

Effect of Organic Versus Inorganic Trace Mineral Supplement on Carcass Characteristics, Blood Mineral and Meat Heavy Metals Concentration in Growing Lambs under Ambient Heat Stress

Arash Abdian Samarin and Mohammad Ali Norouzian*

Department of Animals and Poultry Science, University of Tehran, Tehran, Iran

Abstract

Trace minerals are critical for production and reproduction in livestock. It has been demonstrated that organic mineral can be transported intact from intestinal lumen into mucosal cells, resulting in increased tissue supply of minerals and subsequently improved animal productivity. This study compared an organic amino acid-mineral complex supplement versus a non-organic mineral supplement in term of effect on performance, carcass and meat quality of finishing Zandi lambs under ambient heat stress. Eighteen Zandi male lambs (28.5 ± 1.4 kg BW) were randomly assigned to one of the three dietary treatments. Control, 2 and 3 basal diet supplemented with inorganic and organic trace mineral supplements (Zn, Cu, Mn and Co). Blood samples were collected days 0, 25, 50, and 70 of experiment from jugular vein. At the end of feeding period (70 days), lambs were slaughtered and meat samples from the longissimus thoracis muscle were analyzed for proximate composition (moisture, protein, fat and ash), macro (Ca, K, P, Na, Mg, S), micro (Cu, Zn, Co, Mn, Fe, Mo, Se, Cr) and heavy minerals (As, Al, Pb, and Cd). There was no effect of trace mineral source on lamb growth rate or carcass traits, but the fat content of longissimus muscle was decreased by 22.6% in organic mineral supplement fed lambs ($P < 0.05$). The Zn and Fe contents in muscle was significantly ($P < 0.05$) higher in lambs fed diets containing mineral supplement. Lower contents of heavy metals including Pb and Ni, were observed in muscle samples from lambs fed inorganic minerals ($P < 0.05$). The results of the study indicate that dietary minerals supplementation can decrease carcass fat content and the concentration of heavy metals in muscle tissue of finishing lambs.

Keywords: Organic trace minerals • Meat composition • Heavy metals • Performance • Finishing lamb

Introduction

Heat stress is a significant challenge in livestock production, particularly in regions with high temperatures. It can negatively impact animal performance, carcass characteristics, and overall meat quality. One approach to mitigate the adverse effects of heat stress is through the supplementation of trace minerals in the diet of growing lambs. Trace minerals play essential roles in various physiological processes, including immune function, and antioxidant defense [1].

The choice of trace mineral supplementation, whether organic or inorganic, can have a significant impact on the efficacy and bioavailability of these minerals. Organic trace minerals are bound to organic molecules, such as amino acids or peptides, which can enhance their absorption and utilization by the animal's body. In contrast, inorganic trace minerals are typically in the form of salts or oxides and may have lower bioavailability [2].

Several studies have investigated the effects of organic and inorganic trace mineral supplementation on performance, carcass characteristics, and fecal mineral excretion in different livestock species. For example, research conducted on phase-fed, grow-finish lamb demonstrated that organic micro minerals had higher bioavailability compared to inorganic trace minerals, resulting in improved performance and nutrients digestibility [3].

However, limited research has been conducted specifically on the effect of organic versus inorganic trace mineral supplementation on carcass characteristics, blood mineral levels, and meat heavy metal concentrations in growing lambs under ambient heat stress conditions. Therefore, the objective of this study was compare effect of organic amino acid-mineral complex with a non-organic mineral supplement on performance, carcass and meat quality of finishing Zandi lambs during the summer.

*Address for Correspondence: Mohammad Ali Norouzian, Department of Animals and Poultry Science, University of Tehran, Tehran, Iran; E-mail: manorouzian@ut.ac.ir

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Materials and Methods

Eighteen male Zandi lambs aged between 3–4 months, with an initial weight of 28.5 ± 1.4 kg were randomly allocated to three different dietary treatments using a completely randomized design based on their live body weights. The first group, serving as the control, was fed a basal diet with no trace mineral supplement. The second and third groups, serving as the test groups, were given trace mineral supplements supplied by sulfates (inorganic trace mineral) or a diet containing an organic trace mineral-amino acid complex. The complex was comprised of 25.7 ppm Zn, 14.3 ppm Mn, and 8.9 ppm Cu, which replaced an equivalent amount of Zn, Mn, and Cu from sulfates. The complex also contained 0.86 ppm Co from Co glucoheptonate. The mineral-amino acid complex contained 2.58% Zn as Zn Met, 1.43% Mn as Mn Met, and 0.90% Cu as Cu Lys. All of the lambs were kept in individual pens throughout the experiment. The composition of basal diet is shown in Table 1. The lambs were given the diets ad libitum, with half of the ration being provided at 700 h and the other half at 1600 h for a period of 70 days. To minimize the risk of gastrointestinal disorders, the lambs were gradually introduced to the ration. The experiment was conducted during the hottest months of the year, from May to July. The temperature and humidity of the environment were recorded every minute using a thermo-hydrometer data logger (BENETECH GM1365, China). The Temperature-Humidity Index (THI) was used to estimate the severity of the environment, which was calculated using a specific model.

$$\text{THI} = 0.08 T + \text{RH} \times (T - 14.4) + 46.4$$

All of the lambs were weighed when they entered the feedlot area and again at the end of the experimental period. Blood samples were collected from the jugular vein on day 1, 25, 50, and 70 of the experiment. The serum was separated from the blood samples by centrifugation at $1,800 \times g$ for a duration of 10 minutes. The separated serum was stored at a temperature of -20°C until it was analyzed for mineral profile. Additionally, representative samples of the diet were collected daily and dried in a forced air oven at 105°C to determine the DM content. These samples were then mixed weekly and frozen at a temperature of -20°C for further analysis of mineral and chemical composition.

At the end of the 70 days feeding period, the lambs were slaughtered after a 12 hours period of feed removal in accordance with the standard slaughter protocol at the experimental abattoir of the college of agriculture's farm. After slaughter, the non-carcass components of the lambs, such as the heart, liver, kidneys, subcutaneous and abdominal fats, were removed and weighed. In addition, the fat tails of the lambs were dissected from the hot carcasses and weighed. The weights of the hot carcasses were recorded and their relative dressing percentages were calculated.

The pH of the meat was measured 1 hour and 24 hours after slaughter using a glass penetrating electrode. Prior to the pH measurement, the pH meter was calibrated using pH 4 and 7 buffers. To assess the chemical composition of the meat, muscle samples were collected from the Longissimus thoracis muscle in the loin region (12th rib cut) of each carcass. The muscle samples were homogenized and frozen at a temperature of -40°C until the analyses were performed. The moisture, protein, fat, and ash contents of the meat were measured using the procedures outlined by AOAC. Additionally, the concentrations of macro minerals (Ca, K, P, Na, Mg, S), micro minerals (Cu, Zn, Co, Mn, Fe, Mo, Se, Cr), and heavy minerals (As, Al, Pb, and Cd) in the experimental samples were determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS). Briefly, approximately 1 g of meat samples were digested in a solution containing 5 ml of concentrated nitric acid (Suprapur grade, Merck) and 3 ml of 30% w/v hydrogen peroxide using a microwave digestion system (Ethos Plus; Milestone, Sorisole, Italy). The resulting digested samples were transferred to polypropylene sample tubes and diluted to a total volume of 15 ml with ultrapure water. The sample digests were then kept frozen at a temperature of -20°C until they were analyzed using ICP-MS. The serum vitamin B₁₂ levels were measured using a commercial RIA kit (SimulTRAC B₁₂, MP Biomedicals).

Statistical analysis

The statistical analysis of the data was carried out using the GLM procedure in SAS. Experimental parameters that were determined on different days were analyzed as repeated measures using the MIXED procedure of SAS in a completely randomized design. The following statistical model was used:

$$Y = \mu + t_i + T_j + (t \times T)_{ij} + e_{ijk}$$

Where, Y represents the mean of the observation, μ represents the overall mean, t_i represents the treatment, T_j represents the day of observation, $(t \times T)_{ij}$ represents the interaction between treatment and days of observation, and e_{ijk} represents the random residual. Duncan's multiple range tests were used for comparison of means, with $P \leq 0.05$ considered as the significant level.

Results

Based on the data presented in Figure 1, the environmental temperature in the lamb shed varied between 27.5°C and 32.5°C , the relative humidity varied between 40.4% and 53.2%, and the THI varied between 74.5 and 79.6 throughout the experiment. The average THI for the entire trial period was 75.5 ± 1.5 . It is important to note that animals experience heat stress when the THI is greater than 68 [4]. Using the formula previously described by Collier et al., it can be concluded that the experimental lambs experienced heat stress during the study.

Table 1 provides information on the dietary ingredients and chemical composition of the basal diet. The basal diet had a content

of 12.9 mg Cu, 42.6 mg Zn, 33.7 mg Mn, and 0.7 mg Co per kg DM.

Ingredient	Amount (%)	Nutrient level	Nutrient item
Alfalfa hay	22	ME (Mcal/Kg DM)	2.7
Wheat straw	15	Dry matter (%)	93.8
Barley	45	Crude protein (%)	14.6
Soybean meal	7	Ether extract (%)	8.7
Wheat bran	10	NDF (%)	30.1
Vitamin a and mineral b supplement	0.4	Ash (%)	7.08
Calcium carbonate	0.4		
Sodium chloride	0.2		
Mineral composition			
Calcium (%)	0.67	Ferrous (mg/kg DM)	333.9
Phosphorus (%)	0.41	Molybdenum (mg/kg DM)	1.45
Magnesium (%)	0.15	Selenium (mg/kg DM)	0.05
Sodium (%)	0.34	Chromium (mg/kg DM)	1.77
Sulfur (%)	0.16	Lead (mg/kg DM)	0.19
Potassium (%)	0.7	Arsenic (mg/kg DM)	0.01
Copper (mg/kg DM)	12.9	Cadmium (mg/kg DM)	0.007
Zinc (mg/kg DM)	42.6	Nickel (mg/kg DM)	0.71
Manganese (mg/kg DM)	33.7	Aluminum (mg/kg DM)	381.9
Cobalt (mg/kg DM)	0.7		

Note: ^aProvided 4000 IU of vitamin A, 450 IU of vitamin D₃ and 162 mg of vitamin E per kg of diet.

^bProvided 25 mg of Fe as FeSO₄, 0.5 mg of I as KI, 0.1 mg of Se as Na₂SeO₃, without Cu, Zn, Mn and Co salts per kg of diet.

Table 1. Composition of the basal total mixed ration.

Table 2 presents the results for final body weights, daily weight gain, carcass and non-carcass traits, and meat chemical composition. There were no significant differences among the experimental groups for these parameters. The moisture, protein, ash, and pH levels of the

meat samples also did not differ between the groups. However, there was a significant decrease ($P < 0.05$) in the fat content of the longissimus muscle in lambs fed with organic mineral supplements, with a reduction of 22.6%.

Measurement	Treatment			SEM	P-value
	Control	Inorganic	Organic		
Final shrunk body weight (kg)	35.9	37.8	36.6	1.06	0.15
Final Empty Body Weight (EBW; kg)	32.3	33.2	32	1.03	0.39
Carcass weight (kg)	16.2	16.8	16.5	0.62	0.72
Dressing percentage (%)	48.3	49.4	49.2	0.9	0.22
Fat tail (kg)	2.3	2.45	2.17	0.22	0.68
Visceral fat (kg)	0.17	0.16	0.17	0.04	0.98
Visceral fat+Fat tail (kg)	2.47	2.61	2.34	0.21	0.68
Liver (kg)	0.58	0.6	0.58	0.02	0.62

Kidneys (kg)	0.16	0.18	0.17	0.01	0.65
Heart (kg)	0.2	0.21	0.19	0.01	0.45
Fat tail (% of carcass)	12.9	13.8	12.7	1.21	0.82
Visceral fat (% of carcass)	0.52	0.47	0.54	0.12	0.92
Visceral fat+Fat tail (% of carcass)	13.59	14.64	13.31	0.5	0.89
Liver (% of carcass)	1.79	1.76	1.81	0.04	0.72
Kidneys (% of carcass)	0.5	0.53	0.52	0.04	0.86
Heart (% of carcass)	0.61	0.61	0.58	0.03	0.75
pH (1 hour after slaughtering)	6.37	6.46	6.42	0.05	0.47
pH (after 24 h)	5.43	5.49	5.49	0.05	0.66
pH fall (after 24 h)	0.94	0.97	0.93	0.08	0.95
Longissimus muscle composition (g/kg)					
Moisture	767	779	762	7.2	0.25
Ash	16.1	14.1	16.1	6.2	0.62
Protein	171	175	172	10.5	0.84
Fat	42.4 ^a	36.8 ^b	32.8 ^c	1.5	<0.01

Note: ^aTreatments: control: no mineral supplementation, inorganic: basal diet supplemented with inorganic trace elements (Zn, Cu, Mn and Co) and organic: basal diet supplemented with organic mineral element (Zn, Cu, Mn and Co).

^bMeans with different superscript letters in rows are significantly different (P<0.05).

Table 2. Effect of trace mineral sources on performance, carcass and non-carcass traits and meat chemical composition of Zandi lambs

Table 3 shows that the blood concentrations of Zn and Fe were significantly higher (P<0.05) in lambs that consumed the trace mineral supplement compared to the control group. However, the Co concentration in blood serum was significantly higher (P<0.05) in

lambs fed with organic mineral supplements compared to those supplemented with inorganic trace minerals and the control group. The concentrations of calcium, phosphorus, and copper in blood serum were statistically similar (P>0.05) between the treatments throughout the experimental period.

Elements	Treatment			SEM	P-value	
	Control	Inorganic	Organic		Trt	Time
Ca (mg/dl)	16.2	15.9	16.2	0.3	0.81	<0.01
P (mg/dl)	20.6	20.1	19.9	0.49	0.61	0.02
Cu (µg/dl)	117.7	110.1	121.9	7.31	0.53	0.04
Zn (µg/dl)	116.3 ^b	118.6 ^b	130.3 ^a	6.8	0.05	0.35
Co (µg/dl)	1.26 ^b	1.37 ^b	2.77 ^a	0.26	<0.01	<0.01
B ₁₂ (ng/ml)	2.8 ^b	3.1 ^b	4.3 ^a	1.01	0.04	0.03
Fe (µg/dl)	350.0 ^b	371.3 ^b	468.1 ^a	40.1	0.05	0.2

Note: ^aTreatments: control: no mineral supplementation, inorganic: basal diet supplemented with inorganic trace elements (Zn, Cu, Mn and Co) and organic: basal diet supplemented with organic mineral element (Zn, Cu, Mn and Co).

^bMeans with different superscript letters in rows are significantly different (P<0.05).

Table 3. Effect of trace mineral sources on serum mineral and vitamin B₁₂ concentration in lambs of different groups.

Table 4 shows that the Zn content in the Longissimus muscle was significantly ($P<0.05$) higher in lambs fed diets containing mineral supplements. However, lambs fed with organic minerals had higher

($P<0.05$) concentrations of Fe in the longissimus thoracis muscle compared to lambs fed with inorganic trace minerals and the control group.

Elements	Treatment			SEM	P-value
	Control	Inorganic	Organic		
Macro (mg 100 g ⁻¹)					
Ca	4.7	4.41	4.61	0.103	0.42
P	18	15.3	16.8	0.46	0.35
Mg	12.4	10.4	12	0.64	0.38
Na	33.2	31.8	32.4	1.7	0.76
S	0.51	0.5	0.45	0.06	0.5
K	59.9	55.4	58.7	2.85	0.43
Micro (µg 100 g ⁻¹)					
Cu	78	65.1	76.2	6	0.58
Zn	192.1 ^b	203.2 ^a	221.1 ^a	12.2	0.05
Mn	63.2	57.2	53.1	4.1	0.25
Co	0.6	0.48	0.64	0.12	0.34
Fe	2700.1 ^c	3880.2 ^b	6390.3 ^a	550.2	0.01
Mo	0.07	0.04	0.05	0.002	0.25
Se	0.31	0.26	0.3	0.02	0.36
Cr	26.3	21.6	22.5	1.3	0.07
Pb	7.2 ^a	5.3 ^b	8.5 ^a	0.7	0.01
As	0.069	0.066	0.048	0.004	0.88
Cd	0.11	0.09	0.1	0.02	0.65
Ni	19.2 ^a	10.2 ^c	15.1 ^b	2.1	0.04
Al	1300.2 ^a	1080.2 ^b	980.3 ^c	30.5	<0.01

Table 4. Effect of trace mineral sources on mineral concentration of longissimus thoracis muscle in Zandi lambs.

The tissue samples from lambs fed with inorganic minerals had lower contents of heavy metals, Pb and Ni, compared to lambs fed with organic minerals and the control group. However, the concentration of Al was higher ($P<0.05$) in the control group compared to the mineral supplemented groups. No significant differences were observed in the concentrations of macro minerals (Ca, K, P, Na, Mg, S), micro minerals (Mo, Se, Cr), and heavy minerals (As and Cd) in the longissimus thoracis muscle of the experimental lambs.

Discussion

The meteorological data collected during the study indicated that the lambs experienced mild heat stress conditions, with the THI exceeding 68 units each day [5]. Exposure to elevated ambient temperature can lead to drastic changes in biological functions, including increased body temperature, decreased feed intake, and depression of mineral blood concentrations [6]. The higher

serum concentrations of Zn, Co, and B₁₂ in the lambs fed with organic mineral supplements in this study may be important for sheep husbandry in countries like Iran, which are facing heat stress and climate warming. However, it is noteworthy that in this study, the growth and carcass performance of the lambs were not affected by the addition of organic mineral supplements. Previous studies investigating the effect of organic trace mineral supplementation on the performance of ruminants have reported varying results. Some studies using organic minerals have reported increases in performance, while others have found no differences [7]. This inconsistency in the effects of organic trace mineral supplementation on ruminant performance across studies may be attributed to various factors, including differences in mineral status, breed variations in mineral metabolism, levels and duration of supplementation, and the form of mineral supplementation [8].

This study is the first to investigate the effect of dietary trace mineral supplementation on carcass and meat traits in growing lambs. The results showed that carcass traits (empty body weight, carcass weight, and dressing percentage), non-carcass traits (fat tail, visceral fat, liver, kidneys, and heart weights), and meat characteristics (moisture, ash, and protein) did not differ between the experimental groups. However, the feeding of organic trace elements decreased the fat content of the longissimus muscle samples. Similar observations have been reported by other studies that reported different sources and levels of trace minerals did not affect carcass traits in growing animals. In contrast, some studies have reported a decrease in carcass fat in lambs and steers fed with supplemental organic mineral supplements [9]. It has been suggested that trace elements such as copper, zinc, and chromium can depress the expression of lipid metabolism-related genes, leading to a decrease in fat deposition [10]. In the present study, the feeding of organic mineral supplements resulted in a decrease in intramuscular fat content in the longissimus muscle samples of the lambs, indicating a change in fat deposition and energy expenditure. This decrease in meat marbling fat may have potential health benefits for consumers.

This study found no significant effects of feeding trace minerals on the content of blood macro-minerals (Ca and P). However, feeding the organic form of minerals increased the blood content of trace elements (Zn, Co, and Fe) compared to feeding the inorganic source of minerals and the control group. These blood mineral results are consistent with previous research on lambs, calves, beef cattle and ewes, which have shown that blood trace mineral concentrations increase with organic minerals. The higher gut absorption and bioavailability of trace minerals from organic sources in lambs may explain the increased serum concentrations of Zn, Co, and Fe observed in the experimental group.

The reason for the increased serum Fe in trace mineral supplemented lambs is not clear, but one possibility is that feeding organic sources of trace minerals reduces mineral interactions and therefore increases Fe availability and absorption. Additionally, in this study, supplemental organic Co increased the serum vitamin B₁₂ concentration of growing lambs. Several studies on dairy cows and lambs have shown that Co supplementation increases vitamin B₁₂ concentrations in blood and liver [12].

The present study found that lambs fed with organic trace minerals had higher levels of Zn and Fe contents in the Longissimus thoracis muscle compared to other treatments. The lambs were fed a high concentrate diet, which contains high phytates (Table 1). Phytic acid, can form insoluble complexes with minerals, reducing their bioavailability and retention in the body [13]. The interaction of minerals with phytate when supplemented through inorganic sources can lead to reduced bioavailability and retention of tissue Zn and Fe. On the other hand, feeding organic trace minerals, which are chelated and remain inert in chemical reactions, can improve the bioavailability and retention of minerals [14].

The present study found that the Pb and Ni content in the Longissimus muscle was significantly lower in lambs fed a diet containing an inorganic mineral source. Previous studies have

reported that mineral supplements may reduce the absorption and retention of lead and nickel [15]. It has been experimentally shown that the availability of lead and nickel is inversely related to the zinc and copper content of the diet. Supplementation of 200 ppm dietary zinc decreased tissue lead retention and apparent absorption [16]. Additionally, the influence of copper on Pb toxicity has been reported, and adequate dietary Cu intake could minimize the toxic effect of administered Pb in rats [17]. Furthermore, the addition of 100 mg/kg of Zn and Cu decreased tissue nickel content [18]. Based on our and previous results, it seems that the inorganic source of essential metal nutrients such as zinc and copper decreases tissue heavy metal accumulation, which could be due to the stronger antagonistic effects of inorganic minerals compared to the control and organic mineral supplemented groups [19,20].

Conclusion

In conclusion, the study found that feeding organic trace minerals to lambs did not affect carcass and non-carcass traits, or meat characteristics, but it did decrease the fat content of Longissimus muscle samples. Additionally, feeding organic trace minerals increased the blood content of trace elements such as Zn, Co, and Fe, compared to feeding the inorganic source of minerals and the control group. The study also found that lambs fed with organic trace minerals had higher levels of Zn and Fe contents in the longissimus thoracis muscle compared to other treatments, which may be due to the higher bioavailability and retention of minerals from organic sources. Moreover, the inorganic source of essential metal nutrients such as zinc and copper decreased tissue heavy metal accumulation, which could be due to the stronger antagonistic effects of inorganic minerals compared to the control and organic mineral supplemented groups. These findings provide valuable insights into the effects of trace mineral supplementation on lamb growth and meat quality.

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