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REGULAR ARTICLES

Effect of trace mineral source on biochemical and hematological parameters, digestibility, and performance in growing lambs

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Abstract

To study the efficacy of organic trace mineral supplementation on blood parameters, digestibility, and growth as compared to inorganic sources, 18 Zandi male lambs (with initial body weight, 28.5 ±1.4 kg and 110±5 days old) were divided into three groups of six animals in each in a completely randomized design. Lambs in the control group were fed basal diet containing 63 kg/100 kg of concentrate mixture, 22 kg/100 kg of alfalfa hay, and 15 kg/100 kg wheat straw. Animals in the experimental groups were additionally supplemented with trace minerals supplied by sulfates or a diet in which 25.7 ppm Zn, 14.3 ppm Mn, 8.9 ppm Cu from mineral-amino acid complex, and 0.86 ppm Co from Co glucoheptonate replaced with similar amounts of Zn, Mn, Cu, and Co from sulfates. All lambs were kept in individual pens with cemented floor and provision of individual feeding and watering. Lambs fed with either organic or inorganic trace mineral supplement showed higher dry matter intake and growth rate and better feed conversion efficiency ($P<0.05$) as compared to the control group. Blood glucose, urea nitrogen, cholesterol, and hepatic enzymes were similar among the treatments. Triglycerides ($P<0.01$) concentration was lower for mineral-supplemented groups. Blood vitamin B12 concentration increased with mineral supplementation and was higher for the lambs fed with organic source of trace elements as compared with those fed with inorganic mineral and the control diet ($P=0.04$). The results of this study showed that feeding organic trace elements improves growth performance of finishing lambs but did not affect nutrient digestibility and blood parameters.

Keywords Organic trace mineral · Hepatic enzymes · Vitamin B12 · Growth · Growing lamb

Introduction

Arid and semi-arid regions have consistently been identified as among the most vulnerable regions of the world with respect to climate change. Livestock grazing in these areas is done on mostly semi-dry rangelands, which are often value nutritive and highly influenced by the climate variables. On the other hand, overgrazing has made most of the natural pastures bare and deteriorated. Therefore, during the dry season, most of the animals suffer from severe nutritional deficiencies such as minerals and vitamins (Valizadeh and Sobhanirad 2009). In such regions, heat stress and nutrition deficiency impose severe effects on farm animal performance and health. Farm animals exposed to elevated ambient temperatures are not able to dissipate sufficient body heat, which results in a decrease of DMI due to the need to control body temperature (Naderi et al. 2016). It has also been noted that the leaner and faster-growing an animal (like finishing lambs) is, the higher their basal metabolic rate and thus is more sensitive to heat stress (Nienaber and Hahn, 2007). Supplementing of heat-stressed animals with mineral resources is, therefore, required to correct their negative balances (Marai et al. 2008). Trace minerals can alleviate the effects of high environmental temperatures on animal performance and health by improving nutrient utilization and cell metabolism (Panda et al. 2008; Siciliano-Jones et al. 2008).

Trace elements such as Zn, Cu, Co, and Mn Zinc are used in most metabolic reactions, also essential for enterocyte regeneration and intestinal barrier function. The supply of these trace minerals upregulates tight junction proteins such as occludin, which can help alleviate the degradation of intestinal barrier function during heat stress (Zhao et al. 2019).

According to the literature, the form and level of mineral supplementation also may affect individual animal performance. Trace minerals have been typically supplemented

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as inorganic forms that dissociate in the digestive tract and can interact with other nutrients and form un-digestible or indigestible compounds that decrease bioavailability of these nutrients for animals. On the other hand, it has been reported that organic forms of trace minerals have more effectiveness and increase intestinal absorption and mineral bioactivity (Bach et al. 2015). Also, replacing sulfate minerals with organic trace minerals resulted in improved lactation performance, claw integrity (Siciliano-Jones et al. 2008), and reproduction efficiency (Rabiee et al. 2010) in lactating dairy cows.

In reviewing the literature, most of the reports have been compared different sources of minerals in poultry and dairy cow feeding and study to determine the effects of organic trace mineral (copper, cobalt, manganese, and zinc) supplementation on health and performance of finishing lambs under ambient heat stress is also still limited and is not yet fully understood. The hypothesis is that organic trace minerals may reduce the depletion of lamb mineral reserves and improve performance by reducing the interactions between trace minerals and other dietary nutrients which reduce the availability of trace minerals. The objective of this experiment is to study the effect of organic mineral trace elements supplementation on growth performance and nutrient utilization of finishing lambs reared under warm condition.

Table 1 Participation of ingredients and chemical composition of the basal total mixed ration

	Composition	Amount (% DM)	Ingredient
2.7	ME (Mcal/KgDM)	22	Alfalfa hay
93.8	Dry matter (%)	15	Wheat straw
14.6	Crude protein (% DM)	45	Barley
8.7	Ether extract (% DM)	7	Soybean meal
30.1	NDF (% DM)	10	Wheat bran
7.08	Ash (% DM)	0.4	Vitamin-Mineral mix
0.67	Calcium (% DM)	0.4	Calcium carbonate
0.41	Phosphorus (% DM)	0.2	Sodium chloride
333.9	Ferrous (mg/kg DM)		
12.9	Copper (mg/kg DM)		
42.6	Zinc (mg/kg DM)		
33.7	Manganese (mg/kg DM)		
0.7	Cobalt (mg/kg DM)		

Material and methods

Animals, treatments, and feeding

This study was conducted in Ghezlal Farm (35°12′53.7″N 51°47′42.9″E) in Iran. All animal procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Tehran. The experiment period included the hottest months of the year, May to July. Eighteen Zandi male lambs (initial BW, 28.5 ± 1.4 kg and 110±5 days old) were used as experimental animals. These animals were placed into 3 groups of 6 lambs in completely randomized design based on their body weight. Experimental treatments were (1) control (basal diet without mineral supplementation) or (2) basal diet supplemented with inorganic trace mineral (ITM) and (3) basal diet supplemented with organic trace mineral (OTM). Lambs in control group were fed basal diet containing 63 kg/100 kg of concentrate mixture, 22 kg/100 kg of alfalfa hay, and 15 kg/100 kg wheat straw (Table 1). Animals in the experimental groups were additionally supplemented with trace minerals supplied by sulfates or a diet in which 25.7 ppm Zn, 14.3 ppm Mn, 8.9 ppm Cu from mineral-amino acid complex, and 0.86 ppm Co from Co glucoheptonate replaced with similar amounts of Zn, Mn, Cu, and Co from sulfates.

The diets were formulated according to the NRC (2007) guidelines and animals were fed a total mixed ration ad libitum. All lambs were kept in individual pens with cemented floor and provision of individual feeding and watering. Feed was supplied twice daily at 0800 and 1600 h in amounts that allowed 10% refusal. The amount of supplements was adjusted daily based on DMI of individual lambs. Diets were manually mixed and weighed into each lamb feed trough, and refusals were manually removed each day and weighed. Lambs were gradually introduced to the ration in order to minimize the risk of gastrointestinal disorders. The current experiment lasted for 70 days.

Measurements

Lambs were individually weighed on a digital scale (100 kg capacity with 100 g precision), at the beginning of the study and then weighed bi-weekly. Feed intakes were monitored daily. Body measurements were obtained using a measuring tape calibrated in centimeters (cm). Heart girth (HG), body length (BL), and height at withers (HW) were obtained as reported by Searle et al. (1989).

Blood samples were collected from all the lambs at start and days 25, 50, and 70 of experiment from the jugular vein. Two and a half milliliters of blood anticoagulated was used for cell blood count (CBC). Anticoagulated blood was analyzed for red blood cells (RBC) count, and total leukocyte count (white blood cells; WBC) using micro-hematocrit, cyanmethaemoglobin, and standard manual methods, respectively. Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films.

The whole blood was centrifuged at 1,800×*g* for 10 min followed by removal of serum and frozen at −20°C until analyzed for glucose, urea nitrogen, triglycerides, cholesterol, vitamin B12, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) using commercial kits (Pars Azmoon, Tehran, Iran) and an auto-analyzer (Biotecnica, Targa 3000, Rome, Italy).

During the fifth fortnight of the feeding period, diets, refusals, and feces were sampled daily and composited within lamb over the 4-day collection period for determination of in vivo apparent digestibility using AIA as indigestible internal marker. Feed and fecal samples were ground to pass through a 1 mm screen and then stored in sealed plastic bags at room temperature. The N contents of feed and fecal samples were measured by the Kjeldahl method and crude protein (CP) was calculated as $N \times 6.25$ (AOAC, 1990). Neutral detergent fiber (NDF) in feed and feces were determined by a fiber analyzer using the methods of Van Soest et al. (1991). Ash was determined by complete combustion in a muffle furnace at 450 °C for 8 h (AOAC, 1990). Ash samples were then boiled in 100 mL of 2N HCl for 5 min and filtered through Whatman No. 541 filter paper in a vacuum system. Samples and filter paper were again ashed for 8 h. Dry matter and nutrient digestibilities were calculated using the following equations (Van Keulen and Young, 1977):

$$\text{Dry matter digestibility} = 100 - [100\,(\text{AIA in feed}/\text{AIA in feces})],$$

$$\text{Digestibility of nutrient} = 100 - [(\text{AIA in feed}/\text{AIA in feces}) \times (\text{nutrient in feces}/\text{nutrient in feed})] \times 100.$$

During the experimental period, environment temperature and humidity were recorded per minute with the thermo-hygrometerdata logger (BENETECH GM1365, China). Temperature-humidity index (THI) was used to estimate the environmental severity, which was calculated using the following model:

$$\text{THI} = 0.8\,\text{T} + \text{RH} \times (\text{T} - 14.4) + 46.4$$

where T is dry temperature and RH is relative humidity. Using the temperature-humidity index, the heat stress was calculated based on the Collier et al. (2011) method.

Statistical analysis

Data were analyzed as a completely randomized design. Statistical analyses of data for repeated measures (performance and blood parameters) were conducted using the MIXED procedure of SAS (2004). For both repeated and non-repeated measures, Duncan's multiple range test was used to detect statistical significance between treatments using a significance level of 0.05.

Results

Ambient temperature-humidity index

Diurnal and temporal pattern of ambient temperature, RH, and THI are shown in Figures 1 and 2, respectively. According to Figure 1, the environmental temperature of the shed varied between 32.0 °C and 37.2 °C, the relative humidity varied between 31 and 41%, and the THI varied between 77.45 and 85.50 during the experiment.

Performance

The results of the dry matter intake (DMI), daily weight gain, final live weight, and feed conversion ratio (FCR) are

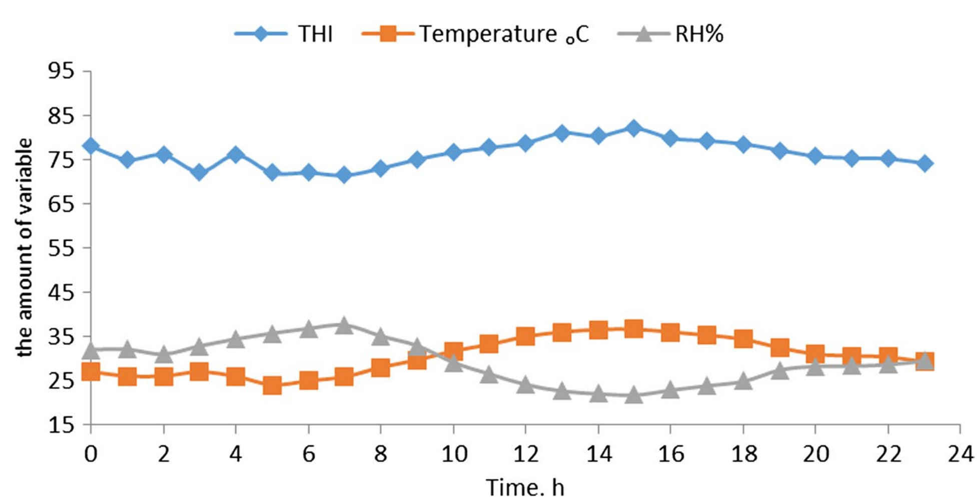

Fig. 1 Diurnal change in relative humidity (RH), temperature, and temperature-humidity index (THI) of the cowshed

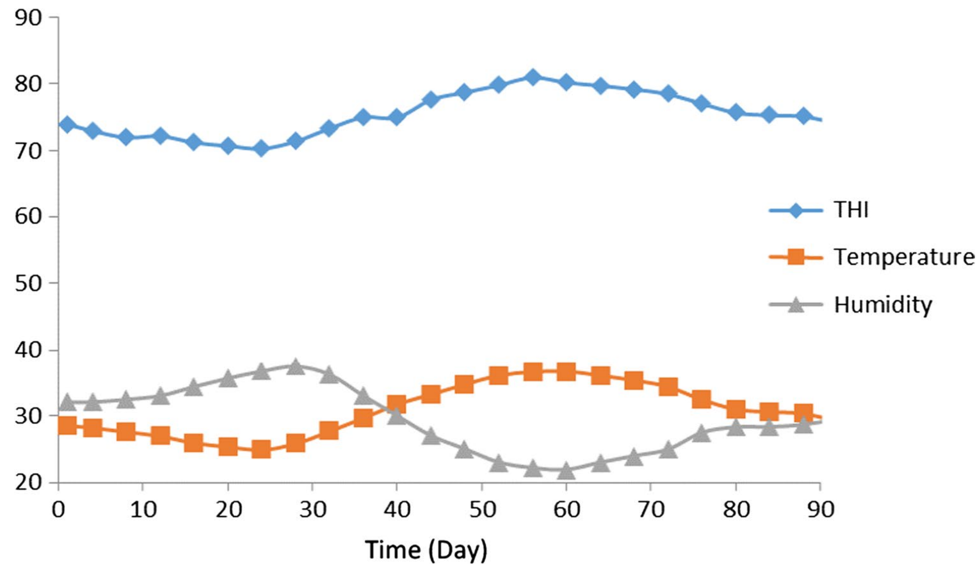

Fig. 2 Change in temperature, relative humidity (RH), and temperature-humidity index (THI) during the experiment

Table 2 Effect of different groups on performance and body measurement of experimental lambs

Measurement	Treatment			SEM	*P*-value	
	Con	ITM	OTM		Trt	Time
Initial body weight (kg)	28.3	28.6	28.8	0.55	0.93	-
Final body weight (kg)	35.9 [b]	37.8 [a]	37.3 [a]	0.82	0.02	-
Average gain (g/day)	106.3[b]	128.8[a]	125.1[a]	10.3	0.03	˂0.01
Average dry matter intake (g/day)	1099 [b]	1132 [a]	1130 [a]	19.4	0.05	˂0.01
Feed conversion ratio (FCR)	10.3[b]	9.1[a]	9.2[a]	0.98	0.04	˂0.01
Body measurements						
Height at withers (cm)	61.8	62.9	60.9	0.94	0.37	˂0.01
Body length (cm)	57.9	59.4	57.4	1.03	0.11	˂0.01
Heart girth (cm)	80.3	79.7	81.4	1.02	0.69	˂0.01
Tail circumference (cm)	47.2	51.6	45.4	1.54	0.06	˂0.01
Tail length (cm)	26.9	27.0	25.3	0.92	0.55	˂0.01
Tail width (cm)	26.6	28.2	27.4	1.09	0.63	˂0.01

Treatments: Con (control: no mineral supplementation), ITM: inorganic trace elements, and OTM: organic mineral element

Means with different superscript letters in rows are significantly different ($P<0.05$)

presented in Table 2. With mineral supplementation, an increase in final body weight (P=0.02), daily weight gain (P=0.03), dry matter intake (P=0.05), and improvement in feed conversion ratio (P=0.04) was found in lambs reared under heat stress. Body measurements of experimental lambs were not affected significantly by all of the studied factors (Table 2).

Blood parameters

The results for the blood metabolites are presented in Table 3. Blood glucose, urea nitrogen, cholesterol, and hepatic enzymes (ALT and AST) were similar among the treatments. Triglycerides ($P<0.01$) concentration was lower for mineral-supplemented groups. Blood vitamin B12 concentration increased (P=0.04) with mineral supplementation and was higher for the lambs fed with organic source of trace elements as compared with those fed with inorganic mineral and the control diet (Table 3).

The data of cell blood count (CBC) test of the experimental animals are presented in Table 4. All values of the estimated hematological parameters of the experimental lambs were in the normal reference range. Results of these parameters did not show any significant differences between the experimental groups.

Digestibility

No significant effect was observed for apparent digestibility of DM, organic matter, ADF, NDF, and ether extract (Table 5). However, supplementation with mineral supplement increased digestibility of CP ($P = 0.03$).

Table 3 Effect of different treatment on blood metabolite of experimental lambs

Measurement	Treatment			SEM	*P*-value	
	Con	ITM	OTM		Trt	Time
Glucose (mg/dl)	50.8	54.9	50.0	4.82	0.83	<0.01
Blood urea nitrogen (mg/dl)	26.9	30.8	30.2	1.35	0.12	0.76
Triglycerides (mg/dl)	19.6^{a}	10.5^{b}	13.2^{b}	3.04	<0.01	0.11
Cholesterol (mg/dl)	57.5	50.7	47.3	3.80	0.27	0.05
Vitamin B12 (ng/ml)	2.8^{b}	3.1^{b}	4.3^{a}	1.01	0.04	0.03
Aspartate transaminase (AST; U/l)	128.1	121.8	108.6	10.1	0.55	<0.01
Alanine aminotransferase (ALT; U/l)	68.2	62.3	63.7	9.6	0.98	<0.01

Treatments: Con (control: no mineral supplementation), ITM: inorganic trace elements, and OTM: organic mineral element

Means with different superscript letters in rows are significantly different ($P<0.05$)

Table 4 Effect of different trace elements sources on blood hematological parameters of experimental lambs

Measurement	Treatment			SEM	*P*-value	
	Con	ITM	OTM		Trt	Time
Red blood cell (RBC; 10^6/µl)	14.2	13.8	13.5	0.64	0.12	<0.01
White blood cell (WBC; 10^3/µl)	14.1	16.9	13.0	1.18	0.11	0.04
Lymphocyte (%)	58.0	62.4	63.6	2.99	0.52	0.39
Neutrophil (%)	41.0	36.3	34.9	3	0.45	0.52
Eosinophil (%)	0.51	0.64	0.52	0.14	0.53	0.16
Monocyte (%)	0.47	0.43	0.60	0.11	0.55	0.45
Basophil (%)	0.17	0.19	0.24	0.07	0.28	0.03

Treatments: Con (control: no mineral supplementation), ITM: inorganic trace elements, and OTM: organic mineral element

Means with different superscript letters in rows are significantly different ($P<0.05$)

Table 5 Effect of different sources of trace minerals on apparent nutrient digestibility in lambs (DM basis)

Measurement	Treatment			SEM	*P*-value
	Con	ITM	OTM		
Dry matter (%)	86.5	88.8	85.6	1.6	0.06
Organic matter (%)	75.1	76.3	73.8	1.2	0.08
CP (%)	75.8^{a}	79.4^{b}	81.8^{b}	1.8	0.03
NDF (%)	40.2	39.8	38.3	0.9	0.33
ADF (%)	56.9	59.3	58.0	2.2	0.12
EE (%)	70.0	68.1	70.6	0.7	0.11

Treatments: Con (control: no mineral supplementation), ITM: inorganic trace elements, and OTM: organic mineral element

Means with different superscript letters in rows are significantly different ($P<0.05$)

Discussion

Ruminants experience heat stress at a temperature-humidity index greater than 68 (Armstrong, 1994; Collier et al., 2011). Ingraham et al. (1974) believed that THI < 72 had no effect on farm animals, between 72 and 78 cause slight heat stress, between 78 and 89 moderate heat stress, and THI > 90 severe heat stress. Therefore, in the current experiment, lambs are in a state of moderate heat stress.

The lambs fed diet supplemented with organic trace elements showed higher feed intake and growth rate and better feed conversion ratio. Similar observations have been reported in lambs (Haddad and Goussous 2005), ewes (Pal et al. 2010a), growing kids (Pal et al. 2010b), and dairy goats (Abd El-Ghani 2004). It is reported that lambs consuming a diet supplemented with Zn-methionine had greater ADG compared with lambs consuming a diet supplemented with $ZnSO_4$ and control lambs fed a basal diet (Garg et al. 2008). In contrast, Bach et al. (2015) reported no improvement in performance of dairy cows when fed diet supplemented with chelated Zn, Cu, and Mn.

In the present study, feed intake was reduced under heat stress in the lambs fed the control diet, but feed intake was maintained in lambs supplemented with trace elements either as organic or inorganic forms. This is in agreement with Chauhan et al. (2014), who showed a 13% decline in feed intake in ewes reared under heat stress. Marai et al. (2008) reported that heat stress decrease feed intake and increase excretion of minerals and feeding trace elements is required to correct mineral balances of heat-stressed animals (Marai et al. 2008). The higher performance in the supplemented groups may be due to optimum essential nutrients such as mineral supplied to these animals. Similar trends were also observed in ewes reared under warm condition as a result of additional multi-nutrient supplementation (Mubi et al. 2011).

In this study, levels of blood biochemical and hematological parameters of experimental lambs were within the physiological range for sheep. Feeding lambs with organic trace elements had no effect on blood concentration of glucose, blood urea nitrogen (BUN), total cholesterol, and hepatic enzymes (AST and ALT) activity. Contrary to our results, growing goats fed by trace mineral supplements showed higher AST activity compared with those of the control (Shi et al. 2018). Hyper activity and necrosis of hepatocytes increase serum AST and ALT activity (Mousaie et al. 2014). In the present study, constant levels of serum activity of AST and ALT in response to trace elements supplementation could propose that trace elements supplementation has no toxic effect on body metabolism.

Lambs fed by organic mineral had low levels of blood triglycerides concentration ($P<0.05$) compared to control. The effects of trace elements supplementation on lipids metabolism in lambs and calves are controversial. Our results agree with Bakalli et al.'s (1995) findings which showed organic copper supplementation decreased blood triglycerides in broilers. However, in the findings of Hosienpour et al. (2014) and Lee et al. (2002), plasma triglycerides were unaffected by trace mineral supplementation. This inconsistency on the effects of mineral supplementation on performance and blood lipid profile may be due to individual animal variability, concentrations and duration of mineral administration, or interactions between mineral and other nutrients in the site of absorption (Solaiman et al. 2006; Datta et al. 2007).

Lambs fed control unsupplemented diet had lower levels of serum vitamin B12, which showed that control lambs were deficient in vitamin B12. Feeding diet supplemented with organic source of cobalt increased blood concentration of vitamin B12 to adequate level for growing lambs. With agreement to our results, other studies showed that feeding organic cobalt increases blood vitamin B12 concentration in sheep (Aliarabi et al. 2019; Run-lian et al. 2010; Wang et al. 2007; Bishehsari et al. 2010) and cows (Griffiths et al. 2007). It seems that organic sources of cobalt provide more available substrates for rumen microorganisms to synthesize vitamin B12 (Aliarabi et al. 2019).

The values of the blood cell count and differential leukocyte parameters (Neutrophil, Lymphocyte, Monocyte, Eosinophil, and Basophil) were not influenced by the source of trace elements. Studies related to the hematological effects of trace elements supplementation in finishing lambs are rather limited and controversial. Hosienpour et al. (2014) and Norouzian et al. (2014) reported that organic mineral supplementation had no effect on hematological parameters in calves and lambs. In contrast, previous studies reported that administration of organic trace elements increases RBC parameters in newborn animals (Rupić et al. 1997; Heidarpour Bami et al. 2008). It appears that in adult animals that fed sufficient amounts of dietary mineral, supplementation of trace mineral does not affect the hematological responses (Norouzian et al. 2014) as observed in this study.

In this study, overall diet digestibility was not affected by experimental treatments. However, apparent total tract digestibility of crude protein increased by the supplementation of trace elements regardless of the mineral source. Previous studies have demonstrated that supplemental trace minerals did not affect apparent total-tract dry matter, NDF, hemicellulose, or starch digestibility (Mallaki et al. 2015; Pino and Heinrichs 2016). In contrast, it has been reported that administration of inorganic trace elements increased digestibility of dry matter, organic matter, and NDF in steer (Mandal et al. 2007), lambs (Garg et al. 2008), and goat (Salama et al. 2005). Also and consistent with our results, higher digestibility of CP in dairy cows (El Ashry et al., 2012) and goats (Salama et al. 2005) given mixed chelated minerals (Mn, Cu, and Zn methionine complexes) has been suggested in previous studies. The increased nutrient digestibility relating to trace elements supplementation could be caused by an increase of metabolic activity of rumen microorganisms or changes in the rumen microbial populations (Gresakova et al. 2018).

Conclusion

Under the dietary conditions of the current study, replacing a portion of inorganic Zn, Mn, Cu, and Co with organic forms of these trace minerals improved feed intake, daily gain, and FCR but had no detectable effect on blood parameters and nutrient digestibility of growing lambs. Also, in this study, lambs supplemented with organic trace minerals had a higher level of blood vitamin B12 than lambs fed basal diet or supplemented with inorganic sources of trace minerals, which suggested better bioavailability through organic sources in finishing lambs.

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Author contribution Arash Abdian Samarin: Project administration, Formal analysis. M.A. Norouzian: Conceptualization, Supervision, Validation, Writing original draft. Ahmad Afzalzadeh: Methodology. All authors read and approved the manuscript.

Data Availability (data transparency) All data are available via the corresponding author.

Code availability (software application or custom code) Not applicable.

Declarations

Ethics approval The study and all procedures involving the animals were approved by the Institutional Animal Care and Use Committee of University of Tehran.

Consent to participate All authors have consented to participate in this article.

Consent for publication The participant has consented to the submission of articles to the journal.

Conflict of interest The authors declare no competing interests.

Informed consent Not applicable

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