



Effect of Natural Zeolite as a Rumen Buffer on Growth Performance and Nitrogen Utilization of Barki Lambs

Wafaa M.A. Ghoneem, Reham R. El-Tanany and Adel E.M. Mahmoud*

Animal Production Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt

ABSTRACT

This study aimed to determine the effect of zeolite addition in comparison to sodium bicarbonate as a rumen buffer on the growth performance of lambs and nitrogen utilization. Thirty growing Barki lambs (live body weight 34 ± 0.1 kg, age: 6 months); divided into three groups (10 in each). Animal groups were randomly divided as follows: G1 (control group): lambs fed clover hay, concentrate feed mixture (CFM) plus sodium bicarbonate (1% of dry matter (DM)), while lambs in G2 and G3 groups fed clover hay, CFM plus natural zeolite (clinoptilolite) at the level of 1 and 2% of DM, respectively. Results showed that there were insignificant ($P < 0.05$) differences among groups in the digestion coefficients (%) of dry matter, crude fiber, ether extract, nitrogen-free extract, and feeding value expressed as digestible crude protein. And there were insignificant ($P < 0.05$) differences between G1 and G2 in the digestibility (%) of OM, CP, ADF, and TDN values. While the highest nutrients digestibility and nutritive value were recorded in G3 (2% zeolite addition) Rumen parameters were in the normal range and with insignificant ($P < 0.05$) differences among groups. The pH values tended to increase but concentrations of ruminal $\text{NH}_3\text{-N}$ and TVFA tended to decrease as the level of zeolite increased. Concentrations of plasma urea were significantly ($P < 0.05$) decreased with lambs fed zeolite rations. Results indicated that zeolite addition had no adverse impact on lambs' health which all blood parameters were within the normal range. Lambs fed 2% zeolite ration had the highest ADG and the best feed conversion and energy utilization efficiency with no significant difference among the different groups. Also, the addition of 2% zeolite decreased nitrogen loss in feces. It could be concluded that zeolite addition especially at level 2% of DM in Barki lamb rations improved productive performance and economic efficiency.

Article Information

Received 07 December 2019
Revised 12 February 2021
Accepted 05 March 2021
Available online 09 June 2021
(early access)
Published 21 February 2022

Authors' Contribution

WMAG, RRE and AEMM presented the idea and designed the study, performed the trial, collected the samples and completed the analyses. AEMM and WMAG did the statistical analysis, data discussion and manuscript writing and editing.

Key words

Zeolite, Sodium bicarbonate, Barki lambs, Digestibility, Growth performance.

INTRODUCTION

Ruminal acidosis is one of the digestive disorders which is associated with consuming high concentrate diets. To alleviate acidosis, the inclusion of ruminal buffers has been suggested. The most commonly exogenous buffer is sodium bicarbonate (NaHCO_3). It helps in ruminal pH stabilization in cows that may suffer from acidosis (Clark *et al.*, 2009). Sodium bicarbonate is characterized by an acid dissociation constant ($\text{pK}_a = 6.25$), which makes the ruminal pH close to the normal value. Erdman (1988) and Russell and Chow (1993) documented the mode of action of NaHCO_3 in the rumen by its high acid consuming capacity. Although NaHCO_3 is considered as an efficient buffer, its inclusion increases the diet cost for the producers (Harrison *et al.*, 1986). Therefore, searching for cheaper mineral buffers which exhibit the same mode of action as NaHCO_3 is necessary.

Natural zeolite (clinoptilolite) is an example of such minerals, which can gain and lose water and other cations such as K^+ , NH_4^+ , Ca^{2+} and Mg^{2+} reversibly (Mumpton, 1999). The ability of zeolite to liberate progressively ions may facilitate the rumen fermentation and regulate ruminal pH by combination with hydrogen ions released from organic acids. In addition, zeolite can be used to improve nitrogen utilization by the animal via increasing microbial protein syntheses because of its ability to release excess ammonia gradually in the rumen (Mumpton, 1999). McCollum and Galyean (1983) observed that zeolite may alter digestion and rumen fermentation of beef steers. Dschaak *et al.* (2010) and Khachlouf *et al.* (2018) concluded that zeolite had no negative effect on lactation production and rumen parameters and could be used as a ruminal buffer with moderate inclusion level, in addition to decrease feeding cost in lactating cows. The European Committee has approved zeolite as an additive in diets of farm animals at inclusion rate of 2% of dry matter (European Commission Regulation, 2005). Eng *et al.* (2003) and Tiwari (2007) indicated that increasing of the fecal N excretion by the animals can be rapidly lost as

* Corresponding author: akkb2010@gmail.com
0030-9923/2022/0003-1199 \$ 9.00/0
Copyright 2022 Zoological Society of Pakistan

NH₃, or may increase nitrate levels in ground water, which may cause nutrients imbalance between N and P in the soil. Zeolite has been demonstrated as a potential to reduce N and P concentrations in pigs' manure (Cromwell *et al.*, 1998) meanwhile, reduce the negative impact of odour and other gaseous such as NH₃ (Bozkurt, 2006). So, zeolite may be considered as environmentally friendly additive, which reduce ammonia volatilization by adsorbing it and reducing fecal N loss (Toprak *et al.*, 2016).

The objectives of this study were to investigate if natural zeolite (clinoptilolite) could replace sodium bicarbonate as a buffer in sheep ration, and to evaluate the effects of sodium bicarbonate and natural zeolite additions on nutrients digestibility, growth performance, blood parameters and ruminal fermentation characteristics of barki lambs and nitrogen loss in feces.

Table I.- Chemical composition of the concentrate feed mixture, clover hay and experimental ration.

Item	Feedstuffs		Experimental ration (calculated)
	CH	CFM	
DM	90.84	91.60	91.41
Chemical composition, % (DM basis)			
OM	88.24	94.91	93.24
Ash	11.76	5.09	6.76
CP	13.60	14.74	14.45
EE	1.65	2.49	2.28
CF	26.86	5.75	11.03
NFE	46.13	71.93	65.48
Fiber fraction, %			
NDF	43.82	31.46	34.55
ADF	31.72	7.50	13.55
ADL	4.95	2.41	3.05
Cellulose	26.77	5.09	10.50
Hemi-cellulose	12.10	23.96	21.00

CFM, Concentrate feed mixture; CH, clover hay; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen free extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

MATERIALS AND METHODS

Experimental animals and ration

Thirty Barki lambs 34±0.10 Kg live body weigh; age 6 months, were used in growth trial for 90 days. Lambs were divided into 3 feeding groups (10 animals each) according

to their live weight, then the groups were randomly divided as follows: G1 (control group): lambs fed clover hay, concentrate feed mixture (CFM) plus sodium bicarbonate (1% of DM), while sodium bicarbonate was replaced by either 1 or 2% natural zeolite (clinoptilolite) in G2 and G3 groups, respectively. The concentrate feed mixture (CFM) consisted of 45% yellow corn, 20% barley, 13% soybean meal, 20% wheat bran, 1% common salt, 0.5% dicalcium phosphate, 0.3% ammonium chloride, 0.1% toxin binder and 0.1% premix, was offered by 3% and clover hay by 1% of body weight to cover the total requirements according to NRC (1985). The chemical composition of concentrate feed mixture, clover hay and experimental ration are presented in Table I.

Feeding procedures

The growing lambs were fed CFM and clover hay twice daily. Clean water was allowed freely all-day round. Orts were collected just before offering the next day feed. Lambs were weighed every two weeks before morning feeding after 15 h of fasting. Rations were adjusted every two weeks according to body weight changes. Body weight gain was recorded, and daily feed intake was calculated. Feed conversion ratio was calculated as dry matter (DM) intake, g/daily gain, g and total digestible nutrients (TDN) intake, g/ daily gain, g.

Digestion trials

At the end of growth trial, three animals from each group were fed individually in metabolic cages for digestion trial. Each trial was divided into two stages: a preliminary period (adaptation) for 21-days and collection period for 7-day where the voluntary feed intake was measured, and total collection of feces was carried out. Then one tenth of daily feces weight was taken and dried at 60-70°C in a hot air oven. The orts were weighted daily, meanwhile feed intake was calculated. The dried samples of feces and feeds were grinded to pass through 1 mm screen, and then these samples of ration and feces were stored for chemical analysis. Consequently, the digestion coefficient and nutritive values of the experimental rations were calculated.

Chemical analysis

Feeds and feces were analyzed for proximate analyses (AOAC, 2000). Nitrogen free extract was calculated by difference. The NDF, ADF and ADL were determined according to Van Soest *et al.* (1991). Cellulose and hemi-cellulose were calculated by difference according to the following equations: cellulose = ADF - ADL and hemi-cellulose = NDF - ADF.

Rumen liquor sampling

Rumen liquor samples were taken just before morning feeding then at three- and six-hours post feeding. Samples of rumen liquor were strained through two layers of cheesecloth and its pH was immediately measured after collection by using pH meter. Strained rumen liquor (SRL) samples were acidified with 0.1 N hydrochloric acid and concentrated orthophosphoric acid and stored by freezing for determination of total volatile fatty acids (TVFA's). Ammonia was determined according to (Preston, 1995). Total VFA was determined by steam distillation according to Cunniff (1997).

Blood parameters

Blood samples were withdrawn from the jugular vein from all the experimental animals 4 h post the morning feeding in dry clean glasses tubes using heparin as anticoagulant and then centrifuged for 15 min at 4000 rpm to obtain plasma. Blood plasma total protein and creatinine were determined according to Tietz (1986) and Tietz *et al.* (1990), albumin was determined according to Dumas *et al.* (1971), blood plasma urea was determined according to Patton and Grouch (1977). Alanine amino transferase (ALT) and activity of aspartate amino transferase (AST) were determined by the methods of Young (1997).

Statistical analysis

Data were analyzed using the general linear model procedure of SAS (2001, Ver.8.02, SAS Institute Inc., Cary, NC, USA). The differences among means were separated according to Duncan New Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Nutrients digestibility and nutritive values

Digestion coefficients of nutrients are presented in Table II. Although, data indicated that there was insignificant ($P < 0.05$) difference in dry matter (DM) digestibility among groups, it increased with zeolite rations either with 1 or 2% compared with 1% sodium bicarbonate ration. Similar results had been recorded in previous research, where DM digestibility was not significantly ($P < 0.05$) affected with addition of 1 or 2% zeolite to finishing diet of beef steers (Cole *et al.*, 2007) or addition of 1.4% zeolite (Dschaak *et al.*, 2010) and 1.25, 2.5 and 5% zeolite (McCullum and Galyean, 1983) to dairy cow rations. Also, addition of 2, 4 and 8% zeolite had no significant effect on *in vitro* DM disappearance (Sanders *et al.*, 1997). However, Ghaemnia *et al.* (2010) noted that DM digestibility decreased insignificantly with 3 and 6% zeolite and significantly with 9% zeolite in lambs' rations.

On the other hand, Forouzani *et al.* (2004) reported a significant ($P < 0.05$) increase in DM digestibility with zeolite rations (30 and 60 g/kg) compared with control.

Table II.- Nutrients digestibility and nutritive values of the experimental rations.

Item	Experimental groups			±SE
	G1	G2	G3	
Apparent digestibility, %				
DM	77.72	78.64	79.55	0.39
OM	78.70 ^b	80.27 ^{ab}	81.32 ^a	0.48
CP	74.66 ^b	75.41 ^{ab}	77.45 ^a	0.52
CF	60.07	61.35	60.79	0.48
EE	70.94	68.12	68.46	0.73
NFE	84.53	85.24	86.61	0.51
Fiber fractions				
NDF	60.90 ^b	61.88 ^a	61.71 ^a	0.18
ADF	51.57 ^b	52.21 ^{ab}	52.82 ^a	0.22
Cellulose	62.30 ^b	65.42 ^a	64.88 ^a	0.50
Hemi-cellulose	66.92 ^b	68.12 ^a	67.46 ^a	0.19
Nutritive values, %				
TDN	76.40 ^b	76.99 ^b	78.13 ^a	0.28
DCP	10.79	10.90	11.19	0.16

^{a, b} Means in the same row with different superscript are significantly different ($P < 0.05$). DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen free extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients; DCP, digestible crude protein. G1 (control group), lambs fed CFM + Clover hay + 1% sodium bicarbonate; G2, lambs fed CFM + clover hay + 1 zeolite; G3, lambs fed CFM + clover hay + 2% zeolite.

Lambs fed 2% zeolite ration had significantly higher ($P < 0.05$) organic matter (OM) and crude protein (CP) digestibility than the control group, with no significant difference between group fed 1% zeolite ration (G2) and other groups (G1 and G3). McCullum and Galyean (1983) showed insignificant increase in OM digestibility with 1.25% zeolite ration of beef steers. While it was not affected either by 1.4% zeolite or sodium bicarbonate when dairy cows fed the diet (Dschaak *et al.*, 2010). The improvement in OM digestibility may be due to higher digestibility of CP and cell wall constituents. In the same context, Forouzani *et al.* (2004) and Ghaemnia *et al.* (2010) recorded higher CP digestibility either with lambs fed 3, 6 and 9% zeolite diets or those fed 30 and 60 g/kg zeolite, respectively. In contrast, McCullum and Galyean (1983) reported that there was no significant effect with zeolite addition on CP digestibility. The increase in CP

digestibility with zeolite addition can be attributed to the ability of zeolite to take up ammonia from the rumen, then release it gradually by replacing it with potassium and sodium, which allow continuous synthesis of microbial protein by rumen microorganisms (Mumpton and Fishman, 1977; Koknarroglu *et al.*, 2006).

Insignificant differences were observed in digestibility of CF, EE and NFE among groups. Significant increases ($P < 0.05$) in digestibility of NDF, ADF, cellulose and hemicelluloses were detected with addition of 2% zeolite (G3) compared with 1% sodium bicarbonate. Group 2 take the same trend of G3 except that there was insignificant ($P < 0.05$) difference in the digestion of ADF compared with G1. In the same context, values of neutral detergent fiber (NDF) digestibility was significantly higher with zeolite addition at 30 g/kg ration (Forouzani *et al.*, 2004) and at 3, 6 and 9% (Ghaemnia *et al.*, 2010). While no significant difference was observed in ADF digestibility with zeolite diets (Forouzani *et al.*, 2004; Dschaak *et al.*, 2010; Ghaemnia *et al.*, 2010). The high attraction of zeolite with water and other active cations may facilitate the interaction between rumen bacteria and fibers which can alter the digesta passage rate through the rumen (Sweeney *et al.*, 1984; Johnson *et al.*, 1988). Also, the same authors found that the increase in ruminal pH with zeolite addition can make the rumen environment more suitable for microbial fermentation which leads to more fiber digestion.

Addition of 2% zeolite significantly ($P < 0.05$) increased the nutritive value as total digestible nutrients (TDN) being 78.13% compared to 76.41% in sodium bicarbonate ration. However, ration contained 1% zeolite had no significant effect on TDN value. Digestible crude protein (DCP) was insignificantly ($P < 0.05$) increased with zeolite addition compared with control group. The improvement in nutritive values as TDN and DCP with 2% zeolite ration may be due to higher digestibility of OM and CP when zeolite added by 2% compared with 1% zeolite and sodium bicarbonate rations.

Rumen liquor parameters

Data in Table III indicated that ruminal pH was significantly higher ($P < 0.05$) just before feeding and 3 h post feeding when zeolite added by 2% compared with 1% zeolite, but the difference between zeolite rations and control was not significant. Ruminal pH at 6 h post feeding and the mean value or ruminal pH were increased insignificantly with zeolite addition compared with sodium bicarbonate.

Results of ruminal pH tended to increase insignificantly with 1.4% zeolite group (6.61) compared to 6.54 for 1.4% sodium bicarbonate group (Dschaak *et al.*, 2010). Also, Eng *et al.* (2003) found that addition

of 1.2% zeolite to steers ration increased ruminal pH significantly. However, ruminal pH was not significantly different when zeolite added to lamb's ration (Kardaya *et al.*, 2012) or when ammoniated zeolite added to goats rations by 2, 4 and 6% (Erwanto *et al.*, 2011). On the other hand, insignificant increase and decrease were observed when zeolite added to lambs rations at 30 g and 60 g/kg, respectively (Forouzani *et al.*, 2004).

The ability of zeolite to bind with water and active cations may improve rumen fermentation and osmotic activity that regulate ruminal pH due to zeolite binding with hydrogen ions released because of organic acids fermentation (Dschaak *et al.*, 2010). Also, the higher aluminum and magnesium silicate contents may contribute to buffering capacity (Khachlouf *et al.*, 2018). The lack of significant effect of zeolite addition on ruminal pH may be attributed to the strong capacity of sodium bicarbonate to neutralize protons (Marden *et al.*, 2008).

Table III.- Effect of zeolite on rumen parameters of growing Barki lambs.

Item	Sampling time (h)	Experimental groups			±SE
		G1	G2	G3	
pH	0	6.67 ^{ab}	6.42 ^b	6.74 ^a	0.06
	3	5.90 ^b	6.14 ^{ab}	6.36 ^a	0.09
	6	6.21	6.55	6.68	0.10
	Mean	6.26	6.37	6.59	0.09
NH ₃ -N (mg/100 ml rumen liquor)	0	4.82	5.10	5.47	0.24
	3	12.97 ^a	10.75 ^c	11.62 ^b	0.34
	6	12.17 ^a	10.32 ^b	8.90 ^b	0.52
	Mean	9.99	8.72	8.66	1.07
TVFA (mleq/100 ml rumen liquor)	0	4.85	4.59	4.34	0.13
	3	10.10 ^a	9.47 ^a	8.25 ^b	0.30
	6	8.18 ^a	8.43 ^a	7.23 ^b	0.22
	Mean	7.71	7.50	6.61	0.72

^{a, b, c}. Means in the same row with different superscript are significantly different ($P < 0.05$). G1 (Control group), lambs fed CFM + Clover hay + 1% sodium bicarbonate; G2, lambs fed CFM + clover hay + 1 zeolite; G3, lambs fed CFM + clover hay + 2% zeolite.

Regarding to sampling time, ruminal NH₃-N concentrations just before feeding were not significantly affected by zeolite addition. While it decreased significantly ($P < 0.05$) with addition of 1 and 2% zeolite either at 3 or 6 h post feeding. Regardless, sampling time, the mean values of ruminal ammonia-nitrogen were decreased insignificantly when zeolite added to lambs rations at 1 and 2%.

The results obtained by [Forouzani *et al.* \(2004\)](#) agreed with our results that revealed insignificant decreases in ruminal $\text{NH}_3\text{-N}$ concentrations with 30 or 60 g zeolite/kg added to lamb's rations. In contrary, insignificant increases in ruminal $\text{NH}_3\text{-N}$ concentrations were observed by [Erwanto *et al.* \(2011\)](#) with 2, 4 and 6% zeolite. Zeolite addition can improve nitrogen utilization because of absorbing high concentrations of ammonia in the rumen by zeolite and releases it gradually when NH_3 concentration in the rumen is declined ([Bosi *et al.*, 2002](#)). The reduction in ruminal NH_3 accumulation after feeding may provide a more consistent environment in the rumen, which affect positively on animal performance ([Stojković *et al.*, 2005](#); [Ghaemnia *et al.*, 2010](#); [Kardaya *et al.*, 2012](#)).

Table IV.- Effect of zeolite on blood plasma parameters of growing Barki lambs.

Item	Experimental groups			±SE
	G1	G2	G3	
Total proteins (g/dl)	6.50	6.44	6.56	0.03
Albumin (g/dl)	2.66 ^a	2.38 ^b	2.50 ^b	0.04
Globulin (g/dl)	3.84 ^b	4.06 ^a	4.06 ^a	0.04
Urea (mg/dl)	41.80 ^a	39.20 ^b	37.60 ^b	0.59
Creatinine (mg/dl)	1.34	1.30	1.26	0.02
AST (IU/L)	68.00 ^a	66.60 ^{ab}	66.00 ^b	0.39
ALT (IU/L)	56.00	55.40	55.80	0.44

^{a, b} Means in the same row with different superscript are significantly different ($P < 0.05$). ALT, alanine amino transferase; AST, Aspartate amino transferase. G1 (Control group), lambs fed CFM + Clover hay + 1% sodium bicarbonate; G2, lambs fed CFM + clover hay + 1% zeolite; G3, lambs fed CFM + clover hay + 2% zeolite.

No significant differences were recorded in ruminal TVFA concentrations among treatments just before feeding. At 3 and 6 h post feeding, lambs fed 2% zeolite ration had the lowest ($P < 0.05$) concentration of ruminal TVFA, while addition of 1% zeolite decreased TVFA concentrations insignificantly compared with sodium bicarbonate. The mean values of ruminal TVFA were decreased insignificantly with zeolite addition especially at 2% level. In the same trend of our results, ruminal TVFA concentrations were decreased insignificantly when zeolite added by 1.4% to dairy cow rations ([Dschaak *et al.*, 2010](#)) or added to lamb's ration ([Kardaya *et al.*, 2012](#)). While concentrations of TVFA were not affected by 1.25, 2.5 and 5% zeolite ([McCollum and Galyean, 1983](#)). In contrast, goats fed 6% ammoniated zeolite ration had higher concentration of TVFA, however goats fed 2 and 4% ammoniated zeolite had the same TVFA concentrations compared with control. The reduction in concentration

of ruminal TVFA may be attributed to faster removal of soluble nutrients before their fermentation ([Vicentin *et al.*, 1995](#)).

Blood parameters

Data concerning blood plasma parameters ([Table IV](#)) indicated that there were no significant ($P < 0.05$) differences in blood plasma total protein, creatinine, and ALT concentrations among different lambs' groups. Also, [Toprak *et al.* \(2016\)](#) found that lambs fed on 1, 2 and 3% micronized zeolite had no significant ($P < 0.05$) effect on blood total protein and creatinine compared with control. Concentrations of blood albumin and urea were significantly ($P < 0.05$) decreased with zeolite addition either at 1 or 2% compared with 1% sodium bicarbonate.

The significant decrease in blood urea concentrations with zeolite addition is paralleled with data obtained by [Forouzani *et al.* \(2004\)](#) who recorded significant decreases in blood urea nitrogen (BUN) concentrations at 4 h after lambs fed 30 or 60 g zeolite/kg. In the same trend, concentration of BUN was decreased significantly when lambs fed 9% zeolite ration, while the reduction was insignificant with 3 and 6% zeolite rations ([Ghaemnia *et al.*, 2010](#)). On the other hand, zeolite addition had no significant influence on plasma urea concentrations ([Kardaya *et al.*, 2012](#)) and BUN ([Toprak *et al.*, 2016](#)).

Lower concentrations of blood urea with zeolite addition may be due to zeolite adsorption of ruminal ammonia and release it gradually, which allows the microorganisms in the rumen to synthesize microbial protein ([Koknarroglu *et al.*, 2006](#)). Also, this decrease may be attributed to supply the optimal level of fermentable carbohydrate in the rumen that enhances the conversion of protein in ration into microbial protein ([Hoover and Miller, 1990](#)).

Lambs fed zeolite rations had higher ($P < 0.05$) blood globulin than those fed sodium bicarbonate ration. The lowest ($P < 0.05$) concentration of blood AST was recorded with 2% zeolite group, while no difference was recorded between 1% zeolite and sodium bicarbonate groups. The increase in concentration of blood globulin may reflect the improvement in immunity with zeolite addition, as mentioned by many authors. [Thilsing-Hansen *et al.* \(2002\)](#) and [Katsoulos *et al.* \(2005\)](#) reported a positive effect of zeolite on animals' health via reducing some metabolic diseases such as hypocalcaemia in dairy cows and diarrhea in lambs ([Stojković *et al.*, 2005](#)). Also, zeolite showed detoxification properties because of its ability to remove many harmful substances such as: poisons, mycotoxins, and radioactive elements ([Katsoulos *et al.*, 2015](#)), which can improve immunity ([Pavelić *et al.*, 2018](#)) and the oxidant status of the animal ([Yarovan, 2008](#)).

The present results indicated no adverse effects on tested blood parameters of growing barki lambs due to zeolite addition, since these values of blood parameters are within the normal physiological ranges which reported by Kaneko *et al.* (1997).

Growth performance

Data in Table V shows that no significant differences among different groups in final body weight and total gain. In the same trend, there were no significant differences among zeolite groups and sodium bicarbonate group in average daily gain (ADG), while ADG was slightly higher for lambs fed 2% zeolite ration. Forouzani *et al.* (2004) observed insignificant increases in ADG for both lambs' groups fed rations contained 30 and 60g zeolite/kg. While, when lambs fed 1, 2 and 3% micronized zeolite rations, ADG did not differ significantly with lower values for 2 and 3% zeolite groups (Toprak *et al.*, 2016). On the other hand, Stojković *et al.* (2005, 2012) recorded significant increases ($P < 0.01$) in ADG for lambs fed zeolite rations. However, McCollum and Galyean (1983), Sanders *et al.* (1997), Eng *et al.* (2003), Sherwood *et al.* (2005) and Koknarroglu *et al.* (2006) reported no effect of zeolite addition to feedlot cattle rations on ADG.

Table V.- Effect of zeolite on growth performance of barki lambs.

Item	Experimental groups			±SE
	G1	G2	G3	
Live body weight				
Initial body weight (kg)	33.90	33.90	34.00	0.96
Final body weight (kg)	48.68	47.72	49.76	1.40
Total weight gain (kg)	14.78	13.82	15.76	0.59
Average daily gain (g)	164.22	153.56	175.11	6.54
Feed intake / day				
Concentrate (kg)	1.12	1.06	1.09	----
Roughage (kg)	0.36	0.34	0.35	----
DMI (kg)	1.48	1.40	1.44	----
TDNI (kg)	1.13	1.08	1.13	----
Feed conversion, g/g				
DMI / daily gain	9.01	9.12	8.22	0.30
TDN intake / daily gain	6.88	7.03	6.45	0.22

^{a, b} Means in the same row with different superscript are significantly different ($P < 0.05$). DMI, total dry matter intake; TDN, total digestible nutrients intake. G1 (Control group), lambs fed CFM + Clover hay + 1% sodium bicarbonate; G2, lambs fed CFM + clover hay + 1 zeolite; G3, lambs fed CFM + clover hay + 2% zeolite.

Total dry matter intake (DMI) tended to decrease with both groups fed zeolite rations compared with those fed

sodium bicarbonate ration. In agreement of these results, feed intake was not affected significantly with zeolite addition in lambs' rations (Stojković *et al.*, 2012; Toprak *et al.*, 2016), and in feedlot cattle rations (McCollum and Galyean, 1983; Sherwood *et al.*, 2005). While feed intake tended to increase insignificantly when zeolite added to lambs' rations (Forouzani *et al.*, 2004; Stojković *et al.*, 2005) or to feedlot cattle rations (Eng *et al.*, 2003; Koknarroglu *et al.*, 2006). On the other hand, significant increase in feed intake was observed when steers fed 2% zeolite ration (Sanders *et al.*, 1997). Although there were no significant differences in feed conversion (DMI/daily gain) among different groups, however, lambs fed 2% zeolite ration had the best feed conversion compared with other groups.

Also, McCollum and Galyean (1983) and Toprak *et al.* (2016) found that zeolite had no significant effect on feed conversion. However, Eng *et al.* (2003), Forouzani *et al.* (2004), Sherwood *et al.* (2005), Koknarroglu *et al.* (2006) and Stojković *et al.* (2012) recorded insignificant decrease in feed conversion with zeolite addition. While the decrease in feed conversion that recoded by Stojković *et al.* (2005) was significant ($P < 0.05$).

The positive effect of zeolite addition on ADG and feed conversion may be due to the ability of zeolite to hold the harmful substances such as: heavy metals, mycotoxins, ammonia, *etc.*, and prevent their negative impact on animal performance and health (Smical, 2011; Stojković *et al.*, 2012). The slight improvement in ADG and feed conversion with 2% zeolite may be due to the significant increase in digestibility of OM and CP compared with other groups.

Data indicated that addition of 2% zeolite improved energy utilization efficiency as (TDN intake/daily gain) by 7.11% compared with control. In the same context, Stojković *et al.* (2012) observed an improvement in energy utilization as (ME intake/gain) by 10.58% when lambs fed zeolite ration.

Fecal dry matter, ash, and nitrogen contents

Table VI represents dry matter (DM), ash and nitrogen contents in feces collected from lambs at the end of the experiment. It shows significant ($P < 0.05$) increase in fecal DM contents with zeolite addition. Sweeney *et al.* (1980) recorded an increase in fecal DM content with zeolite addition. Also, McCollum and Galyean (1983) noted the same trend, but the differences among zeolite groups and control were not significant. The same authors attributed the higher fecal DM content with zeolite addition to the ability of zeolite to hold water and alter rumen osmolality consequently. In contrast, zeolite addition did not alter fecal DM content obtained by Sherwood *et al.* (2005) and

Toprak *et al.* (2016).

Table VI.- Effect of zeolite on dry matter, ash, and nitrogen contents in feces of Barki lambs.

Item	Experimental groups			±SE
	G1	G2	G3	
Dry matter, DM, %	36.29 ^b	34.04 ^a	34.09 ^a	0.16
Ash, % (DM basis)	10.88 ^c	13.88 ^b	14.81 ^a	0.59
Nitrogen, % (DM basis)	2.63 ^{ab}	2.66 ^a	2.55 ^b	0.02

^{a, b} Means in the same row with different superscript are significantly different ($P < 0.05$). G1 (control group), lambs fed CFM + Clover hay + 1% sodium bicarbonate; G2, lambs fed CFM + clover hay + 1 zeolite; G3, lambs fed CFM + clover hay + 2% zeolite.

It was observed that fecal ash content significantly ($P < 0.05$) increased as the level of zeolite in the ration was increased. The increases in fecal ash contents with zeolite rations agreed of results recorded by Toprak *et al.* (2016).

Addition of 2% zeolite decreased significantly ($P < 0.05$) fecal N content (2.55%) compared with 1% zeolite (2.66%), but the difference between the two zeolite groups and sodium bicarbonate group (2.63%) was not significant. In the same context, Bechtel and Hutchenson (2003) observed a decrease in fecal N content after 15 days of the trial when 1.2% zeolite added to ration. The same authors explained this reduction in fecal N by the ability of zeolite excreted in the feces to bind with ammonia and reduce N losses. However, no significant effect of zeolite addition on fecal N loss was reported by Sherwood *et al.* (2005) and Toprak *et al.* (2016). Bozkurt (2006) stated that only 20-50% of dietary N could be converted into final products, then the indigested nitrogen is excreted in the form of ammonia, which accumulates within the livestock farms and negatively affect on animal and human health. Therefore, natural zeolite addition may reduce nitrogen emission, which reflect the improvement in dietary N utilization and reduce environmental pollution.

CONCLUSION

Results indicated that zeolite addition to lambs ration improved nutrients digestibility, average daily gain, feed conversion, energy utilization. The potential to use zeolite in the ration as a buffer compared with sodium bicarbonate because of its ability to increase ruminal pH and decrease blood urea concentration. Meanwhile, zeolite might be considered as environmentally friendly additive by reducing fecal N loss.

Statement of conflict of interest

The authors have declared no conflict of interests.

REFERENCES

- AOAC, 2000. *Official methods of analysis*, 17th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Bechtel, R. and Hutchenson D., 2003. Adding CZ to rations may reduce nitrogen losses. *Feedstuffs*, 7: 75.
- Bosi, P., Creston, D. and Casini L., 2002. Production performance of dairy cows after the dietary addition of clinoptilolite. *Ital. J. Anim. Sci.*, 1: 187. <https://doi.org/10.4081/ijas.2002.187>
- Bozkurt, Y., 2006. The use of zeolite to improve housed beef cattle performance by reducing ammonia accumulation in small farm conditions. *Asian J. Anim. Vet. Adv.*, 1: 60-64. <https://doi.org/10.3923/ajava.2006.60.64>
- Clark, J.H., Christensen R.A., Bateman, H.G. and Cummings, K.R., 2009. Effects of sodium sesquicarbonate on dry matter intake and production of milk and milk components by Holstein cows. *J. Dairy Sci.*, 92: 3354. <https://doi.org/10.3168/jds.2008-1995>
- Cole, N.A., Todd, R.W. and Parker, D.B., 2007. *Use of fat and zeolite to reduce ammonia emissions from beef cattle feed yards*. Int. Symp. Air Quality Waste Mgt. Agric., Broomfield, CO.
- Cromwell, G.L., Turner, L.W., Taraba, J.L., Gates, R.S., Lindemann, M.D., Traylor, S.L., Dozier, W.A. and Monegue, H.J., 1998. *Manipulation of swine diets to reduce odours and harmful gaseous emissions from manure*. Research Report, National Pork Board, Iwoa, USA, pp. 1-12. Available at: <https://porkcheckoff.org/wp-content/uploads/2021/02/97-1791-Cromwell-U-of-KY.pdf> (Accessed on 18 April, 2021).
- Cunniff, P. (ed.), 1997. *Official methods of analysis*. AOAC International, Maryland. USA.
- Doumas, B.T., Watson, W. and Biggs, H.G., 1971. A method for determination of albumin. *Clin. Chem. Acta*, 31: 87-93. [https://doi.org/10.1016/0009-8981\(71\)90365-2](https://doi.org/10.1016/0009-8981(71)90365-2)
- Dschaak, C.M., Eun, J.S., Young, A.J., Stott, R.D. and Peterson, S., 2010. Effects of supplementation of natural zeolite on intake, digestion, ruminal fermentation, and lactational performance of dairy cows. *Profess. Anim. Scient.*, 26: 647-654. [https://doi.org/10.15232/S1080-7446\(15\)30662-8](https://doi.org/10.15232/S1080-7446(15)30662-8)
- Duncan, C.B., 1955. Multiple range and multiple F test. *Biometrics*, 11: 1-42. <https://doi.org/10.2307/3001478>
- Eng, K.S., Bechtel, R. and Hutchenson, D., 2003.

- Adding a potassium, clinoptilolite zeolite to feedlot rations to reduce manure nitrogen losses and its impact on Rumen pH, E-coli and performance.* Proceedings of South West Nutrition and Management Conference, Arizona, USA. Available at: <https://practitioners.vitalitydetoxdrops.com/wp-content/uploads/2017/11/Clino-binding-Nitrogen-Ammonia1.pdf> (Accessed on 18 April, 2021)
- Erdman, R.A., 1988. Dietary buffering requirements of the lactating dairy cow: A review. *J. Dairy Sci.*, **71**: 3246. [https://doi.org/10.3168/jds.S0022-0302\(88\)79930-0](https://doi.org/10.3168/jds.S0022-0302(88)79930-0)
- Erwanto, I.R., Zakaria, W.A. and Prayuwidayati, M., 2011. The use of ammoniated zeolite to improve rumen metabolism in ruminant. *Anim. Prod.*, **13**: 138-142.
- European Commission Regulation, 2005. European Commission Regulation No. 1810/2005 of 4 November 2005 concerning a new authorization for 10 years of an additive in feedingstuffs. *Off. J. Eur. Union*, **291**: 1-7.
- Forouzani, R., Rowghani, E. and Zamiri, M.J., 2004. The effect of zeolite on digestibility and feedlot performance of Mehraban male lambs given a diet containing urea-treated maize Silage. *Anim. Sci.*, **78**: 179-184. <https://doi.org/10.1017/S1357729800053960>
- Ghaemnia, L., Bojarpour, M., Mirzadeh, K., Chaji, M. and Eslami, M., 2010. Effect of different levels of zeolite on digestibility and some blood parameters in arabic lambs. *J. Anim. Vet. Adv.*, **9**: 779-781. <https://doi.org/10.3923/javaa.2010.779.781>
- Harrison, G.A., Hemken, R.W. and Harmon, R.J., 1986. Sodium bicarbonate and alfalfa hay additions to wheat silage diets fed to lactating dairy cows. *J. Dairy Sci.*, **69**: 2321. [https://doi.org/10.3168/jds.S0022-0302\(86\)80671-3](https://doi.org/10.3168/jds.S0022-0302(86)80671-3)
- Hoover, W.H. and Miller, T.K., 1990. *Carbohydrate and protein considerations in ration formulation.* Proceedings of large dairy herd management conference. Cornell Cooperation Extensions, Cornell University, Ithaca, New York, pp. 71.
- Kaneko, J.J., Harvey, J.W. and Bruss, M.L., 1997. *Clinical biochemistry of domestic animals*, 5th Ed. Academic Press, Inc., USA, pp. 890.
- Kardaya, D., Sudrajat, D. and Dihansih, E., 2012. Efficacy of dietary urea-impregnated zeolite in improving rumen fermentation characteristics of local lamb. *Media Peternakan*, **35**: 207-213. <https://doi.org/10.5398/medpet.2012.35.3.207>
- Katsoulos, P.D., Karatzia, M.A., Polizopoulou, Z., Florou-Paneri, P. and Karatzias, H., 2015. Effects of prolonged consumption of water with elevated nitrate levels on certain metabolic parameters of dairy cattle and use of clinoptilolite for their amelioration. *Environ. Sci. Pollut. Res. Int.*, **22**: 9119-9126. <https://doi.org/10.1007/s11356-014-4060-8>
- Katsoulos, P.D., Roubies, N., Panousis, N., Arsenos G., Christaki, E. and Karatzias, H., 2005. Effects of long-term dietary supplementation with clinoptilolite on incidence of parturient paresis and serum concentrations of total Ca, P, magnesium, potassium and sodium in dairy cows. *Am. J. Vet. Res.*, **66**: 2081-2085. <https://doi.org/10.2460/ajvr.2005.66.2081>
- Khachlouf, K., Hamed, H., Gdoura, R. and Gargouri, A., 2018. Effects of zeolite supplementation on dairy cow production and ruminal parameters – a review. *Annls. Anim. Sci.*, **18**: 857-877. <https://doi.org/10.2478/aoas-2018-0025>
- Koknarroglu, H., Tokar, M.T. and Bozkurt, Y., 2006. Effect of zeolite and initial weight on feedlot performance of Brown Swiss cattle. *J. Anim. Vet. Adv.*, **1**: 49-54. <https://doi.org/10.3923/javaa.2006.49.54>
- Johnson, M.A., Sweeney, T.F. and Muller, L.D., 1988. Effects of feeding synthetic zeolite A and sodium bicarbonate on milk production nutrient digestion, and rate of digesta passage in dairy cows. *J. Dairy Sci.*, **71**: 946-953. [https://doi.org/10.3168/jds.S0022-0302\(88\)79640-X](https://doi.org/10.3168/jds.S0022-0302(88)79640-X)
- Marden, J.P., Julien, C., Monteils, V., Auclair, E., Moncoulon, R. and Bayourthe, C., 2008. How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? *J. Dairy Sci.*, **91**: 3528-3535. <https://doi.org/10.3168/jds.2007-0889>
- McCollum, F.T. and Galyean, M.L., 1983. Effects of clinoptilolite on rumen fermentation, digestion and feedlot performance in beef steers fed high concentrate diets. *J. Anim. Sci.*, **56**: 517- 524. <https://doi.org/10.2527/jas1983.563517x>
- Mumpton, F., 1999. La Roca Magica: Uses of natural zeolites in agriculture and industry. *Proc. natl. Acad. Sci. USA*, **96**: 3463. <https://doi.org/10.1073/pnas.96.7.3463>
- Mumpton, F.A. and Fishman, P., 1977. The application of natural zeolites in animal science and aquaculture. *J. Anim. Sci.*, **45**: 1188-1203. <https://doi.org/10.2527/jas1977.4551188x>
- NRC, 1985. *Nutrient requirements of sheep*, 6th ed., National Academy of Sciences, Washington, DC, USA, pp. 45.

- Patton, F.G. and Grouch, S.R., 1977. Colorimetric determination of urea. *Anal. Chem.*, **49**: 464-468. <https://doi.org/10.1021/ac50011a034>
- Pavelic, S.K., Medica, J.S., Gumbarevic', D., Filošević', A., Pržulj, N. and Pavelic', K., 2018. Critical review on zeolite clinoptilolite safety and medical applications *in vivo*. *Front. Pharmacol.*, **9**: 1-15. <https://doi.org/10.3389/fphar.2018.01350>
- Preston, T.A., 1995. *Tropical animal feeding - A manual for research worker*. FAO Animal Production and Health Paper 126, FAO, Rome. Available at: <http://www.fao.org/ag/aga/agap/frg/AHPP126/cont126.htm> (Accessed on 18 April, 2021).
- Russell, J.B. and Chow, J.M., 1993. Another theory for the action of ruminal buffer salts: Decreased starch fermentation and propionate production. *J. Dairy Sci.*, **76**: 826. [https://doi.org/10.3168/jds.S0022-0302\(93\)77407-X](https://doi.org/10.3168/jds.S0022-0302(93)77407-X)
- Sanders, K.J., Richardson, C.R. and Harper, S., 1997. *Effects of zeolites on performance of feedlot cattle*. Texas Technical University Animal Science, UK.
- SAS, 2001. *User's guide statistic*, Ver. 8.02. SAS Institute Inc., Cary, NC, USA.
- Sherwood, D.M., Erickson, G.E. and Klopfenstein, T.J., 2005. *Effect of clinoptilolite zeolite on cattle performance and nitrogen volatilization loss*. Nebraska Beef Cattle Reports, pp. 177. Available at: <https://digitalcommons.unl.edu/animalscinbcr/177> (Accessed on 18 April, 2021).
- Smical, I., 2011. Properties of natural zeolites in benefit of nutrition and health. *Int. J. Bioflux Soc.*, **3**: 51-57.
- Stojković, J., Adamović, M., Lemić, J. and Jašović, B., 2005. The effects of feeds based on natural zeolite on production results for fattening lambs. *Biotech. Anim. Husband.*, **21**: 49-52. <https://doi.org/10.2298/BAH0502049S>
- Stojković, J., Ilić, Z., Ćirić, S., Ristanović, B., Petrović, M.P., Caro Petrović, V. and Kurčubić, V., 2012. Efficiency of zeolite basis preparation in Fattening lambs diet. *Biotech. Anim. Husband.*, **28**: 545-552. <https://doi.org/10.2298/BAH1203545S>
- Sweeney, T.F., Cervantes, A., Bull, L.S. and Hemken, R.W., 1984. Effects of dietary clinoptilolite on digestion and rumen fermentation in steers. In: *Zeolite agriculture use of natural zeolites in agriculture and aquaculture* (eds. W.G. Pond and F.A. Mumpton). Westview Press, Boulder, Colorado, pp. 177-187.
- Sweeney, T.F., Bull, L.S. and Hemken, R.W., 1980. Effect of zeolite as a feed additive on growth performance in ruminants. *J. Anim. Sci.*, **51**(Suppl. 1): 401-409.
- Tietz, N.W., 1986. *Textbook of clinical chemistry*. W.B. Saunders, Philadelphia, pp. 1271.
- Tietz, N.W., Finley, P.R., Pruden, E. and Amerson, A.B., 1990. *Clinical guide to laboratory tests*, 2nd edn. W.B. Saunders Co., Philadelphia, pp. 931.
- Tiwari, J., 2007. *Zeolite as natural feed additives to reduce environmental impacts of swine manure*. M.Sc. thesis, McGill University, Montreal, Quebec, Canada, pp. 100.
- Thilising-Hansen, T., Jorgensen, R.J., Enemark, J.M.D. and Larsen, T., 2002. The effect of zeolite a supplementation in the dry period on periparturient calcium, phosphorus, and magnesium homeostasis. *J. Dairy Sci.*, **85**: 1855-1862. [https://doi.org/10.3168/jds.S0022-0302\(02\)74259-8](https://doi.org/10.3168/jds.S0022-0302(02)74259-8)
- Toprak, N.N., Yılmaz, A., Öztürk, E., Yigit, O. and Cedden, F., 2016. Effect of micronized zeolite addition to lamb concentrate feeds on growth performance and some blood chemistry and metabolites. *S. Afr. J. Anim. Sci.*, **46**: 313-320. <https://doi.org/10.4314/sajas.v46i3.11>
- Van Soest, P.J., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, **74**: 3583-3597. [https://doi.org/10.3168/jds.S0022-0302\(91\)78551-2](https://doi.org/10.3168/jds.S0022-0302(91)78551-2)
- Vicentin J., Rearte, D., Santini, F. and Elizalde J., 1995. Effect of zeolite supplementation on rumen environment and forage digestion in bovine fed temperate pasture. *Ann. Zootech.*, **44**: 160. <https://doi.org/10.1051/animres:199505128>
- Yarovan, N.I., 2008. Effect of zeolites on adaptation processes in cows. *Russian Agric. Sci.*, **34**: 120-122. <https://doi.org/10.3103/S106836740802016X>
- Young, D.S., 1997. *Effects of preanalytical variables on clinical laboratory tests*, 2nd edn. AACC Press, Washington, DC, USA, pp. 1285.