



Interrelationship Between Rumen Fluid Minerals and Biological Tissues of Growing Lambs Fed Complete Feed Supplemented with Clionptilolite

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ABSTRACT

This study aimed to investigate the effect of feeding of complete feed with two levels of Zeolite (1% and 2%) on aluminium (Al), chromium (Cr), manganese (Mn), and potassium (K) status in rumen fluid and different biological tissues of growing Naemi lambs. Twenty-four lambs were randomly selected and divided into three dietary groups and placed in separate pen/lamb (8 lambs/treatment). The three treatments were as follow: control (fed with complete feed as total mixed ratio [TMR]); T1 (TMR with drenching 1% of Zeolite daily); and T2 (TMR with drenching 2% of Zeolite daily). The feeding trial lasted for 56 days. Digestibility trial was conducted at mid of the feeding trial using four lambs from each trial. At end of the trial, four lambs from each treatment were slaughtered and rumen fluid, liver, kidney, and meat were collected for mineral analysis by inductively coupled plasma optical emission spectrometry. Results indicate that there is no significant difference ($P > 0.05$) between the concentration of all the elements in all lambs' tissues, except for the concentration of aluminum in kidney and rumen fluid, as well as Mn in rumen fluid. Moreover, Mn concentration in rumen fluid was significantly ($P < 0.05$) decrease with zeolite supplementation, with significantly lower values for lambs from T2 when compared with T1 and control (339.47 vs. 379.82 and 524.90 $\mu\text{g/g}$ wet weight, respectively). Although the same trend was reported for Mn in the liver, no difference between T1 and T2 groups was observed. Moreover, numerical gradual increase coordinated with increased zeolite level (T2) was detected in concentration of Potassium in the liver, kidney, and rumen fluid, but not in meat. Significant differences ($P < 0.05$) were reported for Mn level between treatments, in which higher values were found for lambs from T1 and T2. Introduction of zeolite at 2% showed greater digestibility of the minerals under investigation. Moreover, the inorganic percentage in liver was significantly higher for lambs supplemented with zeolite when compared with control. This gives an indication that zeolite causes a great effect on mineral absorption, utilization, and accumulation in the liver. Furthermore, zeolite supplementation causes modification in the tested minerals' absorption and metabolism, which clear from the high correlation between the tested minerals in the different tissues. In conclusion, zeolite supplementation causes a variable effect on the absorption and utilization of Al, Cr, and Mn; however, no effect on K levels in different tissues was seen.

INTRODUCTION

The growing period of lambs is a crucial stage for sheep production farming, which requires more focus in terms of dietary supplementation to improve their productivity

and health (Alhidary *et al.*, 2016a, b; Alharthi *et al.*, 2021a, b). The rapid growth of lambs starts after 3 months of age, which requires a high level of nutrients to maximize growth and productivity (Alhidary *et al.*, 2016c). Supplementations of feed with additives may be very crucial during fattening, especially during feeding with complete feed as total mixed ration (Abdelrahman *et al.*, 2019). Complete feed consists of high energy feed ingredient, which negatively affects rumen fermentation

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Authors' Contribution

MA and IA designed the study. MB and MA collected the sampls. RA performed analysis. RUK wrote and edited the manuscript.

Key words

Zeolite, Some minerals, Tissues, Digestibility, ICP-OES

process and utilization of nutrients unless the feed supplements added control certain changes, such as a drop in pH (Varga *et al.*, 1998; Abdelrahman *et al.*, 2017a, b).

Zeolites enclose a group of hundreds of microporous mineral members known for their ion exchange features (Pekov *et al.*, 2008). The structure of zeolites essentially involves aluminosilicates with SiO_4 and AlO_4 structures linked by joint oxygen atoms (Jha and Singh, 2016). Since zeolite is a mineral compound with ion-exchange capacity and porous structure, they can adsorb different gases, humidity, low-level radioactive elements, toxic materials and heavy smells, water, petrochemical substances, and minerals. Furthermore, zeolites are considered the greatest important inorganic cation exchangers used in industrial solicitations for water and waste water treatment, catalysis, nuclear waste, agriculture, animal feed additives, and biochemical applications (Bogdanov *et al.*, 2009).

Synthetic zeolite has no effect recorded on rumen ammonia (NH_3) concentrations (Sweeney *et al.*, 1980). Since 1960s, zeolites were used as a dietary addition to improve ruminant animal performance, given that zeolites have a potential adsorbent and binding properties for positive macroelements, such as total calcium, inorganic phosphorus, magnesium, potassium, and sodium, among others (Bosi *et al.*, 2002). However, one of the main critical concern of using zeolite was the binding of some minerals, making them unavailable to animals, which negatively affects the animals' health, performance, and environment (in terms of pollution) (Papaioannou *et al.*, 2002; Pasteiner, 1998).

Studies have shown that natural zeolite (clinoptilolite) does not affect the physiological homeostasis of trace elements and micronutrients, but acts on heavy metals and toxicants (Mastinu *et al.*, 2019). For instance, clinoptilolite-treated milking goats showed serological differences in fat-soluble vitamins, macro-elements, and trace elements, or activities of hepatic enzymes. Moreover, clinoptilolite supplementation improved milk fat percentage and milk hygiene (Katsoulos *et al.*, 2009), but there was no observed effect of clinoptilolite on physiological mineral levels in cows.

Limited information were reported in literature on the effect of using different levels of natural zeolite with complete feed of growing lambs on availability and utilization of some minerals. In addition, there is also limited information on correlation of the minerals' concentration in different tissues and rumen fluid. Therefore, this study was conducted to investigate the effect of feeding of complete feed as TMR with two levels of Zeolite (1 and 2%) on aluminium (Al), chromium (Cr), manganese (Mn), and potassium (K) status in rumen fluid and different biological tissues of growing Naemi lambs

during fattening.

MATERIALS AND METHODS

A total of 24 growing Naemi lambs (3 month old) were selected randomly and used in this trial. Lambs were housed in individual pens at King Saud University (KSU) research station and injected subcutaneously with 2 ml enterotoxaemia vaccine and Ivomic for internal and external parasites. After 15 days of acclimatization, the lambs were randomly divided into 3 dietary treatments, with eight lambs per treatment as follows: Control (fed complete feed as TMR); T1 (TMR with drenching 1% of Zeolite daily); and T2 (TMR with drenching 2% of Zeolite daily). The feeding trial lasted for 56 days. The dietary ingredients of the complete feed as TMR were as follows: Barley grain (17%); feed wheat (29.92%); wheat bran (5%); sunflower meal (10.05%); soya hulls (11.03%); palm kernel cake (20%); salt (0.47%); limestone (2.58%); molasses (3%); and commercial premix (0.15%). The diets' nutritive values were: dry matter (91.40%); crude protein (13.79%); crude fiber (11.98%); ash (9.09%); metabolizable energy (2.79 Mcal/kg); iron (336 ppm); copper (26.8 ppm); and zinc (269 ppm). Digestibility trials were conducted at mid of the feeding trial using four lambs from each trial. Each lamb was kept in a separate metabolic cage throughout the adaptation and collection periods (3 days adaptation and 5 days fecal collection). Feed intake and fecal output were recorded daily and samples were collected for trace mineral analysis. Data were used to calculate the availability percentage of Al, Mn, Cr, and K, considering the intake and fecal excretion. At the end of the experiment, four lambs were randomly selected from each treatment groups and slaughtered after 16 h of fasting, according to Islamic rules by severing the jugular vein and carotid artery. After slaughtering, liver, kidney, spleen, heart, and lungs were collected and weighed. Samples were taken mainly from the liver, kidney, meat, and rumen fluid from the ventral sac for some trace minerals analysis.

The study protocol, including the use of animals and procedures, was approved by the King Saud University's Animal Ethics Committee, according to "Animal Welfare Act of Practice for the Care and Use of Animals for Scientific Purposes" guidelines.

Digestibility trial and sampling

In the middle of the experiment, four lambs from each treatment were moved into metabolic cages (140 cm × 100 cm × 124 cm) for an 8-day period (3 days for adaptation and 5 days for data collection) in order to be used in a digestibility trial. During this period, weight of feed offered, feed refused, and feces excreted were measured

daily at 08:00 h. Representative samples were collected and pooled (5% each of feed offered and refused and 20% of feces excreted) for subsample and then stored at -20°C for determination of apparent digestibility and subsequent analysis of some minerals.

Tissues samples digestion

Before digestion, all samples were vortexed thoroughly to provide a homogeneous matrix for digestion. Samples were immediately pipetted to prevent settling prior to removing the sample. For trace metals analysis; $0.5000 \pm 0.001\text{g}$ rumen fluid and other tissues were weighed in acid-washed TeflonTM vessel. Afterward, 1 ml HNO_3 (65% Riedel-de Haen, Germany), 1 ml HCl (36% Avonchem, UK), 1 ml H_2O_2 (30% w/v Avonchem, UK), and 1 ml deionized H_2O (Milli-Q quality) were added to the sample before loading on the microwave (Anton Paar Microwave 3000 Microwave, Graz, Austria). The samples were digested according to pre-set temperature program as follow: power initially is ramped at 1250 watts for 15 minute and held for 15 min and then power is reduced to zero watts for zero minute and held for 15 min.

Minerals analysis

Determinations of major and trace metals were performed using a Perkin Elmer Model Optima 7000 DV spectrometer (Perkin Elmer, USA) for inductively coupled plasma optical emission spectrometry (ICP-OES) equipped with a Meinhard Nebulizer type A². Argon (purity higher than 99.999% supplied by AH group (Dammam, Saudi Arabia) was used as carrier gas and to sustain plasma. The operating conditions employed for the ICP-OES determination involves: 1300W RF power; 15 L min⁻¹ plasma flow; 0.2 L min⁻¹ auxiliary flow; 0.8 L min⁻¹ nebulizer flow; and 1.5 mL min⁻¹ sample uptake rate. Axial and radial view was used for metals determination, while 2-point background correction and 3 replicates were used to measure the analytical signal, with the processing mode being the peak area. Emission intensities were obtained for the most sensitive lines free of spectral interference. Calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg L⁻¹) in 0.5% (v/v) nitric acid. Calibration curves for all elements were in the range of 1.0 ng mL⁻¹ to 1.0 $\mu\text{g mL}^{-1}$ (1–1000 ppb).

Statistical analysis

Data were analyzed as a complete randomized design using general linear model of SAS (2002). The dependent variables were Al, Cr, Mn, and K concentration in different tissues, following zeolite supplementation (C, T1, and T2 treatments). Least significant differences test were used to test the difference between mean, taking $P < 0.05$ as

the level of significance. Correlations were determined by Pearson's correlation test for each separate group in order to identify the effect of zeolite treatments on the mineral status.

RESULTS

Effect of zeolite on mineral concentration in rumen fluid and tissues

The impact of zeolite in 1% and 2% concentration of TMR on Al, Cr, Mn, and K, as body trace elements concentration in liver, kidney, rumen fluid, and meat, are given in Table I. The results reported no significant difference ($P > 0.05$) between the concentration of all tested elements in all the lambs' tissues, except for the concentration of aluminum in kidney and rumen fluids and Mn in rumen fluid. There was a significantly higher ($P < 0.05$) Al concentration in kidney of lambs from T1 (1% Zeolite) compared to other groups. Furthermore, there was no significant difference ($P > 0.05$) between the control and T2 lambs, but Al concentration in kidney of lambs from T2 was slightly lower than that of control group (3.36 vs. 4.20 $\mu\text{g/g}$ wet weight). Moreover, Mn concentration in rumen fluid was decrease with zeolite supplementation, with a significantly ($P < 0.05$) lower values for lambs from T2 when compared to T1 and control (339.47 vs. 379.82 and 524.90 $\mu\text{g/g}$ wet weight, respectively). Although the same trend was reported for Mn in the liver, no difference between T1 and T2 groups was observed. Moreover, numerical gradually increases coordinated with increased zeolite level (T2) were detected in the concentration of K in the liver, kidney, and rumen fluid, but not in meat.

Digestibility coefficient of some minerals

The digestibility coefficient of this trial is presented in Table II. There were no significant differences ($P > 0.05$) in the absorption of Al, Cr, and K. However, these minerals were numerically higher in group supplemented with high levels of Zeolite (T2). A significant difference was reported for Mn level between the treatments, in which higher values were found for lambs from T1 and T2. Introduction of zeolite at 2% showed greater digestibility of the minerals under investigation. Moreover, feeding zeolite did not cause any significant effect on feed intake, fecal excretion, and dry matter digestibility, but numerically increased the feed intake (Table II). In contrast to the results presented herein, zeolite addition to lamb diets (Ochodnický *et al.*, 1986) and feedlot steer diets (McCullum and Galyean, 1983) was reported to have no impact on feed intake and feed conversion ratio (FCR). Moreover, several studies have shown that feed intake increases with zeolite addition (Camara *et al.*, 2012;

Table I. Effect of Zeolite treatment on some trace minerals concentration in liver, kidney, meat ($\mu\text{g/g}$ wet weight), and rumen fluid ($\mu\text{g/ml}$) of growing lambs fed complete feed.

	Liver				Kidney				Rumen fluid				Meat			
	Al	Cr	Mn	K	Al	Cr	Mn	K	Al	Cr	Mn	K	Al	Cr	Mn	K
C	5.25	0.24	3.58	62.36	4.20 ^a	0.263	1.29	59.03	17.34a	0.49	524.90a	30.83	4.93	0.138	0.151	91.33
T1	5.16	0.17	2.67	65.56	4.99 ^b	0.171	1.48	64.20	38.06b	0.43	379.82b	31.63	3.98	0.095	0.133	89.20
T2	4.87	0.19	2.82	63.00	3.63 ^a	0.115	1.43	66.96	22.05a	0.42	339.47c	32.10	4.04	0.127	0.124	90.93
Pr<F	0.64	0.12	0.49	0.69	0.036	0.064	0.84	0.13	0.05	0.26	0.05	0.24	0.13	0.482	0.67	0.64
SEM	± 0.24	± 0.01	± 0.31	± 1.44	± 0.36	± 0.03	± 0.12	± 1.66	± 11.78	± 0.05	± 37.24	± 1.34	± 0.22	± 0.03	± 0.01	± 0.88

C, Control group; T1, level 1 of zeolite; T2, level 2 of zeolite; Pr > F, Probability of the F statistic; S.E, stander error. Al, aluminum; Cr, Chromium; Mn, Manganese; K, Potassium.

Koknaroglu *et al.*, 2006; Stojkovic *et al.*, 2012) and, at the same time, improves FCR (Nowar *et al.*, 1993), which closely agrees with our finding in this study.

Table II. Digestibility coefficient of Al, Cr, Mn, and K of Naemi lambs fed complete feed with or without Zeolite supplementation.

Parameters	C	T1	T2	SEM	P-Value
T Feed intake, Kg	7.915	8.590	8.610	0.058	0.345
T Fecal excreted, Kg DMDC, %	4.363	3.878	3.905	0.113	0.806
Al digestibility, %	55.67	45.95	45.86	5.11	0.085
Cr digestibility, %	78.25	75.66	80.11	3.22	0.618
Mn digestibility, %	80.13	78.30	80.90	4.81	0.541
K digestibility, %	69.85a	82.35b	79.85b	5.54	0.048
	76.21	79.55	77.61	7.21	0.853

^{ab} Means with same superscript do not differ ($p > 0.05$) significantly; T1= zeolite level 1 (1%); T2= zeolite level 2 (2%). Al, aluminum; Cr, Chromium; Mn, Manganese; K, Potassium; DMDC, Dry matter digestibility coefficient.

Effect of zeolite on growing lambs tissue weights and liver ash percentages

Table III shows the tissues weight of the growing lambs and liver inorganic matter percentage, as an indication of the minerals' accumulation. The liver, kidney, spleen, heart, and lung weight were not significantly affected by zeolite supplementation, but the liver weight was higher for lambs supplemented with zeolite (T1 and T2) compared to control (0.793, 0.773 vs. 0.725 kg). Furthermore, the inorganic percentage in liver was significantly higher for lambs supplemented with zeolite compared to control (Table III). This give an indication that zeolite causes a great effect on absorption, utilization, and accumulation of minerals in the liver.

Table III. Effect of zeolite on tissues weight (Kg) and liver ash percentage.

Tissues	Control	T1	T2	SEM	P value
Liver	0.725	0.793	0.773	0.02	0.39
Kidney	0.115	0.375	0.123	0.08	0.36
Spleen	0.075	0.218	0.063	0.05	0.35
Heart	0.165	0.168	0.190	0.01	0.26
Lungs	0.605	0.635	0.630	0.01	0.50
Liver inorganic %	1.05a	1.18b	1.165b	0.02	0.001

Correlation coefficients between trace mineral and different tissues

Table IV shows the correlation coefficient (R^2) between tested minerals in liver, kidney, meat, and rumen fluid for the control group (without zeolite supplementation). A significantly ($P < 0.05$) positive correlation was reported for Al ($R^2=0.96$) and Cr ($R^2=0.99$) content of rumen fluid and meat. Furthermore, no significant correlation was reported for other minerals in different tissues. This indicated that is a limited correlation between the studied minerals in different tissues when fed regularly with TMR, without zeolite.

Table V shows the effect of zeolite (1%) on correlation of Al, Cr, Mn, and K concentrations in liver, kidney, meat, and rumen fluid. Significantly positive correlations ($P < 0.05$) were found between rumen fluid and kidney for Al ($R^2= 0.97$) and K ($R^2= 0.99$) and between kidney and meat for Cr ($R^2=0.99$). Furthermore, a significant ($P < 0.05$) positive correlation between rumen fluid and meat was found for Mn ($R^2=0.97$) and K ($R^2=0.94$).

The correlations between the different tested minerals in different tissues of lambs fed high zeolite supplementation (T2) are reported in Table VI. The concentration of rumen fluid Al was significantly ($P < 0.05$) and positively correlated with liver Al ($R^2=0.94$),

meat Al ($R^2=0.99$), and meat K ($R^2=0.93$). Moreover, kidney Mn was significantly ($P < 0.05$) and negatively correlated with meat Mn ($R^2=-0.98$) and rumen fluid Cr ($R^2= -0.95$). Liver K is significantly correlated ($P < 0.05$) with rumen fluid K ($R^2=0.96$). It is very clear that 2% zeolite supplementation causes a modification in the absorption and metabolism of the tested minerals, which is due to the high correlation between the tested mineral in the different tissues.

Table IV. Correlation matrix of some minerals between rumen fluid ($\mu\text{g/ml}$), liver, kidney, and meat ($\mu\text{g/g}$ wet weight) of growing lambs fed complete feed without zeolite.

Element	Tissue	liver	Kidney	Rumen fluid	Meat
Aluminum	liver	1			
	Kidney	0.16	1		
	Rumen fluid	-0.79	0.48	1	
	Meat	-0.92	0.23	0.96*	1
Chromium	liver	1			
	Kidney	-0.83	1		
	Rumen fluid	-0.62	0.95	1	
	Meat	-0.66	0.97	0.99*	1
Manganese	liver	1			
	Kidney	0.67	1		
	Rumen fluid	-0.75	-0.99	1	
	Meat	-0.07	0.69	-0.61	1
Potassium	liver	1			
	Kidney	0.47	1		
	Rumen fluid	-0.71	-0.95	1	
	Meat	0.54	-0.48	0.2	1

DISCUSSION

Zeolites are three dimensional frames that consist of SiO_4 and AlO_4 , which form uniform pores and act as an absorbent agent. In the last fifty years, zeolites are undisputed as excellent catalysts for a wide variety of reactions. However, one of the main critical concern of using zeolite was the binding of some minerals, which make them unavailable to animals, thus negatively affecting the animals' health, performance, and environment (in terms of pollution) through fecal mineral loss (Papaioannou *et al.*, 2002; Pasteiner, 1989). Natural and synthetic zeolite has been shown to reduce blood and tissues mineral concentrations in different species, such as pigs (Pond *et al.*, 1988) and broilers (Scheideler, 1993);

however, it is not well documented in ruminants. Although there is limited data in literature regarding the effect of different levels of zeolite on minerals profiles in serum and tissues of growing lambs, valuable conclusions have been obtained from this study.

Table V. Correlation matrix of some minerals between rumen fluid ($\mu\text{g/ml}$), liver, kidney, and meat ($\mu\text{g/g}$ wet weight) of growing lambs fed complete feed with 1% zeolite supplementation (T1).

Element	Tissue	Liver	Kidney	Rumen fluid	Meat
Aluminum	liver	1			
	Kidney	0.49	1		
	Rumen fluid	0.68	0.97*	1	
	Meat	-0.91	-0.81	-0.63	1
Chromium	liver	1			
	Kidney	0.21	1		
	Rumen fluid	-0.99	0.37	1	
	Meat	-0.16	0.99*	0.31	1
Manganese	liver	1			
	Kidney	0.91	1		
	Rumen fluid	0.5	0.08	1	
	Meat	0.27	-0.16	0.97*	1
Potassium	liver	1			
	Kidney	-0.07	1		
	Rumen fluid	0.01	0.99*	1	
	Meat	-0.33	0.92	0.94*	1

The change in Al concentration in some lambs' tissues with zeolite supplementation may be related to the fact that part of the zeolite is hydrolyzed and released as silicic acid, amorphous aluminum silicates, and Al at acidic pH (Cook *et al.*, 1982; Mohri *et al.*, 2008). Therefore, zeolite may possibly supply a significant amount of Al to the diets of lamb, provided that Al is present in a form that can be utilized by the body. The results of this study reveal some numerical Al increases in lamb body tissues, which were supplemented with natural zeolite (especially 1% natural zeolite), such as the kidney and rumen fluids, which confirm the above hypothesis. Rumen Al was highly correlated with that of meat in the group without zeolite supplementation, but correlated with kidney when supplemented with 1% zeolite. Moreover, lambs supplemented with 2% zeolite showed a significantly high correlation of rumen fluid Al with those of liver and meat. This gives an indication that zeolite supplementation plays a role in Al absorption and metabolism according to the level supplemented with the complete feed.

Table VI. Correlation matrix of some minerals between rumen fluid ($\mu\text{g/ml}$), liver, kidney, and meat ($\mu\text{g/g}$ wet weight) of growing lambs fed complete feed with 2% zeolite supplementation (T2).

Element	Tissue	Liver	Kidney	Rumen fluid	Meat
Aluminum	liver	1			
	Kidney	0.73	1		
	Rumen fluid	0.94*	0.46	1	
	Meat	0.88	0.32	0.99*	1
Chromium	liver	1			
	Kidney	-0.27	1		
	Rumen fluid	0.57	-0.95*	1	
	Meat	-0.56	0.95	-0.99*	1
Manganese	liver	1			
	Kidney	0.82	1		
	Rumen fluid	0.17	-0.43	1	
	Meat	-0.89	-0.98*	0.29	1
Potassium	liver	1			
	Kidney	-0.42	1		
	Rumen fluid	0.96*	-0.66	1	
	Meat	0.78	-0.89	0.93*	1

The mechanism of Cr intestinal absorption is determined by passive diffusion (Stoecker, 1999). Cr tends to accumulate in epidermal tissues, bones, liver, kidney, spleen, lungs, and large intestine. Cr concentration was not significantly different in the lamb body tissues, but lower concentrations were reported in all the tissues investigated. This study reported a highly significant negative correlation between rumen fluid and kidney and meat for lambs fed 2% zeolite (T2). The control group (without zeolite supplementation) showed a positive correlation between rumen fluid and meat. Moreover, there was a positive correlation between kidney and meat in the group fed 1% zeolite. Wallach (1985) reported that accumulation in other tissues, especially muscles, seems to be strictly limited or non-existent. This means that zeolite causes a noticeable change in Cr digestion, absorption, and accumulation in tissues by reducing levels, but made changes in the correlation between them.

For Mn status, our result is in agreement with the results of Gowda *et al.* (2007), which concluded that addition of zeolite (in forms of hydrated sodium calcium aluminosilicate) at the rate of 5 g/kg concentrate mixture in lambs resulted in lower absorption of Mn. This completely supports our finding in which a significant decrease was reported in rumen fluid Mn with increasing level of zeolite

supplementation. Moreover, there was a decrease in liver and meat Mn and an increase in kidney Mn. Thus, it is highly recommended that Mn supplementation be increased when using absorbent materials, such as zeolite. This study reported a higher significant Mn digestibility using zeolite when compared to control (without zeolite supplementation), even though no significant differences in levels of Mn in liver, kidney, and meat were found. This may be explained by the reports of Miranda *et al.* (2006) regarding a proper homeostatic mechanism in animal body maintain tissues Mn within a limited range. On the other hand, there was no significant correlation between Mn concentration in different tissues; however, zeolite supplementation (1%; T1) to growing lambs led to a positive significant correlation between rumen fluid and meat Mn. Lambs supplemented with 2% zeolite reported a significant negative correlation between kidney and meat Mn.

For our result regarding K accumulation in tissues and rumen fluid, very minor increase were reported in all tissues and rumen fluid, but it was not significant. This finding agrees with Pond and Yen (1983), Pond *et al.* (1984), and Ronald *et al.* (1993), who reported no effect on blood plasma and liver K with zeolite supplementation for growing lambs, hens, and pigs. Adversely, mice that were on clinoptilolite supplementation was reported have a 20% increase in serum K (Martin-Kleiner *et al.*, 2001). A decrease in K concentration in plasma blood of dairy cattle fed zeolite was observed (Sweeney *et al.*, 1980). This may be due to the high K-binding capabilities of zeolite in the small intestinal tract (Pond *et al.*, 1988). In contrast with findings from previous studies, the present study recorded a gradual increment in K concentration in most of the tested body tissues. Additionally, we registered a very strong correlation of K concentration between rumen fluids and kidney when fed 1% zeolite; however, 2% zeolite supplementation showed a significantly positive correlation between rumen fluid and liver K. Therefore, a change in K accumulation in tissues was mainly affected by the supplemented levels of zeolite, animal species, and physiological status.

CONCLUSIONS

Short-term natural zeolite (Clinoptilolite) supplementation for growing lambs fed complete feed as TMR has variable effects on bioavailability of Al, Cr, and Mn by increasing or decreasing the absorption and utilization efficiency and consequent accumulation in tissues. Therefore, the change in in absorption and accumulation of some minerals in tissues, especially kidney, with zeolite supplementation, require special attention for

the dietary needs of ruminant animal in order to avoid deficiencies or toxicities. Conducting an experimental research using ruminal and duodenal fistulated lambs with minerals isotopes is highly recommended to justify the findings obtained from zeolite supplementations.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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