concentration in three weeks before and after the birth of ewes, one and three weeks after the birth of the lambs, blood samples were taken from the jugular vein three hours after morning feeding.

Blood sampling was done using venoject tubes with and without heparin and the samples were immediately centrifuged at 3000 rpm for 10 minutes to separate the plasma and kept at -40°C until the day of the experiment. To measure blood metabolites including glucose, urea, cholesterol, malondialdehyde, ferric reducing antioxidant power, superoxide desmutase and cortisol, Pars Azmoun chemical kits and autoanalyzer (Spain BT 3500) were used (Cortas and Wakid, 1990). Materials such as potassium chloride salt, tris maleate (Sigma, America), thiobarbituric acid (Sigma, America), sodium hydroxide, pyridine (Merck, Germany) and n-butanol (Merck, Germany) solutions were used to synthesize malondialdehyde. At the beginning of the work, solutions of potassium chloride 0.16 mol/liter, tris maleate 0.2 mol/liter, thiobarbituric acid 0.8 mol/liter, sodium hydroxide 1 normal and pyridine-n-butanol solution with a volume ratio of 3:1 were prepared. Then, the optical absorption of the samples was read with a spectrophotometer at a wavelength of 528 nanometer (Asadi et al., 2023).

In order to measure FRAP, the ferric tripyridyl triazine complex is reduced by the antioxidants in the sample and turns into Fe^{2+} ferrous form, which is dark blue in color. The intensity of the color created in the spectrophotometric device is measured at a wavelength of 593 nm. The intensity of the created color is directly related to the total reducing power of the antioxidants in the reaction mixture. Iron sulfate (Merck, Germany), sodium acetate (Merck, Germany) and tripyridyltriazine (Sigma, USA) were used in this experiment. In this experiment, iron sulfate solution containing Fe^{2+} was used as a standard (Asadi et al., 2022).

Statistical Analysis

The present study was conducted with four treatments and ten repetitions in the form of a completely randomized design. GLM software (SAS, 2003) was used for data analysis and Duncan's multiple test 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:

$$Yij = \mu + Ti + eij$$

Yij = dependent variable (each observation), μ = mean of all observations, Ti= treatment fixed effect, eij= experimental error.

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

$$Yijk = \mu + Ai + Eaik + Bj + ABij + Ebijk$$

Yijk = observation related to treatment i and measurement time j in replication k, μ = mean of all observations, Ai = treatment fixed effect, Eaik = experimental error, Bj = effect of measurement time j, ABij = interaction of treatment i and measurement time j, Eaik = sub-error.

Temperature-humidity Index

To calculate the temperature-humidity index, the data of the Meteorological Department of Bandargaz city were used. This information was obtained from the station inside the educational and research complex, which was adjacent to the sheep farming unit. Information related to dry air temperature and relative humidity, wind speed, and rainfall amount help a lot to detect heat stress. But the thermal-humidity index (THI) 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 (2011) formula:

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

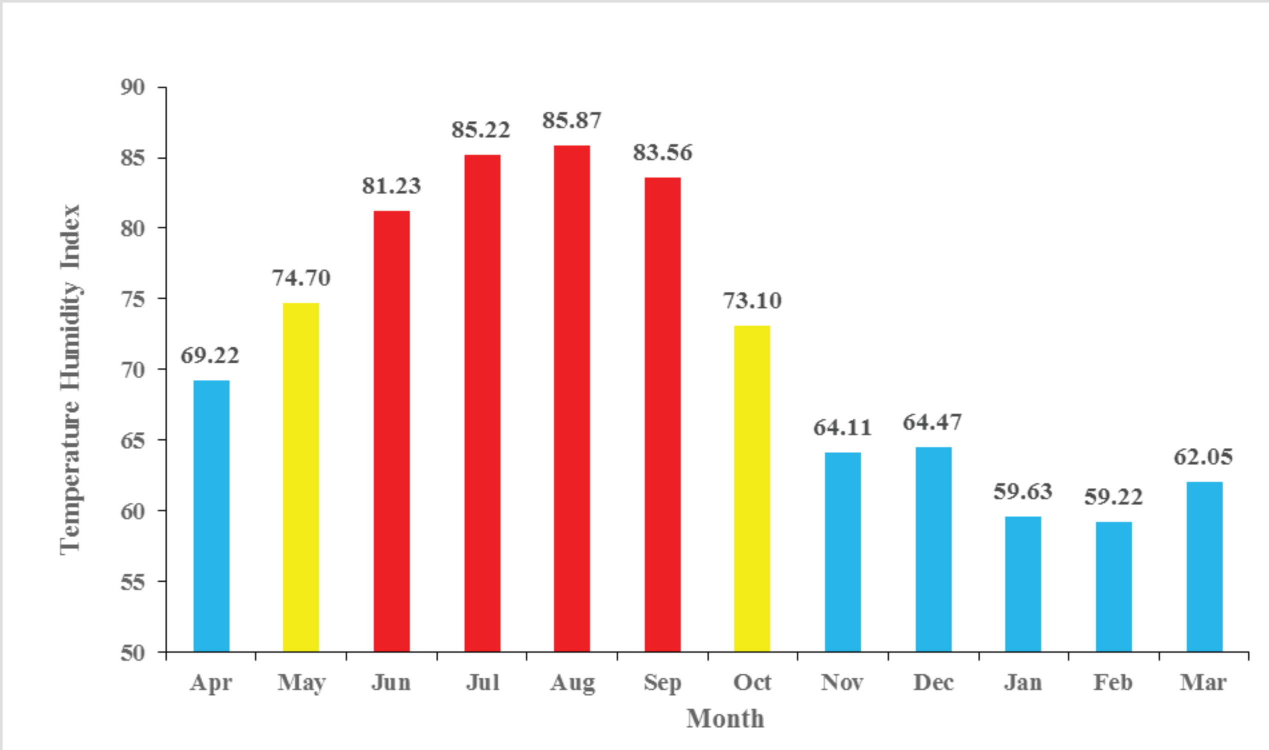

Figure 1. Temperature-humidity index of sheep farming

RESULTS

The results related to the performance of ewes and their lambs are shown in Table 2. Also, in Figure 2 and 3 the results related to the performance and dry matter intake of the ewes by week are presented. As shown, there is a significant difference in terms of birth weight and final weight of ewes between the treatments receiving different forms of chromium and the control treatment ($P<0.05$). So that the ewes receiving methionine cream and cream nanoparticles have the highest weight compared to the other two treatments at the time of birth and final weight. The results of the present study showed that adding cream to the diet of ewes during the transition period increased dry matter consumption before and after lambing ($P<0.05$), and the treatments receiving chromium methionine and chromium nanoparticles had the highest consumption of dry matter compared to the two treatments. The results of the present study show that the weight of lambs born from ewes receiving chromium nanoparticles was higher than the control treatment ($P<0.05$), although there was no significant difference between the treatments receiving different forms of chromium. Also, the weight of the lambs receiving chromium nanoparticles on the 42nd day of the experiment was more than the lambs of the control group ($P<0.05$).

Table 2. The effect of consumption of different forms of chromium on performance of ewes and lambs

Weight performance traits	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Initial weight of ewes (kg)	54.54	54.22	55.22	54.95	1.553	0.7237
Parturition weight of ewes (kg)	46.82 ^{b}	47.25 ^{ab}	48.89 ^{a}	49.10 ^{a}	0.987	0.0466
Final weight of ewes (kg)	44.78^{b}	45.97^{ab}	46.79^{a}	47.08^{a}	1.546	0.0484
Average of DMI before parturition (g)	1354.27^{b}	1349.85^{b}	1404.70^{a}	1397.30^{a}	36.014	0.0202
Average of DMI after parturition (g)	1446.45^{b}	1528.86^{ab}	1605.47^{a}	1581.26^{a}	44.128	0.0341
Birth weight of lambs (kg)	3.40^{b}	3.90^{ab}	4.05^{ab}	4.40^{a}	0.748	0.0267
Weight of lambs on 42nd day (kg)	11.37^{b}	12.70^{ab}	13.08^{ab}	14.40^{a}	1.095	0.0117

SEM= standard error of means;
^{a,b} Means with different superscripts in the same row differ significantly ($P< 0.05$).

The results related to the effect of different forms of chromium on the composition of ewes' milk are presented in Table 3. The results of the present study show that adding chromium to the diet of ewes under heat stress increases daily milk production and chromium concentration in milk ($P<0.05$).

The information related to the effect of different forms of chromium on the antioxidant indices of the blood of ewes and their newborn lambs under heat stress is presented in Table 4. According to the obtained results, blood malondialdehyde and cortisol concentrations in the treatments receiving different forms of chromium also decreased compared to the control group ($P<0.05$). Also, by adding chromium supplement to the diet, the concentration of superoxide desmutase also increased ($P<0.05$). On the other hand, addition of chromium supplement had no effect on FRAP concentration of ewes and their lambs. Also, there was no significant difference in cortisol hormone of lambs.

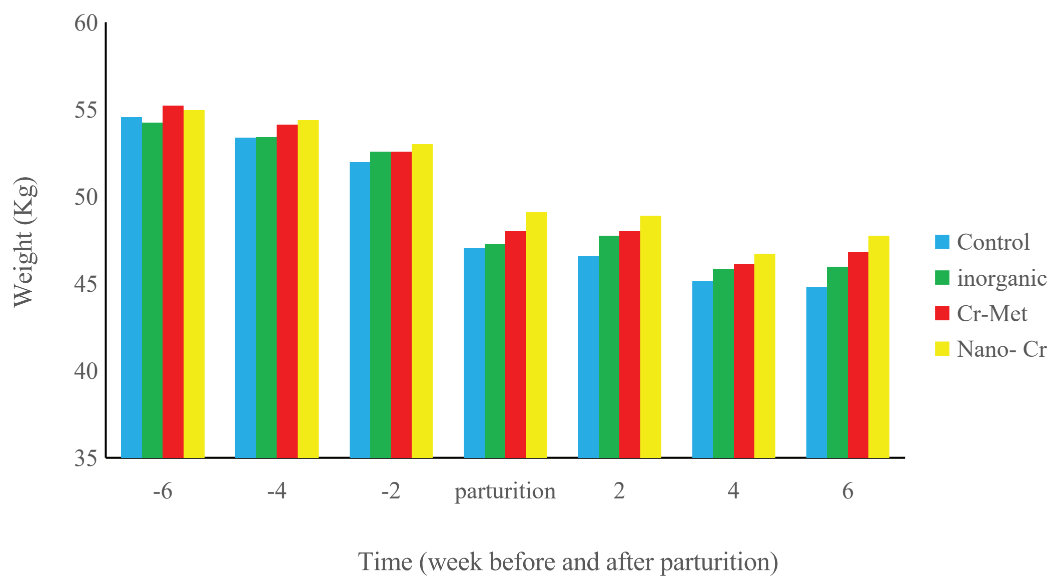

Figure 2. The effect of consumption of different forms of chromium on weight performance of ewes (by week)

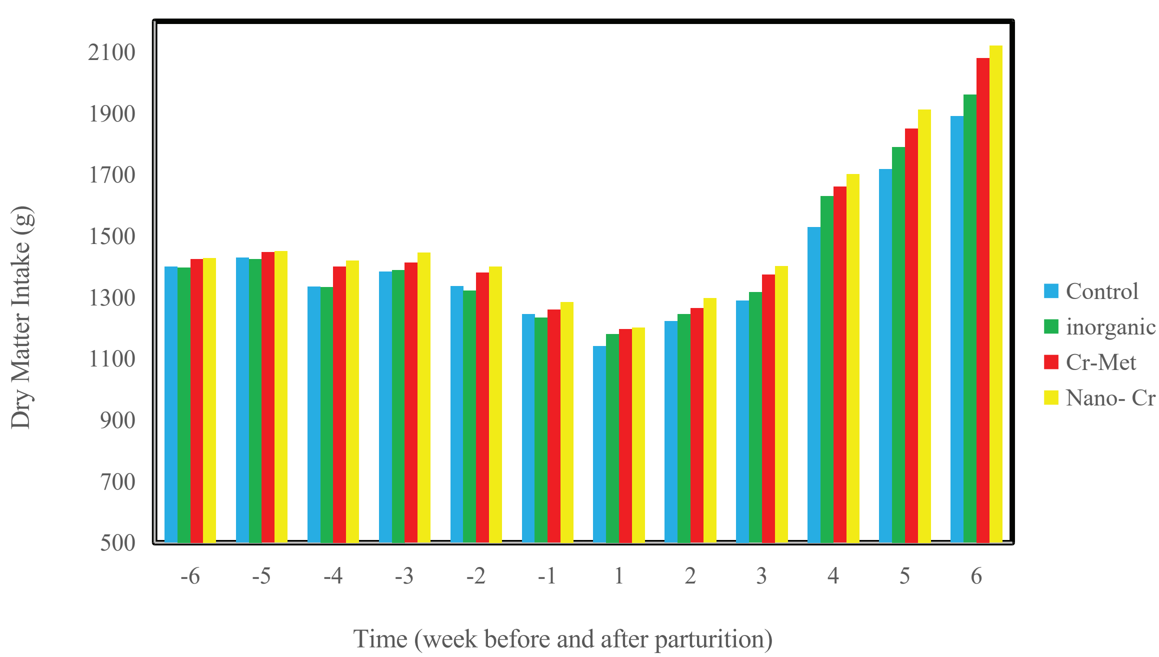

Figure 3. The effect of consumption of different forms of chromium on dry matter intake of ewes (by week)

Table 3. The effect of consuming different forms of chromium on the milk composition of ewes

Milk composition	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Daily milk yield (kg)	2.29[b]	2.35[b]	2.49[ab]	2.64[a]	0.052	0.0062
Fat (%)	6.56	6.85	6.84	6.87	0.126	0.3653
Protein (%)	3.29	3.25	3.27	3.35	0.064	0.7200
Fat: protein	1.99	2.11	2.09	2.06	0.051	0.4134
Lactose (%)	4.82	4.84	4.93	4.95	0.077	0.2665
Total solid matter (%)	14.67	15.05	15.04	14.98	0.206	0.5622
Solids not-fat (%)	8.11	8.20	8.20	8.10	0.117	0.9033
Chromium (µg/L)	35.90[d]	74.50[c]	117.00[b]	166.10[a]	2.152	0.0001

SEM= standard error of means;
[a,b] Means with different superscripts in the same row differ significantly (P< 0.05).

Table 4. The effect of consumption of different forms of chromium on blood antioxidant indices of ewes and their lambs

Antioxidant indices	Control	Different forms of chromium			SEM	P-Value
		Inorganic	Cr-Met	Nano- Cr		
Blood antioxidant indices (Ewes)						
MDA (nmol/ml)	1.36[a]	1.25[ab]	1.14[b]	1.02[b]	0.042	0.0001
SOD (U/mg of proteins)	19.11[c]	22.01[b]	27.79[a]	29.02[a]	3.896	0.0001
FRAP (µmol/ml)	3.62	3.58	3.67	3.70	0.064	0.6847
Cortisol (µg/dl)	5.26[a]	4.85[ab]	4.01[b]	3.87[b]	0.524	0.0001
Blood antioxidant indices (lambs)						
MDA (nmol/ml)	1.42[a]	1.30[ab]	1.23[b]	1.20[b]	0.037	0.0001
SOD (U/mg of proteins)	14.66[c]	17.03[b]	21.66[a]	23.00[a]	3.017	0.0479
FRAP (µmol/ml)	5.19	5.25	5.31	5.28	0.278	0.7849
Cortisol (µg/dl)	4.92	4.88	4.79	4.71	0.689	0.8142

SEM= standard error of means;
[a,b] Means with different superscripts in the same row differ significantly (P< 0.05).

The results related to the effect of different forms of chromium on the blood parameters of ewes and lambs born from them are presented in Figures 4 and 5. The results showed that the addition of different forms of chromium supplements to the diet of ewes caused a significant decrease in blood glucose concentration in three and one weeks before and after parturition compared to the control group ($P<0.05$). The highest amount of glucose is related to the control treatment and the lowest amount is related to chromium nanoparticles. But the blood glucose concentration of the lambs was not affected by the experimental treatments.

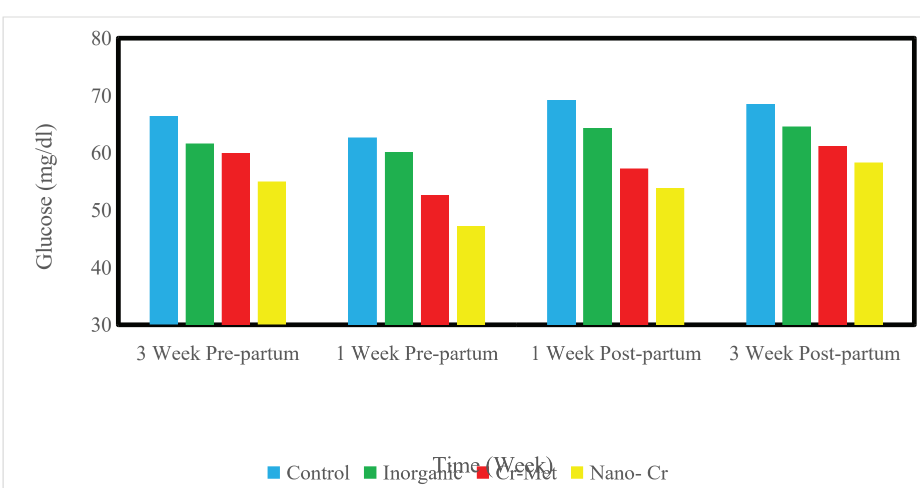

Figure 4. The effect of using different forms of chromium on blood glucose concentration of ewes

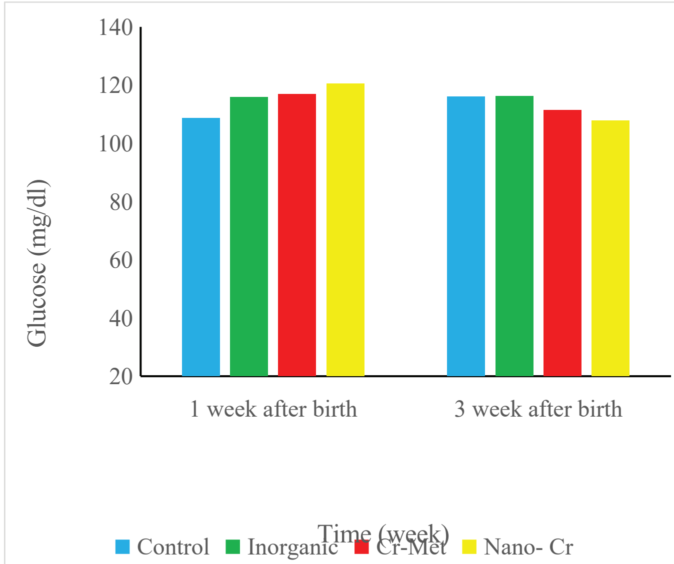

Figure 5. The effect of using different forms of chromium on blood glucose concentration of lambs

The information related to the effect of different forms of chromium on blood urea nitrogen concentration of ewes and their lambs is presented in Figures 6 and 7. According to the obtained results, blood consumption of different forms of chromium had no effect on blood urea nitrogen concentration in three weeks before and after the birth of ewes and one and three weeks after the birth of lambs. But by adding chromium supplement to the diet of ewes, blood urea nitrogen concentration decreased in one week before and after lambing compared to the control treatment (P<0.05).

The results related to the effect of different forms of chromium on blood cholesterol concentration of ewes and lambs born from them are presented in figures 8 and 9. The results showed that the addition of different forms of chromium supplements to the diet of ewes caused a significant decrease in blood cholesterol concentration in three and one weeks before and after parturition compared to the control group (P<0.05). Addition of chromium to the diet of transition period ewes did not affect the cholesterol concentration of their lambs after one week of birth. However, in the third week of birth, the treatments receiving chromium-methionine and chromium nanoparticles had lower cholesterol concentration than the other two treatments (P<0.05).

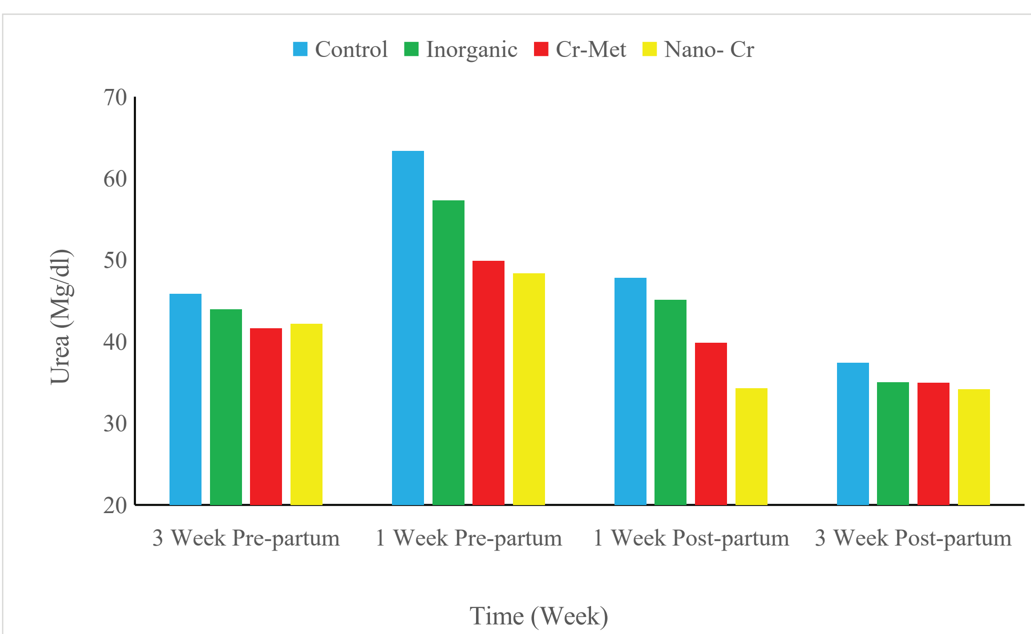

Figure 6. The effect of using different forms of chromium on blood urea concentration of ewes

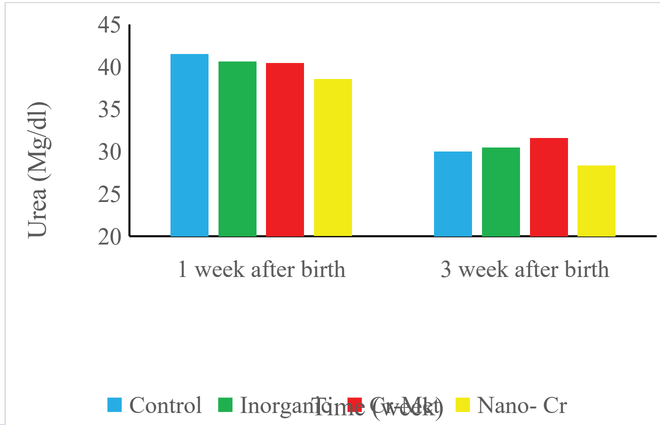

Figure 7. The effect of using different forms of chromium on blood urea concentration of lambs

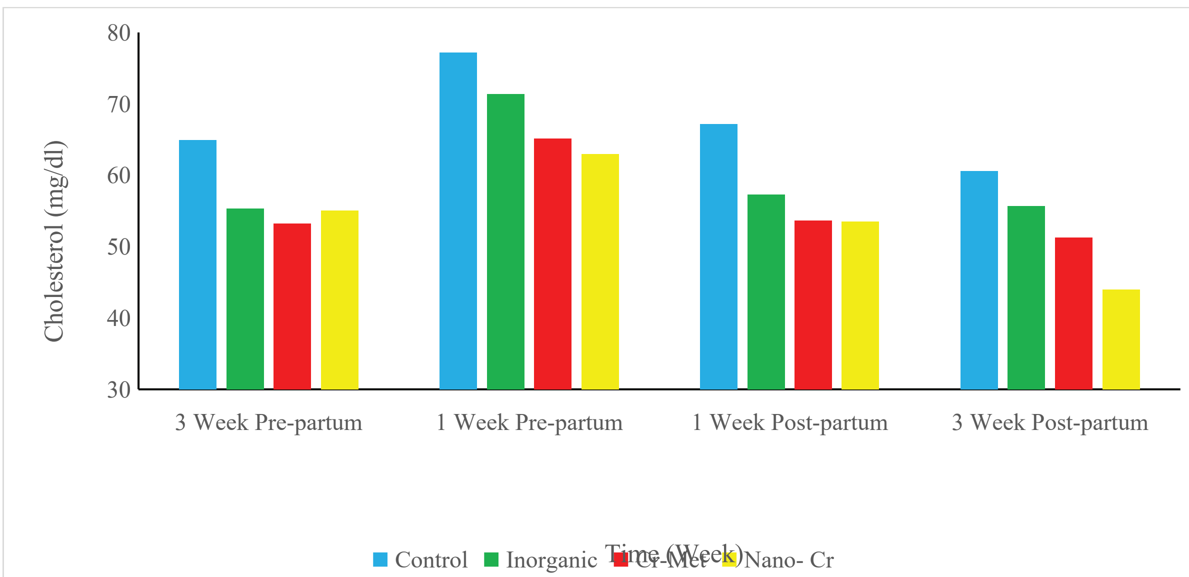

Figure 8. The effect of using different forms of chromium on blood cholesterol concentration of ewes

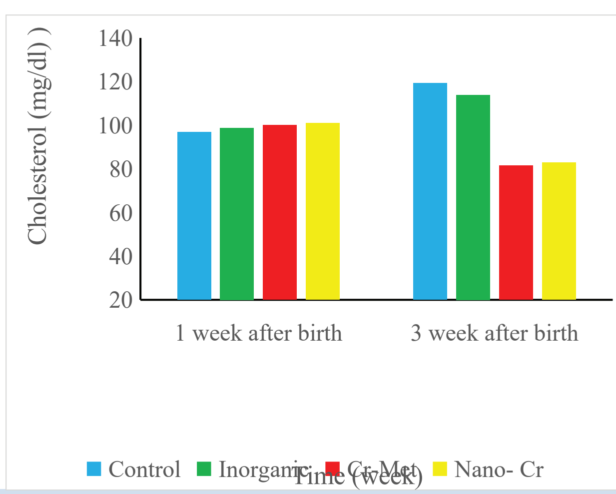

Figure 9. The effect of using different forms of chromium on blood cholesterol concentration of lambs

DISCUSSION

For this purpose, in line with the results of the present study, it was shown in a report that ewes receiving chromium-methionine and chromium nanoparticles had less weight loss than the other two treatments after delivery (Yari et al., 2018). Calves fed with chromium-methionine supplemented diet had heavier body weight compared to control group calves. Lambs fed with three milligrams of chromium per kilogram of feed had numerically higher final weight compared to the control group and lambs receiving 1.5 milligrams of chromium (Seifalinasab et al., 2019). As Figure 3 shows, chromium supplementation had no effect on the dry matter intake of ewes from six weeks before lambing to two weeks after lambing. But from the third to the sixth week after giving birth, the dry matter consumption of the ewes had an upward trend ($P<0.05$) and the highest dry matter consumption was related to the treatments receiving chromium-methionine and chromium nanoparticles. According to the present research, the results show that the consumption of different levels of chromium supplements in goats (Haldar et al., 2009), female lambs (Mousaie et al., 2014), buffaloes (Deka et al., 2015) and dairy cows also cause an increase in consumption. Feed has been reported (Soltan, 2010). In another study, levels of zero to 0.5 mg chromium in lambs did not affect feed intake (Dallago et al., 2011). These variable results may be differences in the type of chromium supplementation, the amount of stress to which the animals are exposed, the physiological stage, body weight and age, the amount and bioavailability of chromium in the basal diet, or the bioavailability of supplements (Yari et al., 2010). ; Spears, 2019). The positive effect of chromium supplementation on the performance of ewes can be related to the role of chromium in the metabolism of carbohydrates and proteins. Chromium enhances the action of insulin by improving its binding to its cell receptor. Enhancing insulin signaling increases the production of proteins, improves the efficiency of amino acid transfer, decreases the rate of protein breakdown, and increases the use of lipids and carbohydrates (Yari et al., 2010). The energy released from these processes can be responsible for the positive effects of chromium on the performance of ewes (Domínguez-Vara et al., 2009).

Changes in milk composition parameters may have economic consequences on the farm. Therefore, several studies investigated the effect of chromium supplementation on milk composition and reported that the percentage of fat, lactose, protein, and solid fat in early lactation was not affected by adding chromium to cows' diets (Soltan, 2010; Vargas-Rodriguez et al., 2014; Yasui et al., 2014). Also, the research of Al-Saiady et al (2004) shows that the addition of chromium to the diet did not affect the milk composition of dairy cows under heat stress in the middle of the lactation stage. Bryan et al. (2004) and Smith et al. (2005) investigated the effect of chromium supplementation on the milk composition of primiparous dairy cows and confirmed that milk composition including fat, protein and lactose percentage did not change with chromium supplementation. In line with the current research, the statistical analysis of data related to milk composition of dairy cows showed that adding chromium to the diet had no significant effect on the amount of milk composition (Shahbedini et al., 2019). Also, the obtained results showed that the percentage of protein, lactose, fat and total solid matter was not significantly different between the treatments (Sobhanirad et al., 2010). Contrary to the results obtained in this research, they reported that increasing chromium-methionine increases lactose and milk fat (Hayirli et al., 2001). This difference could be due to the fact that chromium may increase glucose utilization by insulin-dependent organs such as muscle and adipose tissue (McNamara and Valdez, 2005). Also, higher lactose content may be explained by increased gluconeogenesis (Kafilzadeh et al., 2012). An increase in gluconeogenesis leads to an increase in the content of lactose in the mammary glands, because glucose is the only source of lactose synthesis in milk. The increase in milk lactose in cows supports the hypothesis that chromium supplementation alters blood glucose by increasing gluconeogenesis (Lashkari et al., 2018; Shahbedini et al., 2019).

Blood parameters can be used to evaluate animal welfare. Stress and cortisol are known suppressors of the immune system. Researchers reported that the response of glucose and insulin concentrations to chromium supplementation may be partially attributed to the reduction of blood cortisol levels (Khansari et al., 1990; Lashkari et al., 2018; Seifalinasab et al., 2019). Although the specific pathway by which chromium improves the immune system has not yet been determined, studies have shown that chromium decreases serum cortisol levels (Besong et al., 2001; Kafilzadeh et al., 2012). Higher cortisol has been shown to be immunosuppressive by inhibiting the production and function of antibodies and reducing the number and activity of lymphocytes (Sordillo et al., 2009). Also, insulin and cortisol act antagonistically on metabolism and it is well established that chromium supplementation has the potential to increase insulin levels. Therefore, this antagonist and the change of the body's general metabolism by chromium become 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, an increase in 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, enhances the production of lipid peroxides in the cell membrane and weakens the antioxidant defense function, thus causing oxidative stress (Marc´en et al., 2017). Studies show that rearing 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, predisposing animals to metabolic diseases (Gong and Xiao, 2016). Fortunately, the body has antioxidant enzymes such as superoxide desmutase and glutathione peroxidase that can protect against the negative effects of ROS (Das et al., 2016). Superoxide dismutase is one of the important markers that shows the antioxidant status of animals (Gong and Xiao, 2016). It is the primary substance for eliminating free radicals and can resist and block the damage caused by oxygen free radicals and repair damaged cells in time (Gong and Xiao, 2016; Sun et al., 2017). In contrast, malondialdehyde is an indicator of lipid peroxidation, which mainly changes depending on the availability of polyunsaturated fatty acids and antioxidant defense (Kargar et al., 2018). Found that chromium supplementation to dairy calves weaned in summer effectively improves antioxidant capacity and prevents lipid peroxidation (Mousavi et al., 2019). In the present study, superoxide desmutase activities increased in serum, but malondialdehyde concentration decreased, indicating that chromium supplementation improves the antioxidant capacity of heat-stressed ewes, which was consistent with research (Moeini et al., 2018). In another study, when lambs under transport stress were fed with chromium-methionine, the concentration of malondialdehyde decreased, which is consistent with the results of the present study (Mousaie et al., 2014). Also, the results show that the addition of 0.8 mg of chromium supplement in the form of mineral, organic and nano chromium cream has reduced the amount of malondialdehyde enzyme in Mehraban breed lambs (Ghasemi Kasmaei et al., 2022).

Chromium plays a role in stimulating insulin receptors, increasing membrane permeability, and ultimately insulin acceptance by cells in cattle, and in this way, it is effective in increasing the efficiency of glucose absorption by cells (Mayorga et al., 2019). In addition to playing the main role in glucose metabolism, insulin plays a role in the uptake of amino acids by muscle cells; Therefore, it is effective in regulating energy production, increasing volume, protein and fat metabolism and improving immune system function (Nonaka et al., 2008; Soriani et al., 2013). A low level of insulin in the blood means a decrease in the burning of glucose and, as a result, its conversion and storage in the form of fat in the cell. Also, the low level of insulin leads to a decrease in the absorption of amino acids by muscle cells, and in this way, the speed of muscle growth decreases. By binding to the chromodulin protein, chromium improves and increases the tyrosine kinase property of the insulin receptor in the inner part of the cell membrane, and by activating the transporter, it facilitates the entry of glucose into the cell and ultimately leads to a decrease in blood sugar levels. Vincent, 2015). In a research, adding chromium (4 mg/day) to the ration of dairy cows had no effect on serum blood glucose level (Soltan, 2010). Recently, in calves under heat stress, the use of chromium-methionine had no significant effect on glucose concentration. In a research, Kargar et al. (2018) showed that the addition of 0.05 mg of chromium to the diet of calves increased the concentration of glucose and the ratio of insulin to glucose. Similar results were reported in the studies of Ghorbani et al. (2012) and Yari et al. (2010). Also, Jin et al. (2017) reported that the addition of 20 and 40 mg/kg of dry matter of chromium picolinate to the diet of lactating cows under heat stress conditions did not show any changes in blood glucose. The inconsistency between studies seems to be related to factors such as the type and intensity of the stressful situation the animal is placed in, the basal diet, the type of livestock and the chemical form of the supplement. Chrome has to be connected.

Blood urea nitrogen is the final product of animal feed nitrogen. The digestive system is the main place of absorption of ammonia or blood urea, but other tissues such as muscle and kidney have the ability to produce ammonia (Stewart et al., 2012). Therefore, it is expected that with an increase in the nitrogen source of the feed, the concentration of blood urea will also increase. In a situation where there are many changes in rumen ammonia concentration, changes in blood urea are also expected. In accordance with these results, it has been reported that chromium supplementation did not have a significant effect on plasma urea concentration of lambs (Dominguez-Vara et al., 2009). The lack of effects of chromium on blood urea concentration is consistent with other reports (Kitchalong et al., 1995; Bunting et al., 2000) and suggests that liver function in ammonia detoxification is not compromised by chromium intake (Jin et al., 2017). The lower concentration of blood urea in sheep receiving chromium treatments one week before and after birth, in the present experiment, is consistent with the results of (Amoikon et al., 1995; Wang et al., 2007).While (Page et al., 1993; Matthews et al., 2001) reported no effect of chromium on blood urea concentration. The role of chromium in protein storage and reducing its breakdown can also be the reason for the decrease in blood urea concentration (Wang et al., 2009). As the results showed, the concentration of blood urea in the treatments receiving chromium was lower than the control group, which could be due to the consumption of more feed and less weight loss in these groups compared to the control treatment.

In the situation where the animal is in a negative energy balance, increasing the breakdown of lipids to provide energy, causes an increase in the mobilization of fat from the adipose tissue and an increase in non-esterified fatty acids in the blood and their entry into the liver for beta-oxidation. The increase in the entry of fatty acids into the liver causes an increase in the oxidation of fatty acids and, as a result, an increase in the production of acetyl coenzyme A, which increases the synthesis of ketone bodies and blood cholesterol, a decrease in the amount and function of insulin and an increase in glucagon are among the factors that increase the production of ketone bodies. (Kaneko et al., 1997). As it was said, in the present study, the concentration of blood cholesterol before and after delivery in the groups receiving chromium supplementation was lower than the control treatment.

Regarding the effect of chromium on cholesterol, human experiments have shown that chromium reduced blood cholesterol (Anderson, 1995). Kitchalong et al. (1995) and Domínguez-Vara et al. (2009) in lambs and Bunting et al. (1994) in fattening calves reported the reduction of blood cholesterol as a result of chromium consumption. The mode of action of chromium is explained as follows: chromium improves the transfer of glucose through glucose transporter-4 to fat tissue, which is done through a cholesterol-dependent mechanism. They found that chromium treatment reduced plasma membrane cholesterol, which is in agreement with the results related to the improvement effect of chromium on glucose tolerance (Mousaie et al., 2014). Chen et al. (2006) and Pattar et al. (2006). On the other hand, the reduction of cholesterol by chromium can be caused by the reduction of lipolysis. By increasing insulin sensitivity, chromium reduces NEFA and lowers blood cholesterol (Mertz, 1993). The lower weight loss of sheep in the chromium group in the present experiment may be a proof of the truth of this case, which is supported by the improvement of feed intake by chromium.

CONCLUSIONS

Adding chromium to the diet of ewes under heat stress increases Dry matter intake daily milk production. In general, it is recommended to receive chromium, especially in the form of chromium-methionine and chromium nanoparticles, during the transition period of ewes under the influence of heat stress.

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