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The Effect of Trace Mineral Source on Nutrients Digestibility and Ruminal Fermentation Parameters

Research Article

A. Abdian Samarin[1], M.A. Norouzian[1*] and A. Afzalzadeh[1]

[1] Department of Animal and Poultry Science, College of Aburaihan, University of Tehran, Tehran, Iran

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*Correspondence E-mail: manorouzian@ut.ac.ir

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ABSTRACT

This study was conducted to compare the effect of organic versus inorganic sources of trace elements (Zn, Cu, Mn and Co) on nutrient digestibility in lambs and *in vitro* gas production parameters. In experiment 1, 18 Zandi male lambs (initial body weight (BW), 28.5±1.4 kg) were randomly assigned to either a basal diet with no trace mineral supplement (control diet), basal diet supplemented with trace minerals sulfates, basal diet supplemented with mineral-amino acid complex. In experiment 2, *in vitro* gas production was used to estimate *in vitro* fermentation parameters of the experimental treatments. The digestibility of dry matter, organic matter, and crude protein (CP) were not affected by treatments. However, supplementation with either mineral supplements decreased digestibility of neutral detergent fiber (NDF) (P<0.05). Organic mineral supplementation decreased rate of gas production (P<0.05), however asymptotic gas production (b) and effective digestibility were not different among the groups. Results of this study show that supplementation of trace elements does not affect the *in vitro* ruminal fermentation parameters and nutrients digestibility.

KEY WORDS gas production, lamb, nutrient digestibility, trace element.

INTRODUCTION

Trace elements have important roles in different metabolic processes. They are involved in protein synthesis, utilization of metabolic energy, vitamin metabolism, enzymes, hormones and redox reactions, formation of connective tissue, and immune function (Siciliano-Jones *et al.* 2008). Different sources of trace elements are supplemented in livestock diets including organic and inorganic mineral supplements. It has been shown that trace minerals from inorganic salts are not fully absorbed due to antagonism and anti-nutritional factors present in the diet. However, organic minerals have been shown to be easily absorbed by the body which in turn enhance growth and reproductive performance (Bhanderi *et al.* 2010; Seifalinasab *et al.* 2019).

Furthermore, studies on the effects of trace mineral source on nutrients digestibility and ruminal fermentation are scarce and, where available, results are contradictory. However, in this regard, Salama *et al.* (2003) in their study found that digestibility of dry matter (DM), organic matter, and crude protein (CP) increased when the diets were supplemented with 1 g/day organic Zn. Moreover, Faulkner and Weiss (2017) recently reported that dairy cows supplemented with hydroxy trace mineral had greater neutral detergent fiber (NDF) digestibility than those supplemented with sulfate trace mineral sources. In contrast, Mandal *et al.* (2007) and Jia *et al.* (2008) indicated that DM, CP, acid detergent fiber (ADF), and NDF digestibility were not affected by dietary Zn in cattle and goats, respectively. This phenomenon could be related to the fact that organic sources of mineral have a relatively low solubility in the rumen (Caldera *et al.* 2019). In fact, high concentrations of soluble of trace elements in the ruminal environment can decrease nutrients digestion in the rumen. Our hypothesis for the present study was that supplementing organic trace mineral would result in lower soluble mineral concentration in the rumen, and thus influence fermentation and nutrients digestion differently from that of inorganic sources. The objective of this study was to compare different mineral sources (organic versus inorganic) on diet digestibility and rumen fermentation parameters. To accomplish this, sheep diets were supplemented with trace elements (Zn, Cu, Co and Mn) from organic (mineral-amino acid complex) or inorganic (trace minerals sulfates) sources and then nutrients digestibility, amount and rate of *in vitro* gas production and utilization of metabolizable energy (ME) were evaluated. Ruminal fermentation patterns were also studied as the key parameter for the feeding value of these mineral sources.

MATERIALS AND METHODS

In experiment 1, 18 Zandi male lambs (initial BW, 28.5±1.4 kg and 110±5 days old) were fed by 3 different source of trace elements (Zn, Mn, Cu and Co), as lambs in control group were fed basal diet (Table 1) and 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.

Table 1 Composition of the basal total mixed ration

Ingredient	Amount (%)
Alfalfa hay	22
Wheat straw	15
Barley	45
Soybean meal	7
Wheat bran	10
Vitamin[1] and mineral[2] supplement	0.4
Calcium carbonate	0.4
Sodium chloride	0.2
Chemical composition	
Metabolizable energy (ME) (Mcal/kg DM)	2.7
Dry matter (%)	93.8
Crude protein (%)	14.6
Ether extract (%)	8.7
Neutral detergent fiber (NDF) (%)	30.1
Ash (%)	7.08

[1] Provided per kg of diet: vitamin A: 4000 IU; vitamin D_3: 450 IU and vitamin E: 162 mg.
[2] Provided per kg of diet: Fe as $FeSO_4$: 25 mg; I as KI: 0.5 mg; Se as $Na2SeO_3$: 0.1 mg.

For determination of *in vivo* digestibility of nutrient, diets, refusals and feces were sampled daily, and composited within lamb over the for 4-day collection period. Briefly feed and fecal samples were ground and after determination of DM, CP, NDF, and ether extract (EE), samples were ashed at 450 ˚C for 8 h. 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):

Dry matter digestibility= 100 − [100 (AIA in feed/AIA in feces)]

Digestibility of nutrient= 100 − [(AIA in feed/AIA in feces) × (nutrient in feces/nutrient in feed)] × 100

In experiment 2, *in vitro* gas production (Menke *et al.* 1979) was used to estimate *in vitro* DM digestibility (IVDMD), ME, and short-chain fatty acid (SCFA) production.

Ruminal fluid obtained from 4 animals 3 h after morning feeding was mixed and strained through 4 layers of cheesecloth into a prewarmed thermos and transported to the laboratory. The lambs were fed a TMR (60:40 forage:concentrate; DM basis) and 0.6% mineral and vitamin premix (without Zn). The TMR contained 20.1 mg Zn/kg DM from forage and grain. The lambs were fed twice daily at 07:00 and 19:00 hours and had free access to water. Incubation medium was prepared as described by Menke *et al.* (1979). Samples, each of 200 ± 0.2 mg DM, were incubated in 100-mL glass bottles in which 30 mL of the incubation medium was added. To each bottle, one of the following treatments was applied: 1) control (C; no supplemental zinc), 2) 20 ppm of Zn as ZnS, or 3) 20 ppm of Zn as ZnP. Samples were incubated in triplicate and cumulative gas production was monitored at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, and 96 h after incubation. Three bottles with incubation medium only were used as blanks to correct the gas production values for gas release from the rumen contents. The gas production data were fitted to the following model of France *et al.* (2000):

$$A= b \times [1 - e^{-k\,(t-L)}]$$

Where:
A: volume of gas production at time t.
b: asymptotic gas production (based on mL/200 mg DM).
k: rate of gas production per hour from the slowly fermentable feed fraction b.
time lag (L): discrete lag time prior to gas production.

The rate of gas production (RGP) at 4 and 6 h was calculated from recorded volumes of gas produced before and after these times (Vázquez-Armijo *et al.* 2011). For example, RGP at 4 h was calculated as:

RGP4h [(mL/Gdm/h)]= (volume of gas produced at 6 h-volume of gas produced at 2 h) / 4 × sample weight (mg)

Metabolizable energy (ME, in MJ/kg DM) and short chain fatty acids (SCFA) were estimated according to the equations by Makkar (2005):

ME= 2.20 + 0.136 $IVGP_{24}$ (mL/200 mg DM) + 0.057 CP (% DM)

SCFA (mmol/g DM)= 0.0222 $IVGP_{24}$ (mL/200 mg DM) - 0.00425

Where:
$IVGP_{24}$: 24 h gas volume.
CP: crude protein content of the feed sample.

Data were analyzed as a completely randomized design using the GLM procedure of SAS (2004). Duncan's multiple range test was used to detect statistical significance between treatments using a significance level of 0.05.

RESULTS AND DISCUSSION

Apparent nutrients digestibility of lambs fed by different sources of minerals are presented in Table 2. The digestibility of DM, organic matter, and CP were not affected by dietary treatments. However, our findings revealed that supplementation of the diet with mineral supplemented groups decreased digestibility of NDF (P<0.05). Gas production volumes (mL/200 mg DM) in different incubation times of experimental groups are shown in Table 3 and Figure 1. Supplementation of ruminal medium with mineral supplements had no effect on gas production except for 4, 6 and 16 h of incubation.

The effects of different sources of trace minerals on *in vitro* gas production parameters (b, c) and calculated rate of gas production at different time of incubation (4, 6 and 12 h) and effective digestibility (ED) are shown in Table 4. Organic mineral supplementation decreased rate of gas production (P<0.05), however asymptotic gas production (b) and ED were not different among the groups. There were no differences among treatments for IVDMD, ME and net energy for lactation (NE_L) contents and the production of SCFAs (Table 5).

Trace minerals supplementation are essential for proper health and growth of livestock and to prevent deficiency to optimize livestock production.

Minerals are usually supplied to the livestock diets through mineral mixture in its inorganic form, but interactions with other dietary factors, can reduce absorption and decrease bioavailability. Supplementation of mineral bound to organic compound (organic sources) that is easily absorbed by the body is one of the important solutions to overcome insufficient availability of inorganic sources of minerals.

In this study, effects of trace mineral supplementation from different sources on nutrients digestibility and ruminal fermentation parameters were evaluated. Overall dry and organic matter and CP digestibility were not affected by trace mineral supplementation when compared with lambs not receiving supplemental Cu, Mn, Co and Zn. These results were in agreement with Mandal *et al.* (2007) who indicated that DM and CP digestibility were not affected by dietary Zn in bulls fed a basal diet (32.5 mg Zn/kg DM) supplemented with 35mg Zn/kg DM. By contrast, supplementing 12.22 mg of Cu/kg of diet DM from $CuSO_4$ to heifers actually improved DM digestibility of alfalfa hay and corn cobs (Lopez-Guisa and Satter, 1992). Wei *et al.* (2019) reported that the apparent digestibility of DM and CP were higher in dairy cows receiving a diet supplemented with organic trace element.

In this study trace mineral supplementation, regardless of the source, decreased NDF digestibility of the diet.

Table 2 Effect of different sources of trace minerals on apparent nutrient digestibility in lambs (DM basis)

Measurement	Treatment[1]			SEM	P-value
	T1	T2	T3		
Dry matter (%)	85.5	88.8	87.0	1.64	0.05
Organic matter (%)	75.1	75.3	76.1	0.06	0.11
Crude protein (%)	83.8	81.4	82.4	1.79	0.13
Neutral detergent fiber (%)	42.2a	38.8b	38.3b	0.86	0.33

T1: control; T2: zinc sulfate and T3: zinc peptide.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effect of trace mineral sources on gas production in different groups

Incubation time (hours)	Treatment			SEM	P-value
	T1	T2	T3		
2	6.0	4.9	4.7	0.44	0.06
4	13.0^{a}	13.6^{a}	11.6^{b}	0.60	0.04
6	18.2^{a}	19.4^{a}	16.9^{b}	0.66	0.05
8	22.7	24.3	21.4	0.73	0.08
12	32.2	33.1	30.5	0.70	0.13
16	40.5^{a}	41.5^{a}	37.8^{b}	0.72	0.03
24	51.4	52.8	49.4	1.01	0.22
48	61.1	62.4	59.6	1.22	0.50
72	64.3	65.7	62.8	1.24	0.47
96	65.0	66.8	63.8	1.23	0.40

T1: control; T2: zinc sulfate and T3: zinc peptide.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

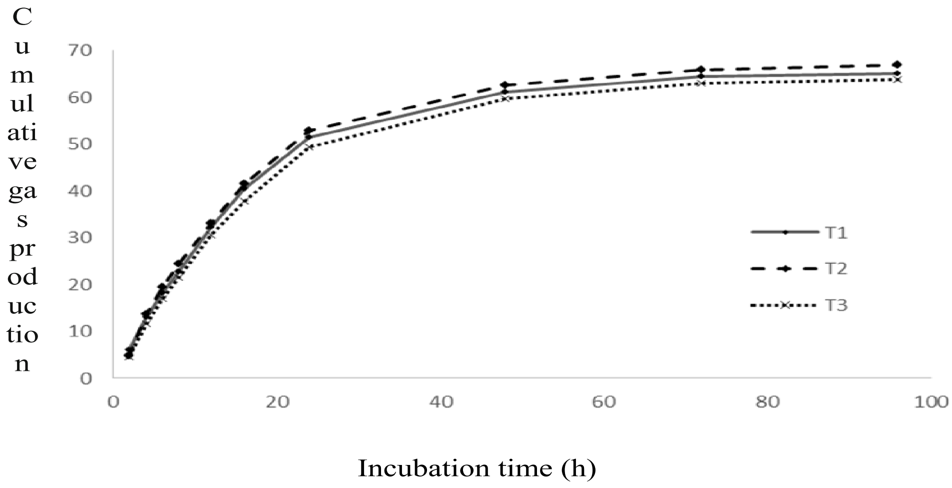

Figure 1 Gas production profiles of the diets of lambs supplemented with different sources of trace minerals
T1: control; T2: zinc sulfate and T3: zinc peptide

Table 4 Effect of trace mineral sources on *in vitro* ruminal fermentation parameters of Zandi sheeps

Measurement	Treatment			SEM	P-value
	T1	T2	T3		
b	65.6	67.1	64.6	1.26	0.53
c	0.057^{a}	0.058^{a}	0.054^{b}	0.001	0.01
ED	27.5	28.4	26.0	0.57	0.09
RGP4h	15.2	15.9	15.2	0.37	0.14
RGP6h	12.4	12.3	12.3	0.31	0.15
RGP12h	11.1	10.8	10.3	0.39	0.38

T1: control; T2: zinc sulfate and T3: zinc peptide.
RGP: rate of gas production (in milliliters per 200 mg DM per hour at 4 and 6 h); GP: gas production (in milliliters per 200 mg at 24 and 48 h); b: asymptotic gas production (in milliliters per 200 mg DM); c: fractional rate of gas production (per hour) and ED: effective digestibility.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In contrast to our results, supplementation with organic trace mineral sources resulted in improved ADF and NDF digestibility in lambs (Garg *et al.* 2008) and dairy cows (Wei *et al.* 2019). Conversely, several *in vitro* studies showed that trace mineral supplementation may decrease fiber digestion.

Table 5 Effect of trace mineral sources on chemical and digestive parameters based on gas production system

Measurement	Treatment			SEM	P-value
	T1	T2	T3		
IVOMD (%)	66.4	67.5	64.6	0.90	0.12
ME (MJ/kg DM)	9.9	10.1	9.6	0.14	0.22
NEL (MJ/kg DM)	5.0	5.1	4.8	0.10	0.24
SCFA (mmol/g DM)	1.14	1.17	1.10	0.02	0.27

T1: control; T2: zinc sulfate and T3: zinc peptide.
IVOMD: *in vitro* organic matter degradability; ME: metabolizable energy (in MJ per kilogram DM); NE_L: net energy for lactation (in MJ per kilogram DM) and SCFA: short chain fatty acid (millimole per gram DM).
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Bonhomme *et al.* (1979) found that 10 µg of Zn/mL decreased *in vitro* cellulose digestion. In another study, adding high levels of Zn reduced cellulose digestion in 24 and 48 h incubations (Eryavuz and Dehority, 2009). Also Arelovich *et al.* (2000) found that when prairie hay was incubated *in vitro* for 24 h with five concentrations of supplemental Zn (0, 5, 10, 15, and 20 ppm) provided as the chloride salt, *in vitro* DM digestibility decreased linearly; 0.447, 0.443, 0.438, 0.432, and 0.409, respectively. The authors suggested that Zn may inhibit the activity of fibrolytic bacteria in the rumen. Similarly, Genther and Hansen (2015) suggested that excessive trace elements such as Zn and Cu might inhibit bacterial attachment to cellulose by binding to the surface of bacteria, decreasing NDF components hydrolysis. In this study, supplementation of either organic or inorganic trace elements had no effect on most gas production parameters except for rate of gas production (c), which was lower with organic trace elements compared to other inorganic supplements.

Similarly, Sobhanirad *et al.* (2008) reported that supplementation of rumen fluid with Zn supplements had no effect on gas production parameters during wheat straw fermentation. Salama *et al.* (2003) and Jia *et al.* (2008) found no differences in the *in vitro* organic matter degradability (IVOMD) with administration of Zn. In contrast to our results, Sofyan *et al.* (2017) reported higher levels of volatile fatty acids (VFA) and improvement of fermentation parameters with organic trace elements supplementation. Run *et al.* (2013) reported higher levels of VFA production at Zn addition as either of the three organic Zn sources compared to the control or Zn sulfate. However, Spears and Kegley (2002) found that the VFA concentration was lowered in steers fed diets supplemented with Zn–Met or Zn-Gly compared to the control or Zn sulfate. According to Fathul and Wajizah (2010), the addition of copper and manganese did not alter *in vitro* ruminal VFA and NH_3-N concentration. Similarly, mineral compounds did not affect ruminal pH, VFA, and NH_3-N in the study of Galyean and Chabot (1981).

A likely reason for the lack of effect on *in vitro* nutrients digestibility and fermentation parameters with trace elements supplementation could that the mineral requirement of rumen microbes was met from the basal diet.

CONCLUSION

In conclusion, supplementation of trace elements does not affect *in vitro* ruminal fermentation parameters and trace elements supplementation from either inorganic or organic sources with different chelation strengths did not impact the rumen fermentation differently.

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