

Dietary effects of Ca-zeolite supplementation on some blood and tibial bone characteristics of broilers

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Abstract

This study was conducted to investigate the effects of differing amounts of natural Ca-zeolite on bone and some blood parameters. A total of 240 day-old Ross 308 broiler chicks were assigned to four treatments with three replicates, each containing 20 day-old chicks of mixed sex. A clinoptilolite+mordenite type of zeolite was added in the broiler diets at levels of 0 g/kg, 10 g/kg, 30 g/kg, and 50 g/kg. Stocking density was 14 broilers/m². During the six-week trial, blood parameters and bone characteristics were monitored. The inclusion of Ca-zeolite, at various levels, did not have any significant effect on the concentration of blood serum biochemical parameters; serum glucose, cholesterol, total protein, uric acid; concentrations of Ca, P, Na, K, Cl, and on tibial bone characteristics (tibia weight, ash, volume, specific gravity, and Ca and P contents) in the two sexes and mixed-sex between the groups at 21 and 42 days of age.

Keywords: Clinoptilolite+mordenite, serum biochemistry, tibia parameters

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Introduction

Zeolites are important minerals of hydrated aluminotectosilicates of alkali and alkaline-earth cations with three-dimensional structures of interconnecting channels and large pores, capable of trapping molecules in proper conditions. Each zeolite species has its own unique crystal structure and, hence, its own set of chemical and physical properties. Among many properties attributed to zeolites, most characteristics that relate to their effectiveness in animal nutrition are their ability to lose or gain water alternatively, and being capable of exchanging a variety of cations selectively without much major changes in their structure (Mumpton & Fishman, 1977; Waldroup *et al.*, 1984; Elliot & Edwards, 1991; Mumpton, 1999; Shariatmadari, 2008; Safaeikatouli *et al.*, 2010).

Zeolites have cationic binding features that could protect animals from the tissue accumulation of toxic materials (Pond & Yen, 1983; Jain, 1999) and influence Ca and P utilization (Leach *et al.*, 1990; Watkins & Southern, 1991; Frost *et al.*, 1992). Beneficial effects may also be attributed to the Si, Al or Na content of zeolites because it has been established that these minerals can influence Ca-metabolism (Edwards, 1987; Öztürk *et al.*, 1998) and bind nitrogenous cations such as NH₄⁺ (Nakaue *et al.*, 1981). It has also been suggested that zeolites may selectively retain or release Ca as it passes through the digestive system (Quarles, 1985; Roland *et al.*, 1985) and that they can absorb the nitrogen of some amino acids and stabilize them, thus reducing the energy required for the production of meat.

Previous studies, in different animal diets, indicated that the dietary supplements of zeolites had no major effect on serum biochemistry. Supplementing broiler diets with hydrated NaCaAl-silicate (Dwyer *et al.*, 1997; Başalan *et al.*, 2005; Miles & Henry, 2007) and with NaAl-silicate (Kurtoğlu *et al.*, 1998), compared with hydrated NaCaAl-silicate, bentonite, and polyvinylpyrrolidone (Keçeci *et al.*, 1998), and with Na-bentonite (Santurio *et al.*, 1999; Tauqir & Nawaz, 2001; Tauqir *et al.*, 2001; Eraslan *et al.*, 2005) did not affect most of the serum biochemical parameters.

The effects of dietary zeolites in poultry have been investigated extensively and a growth-promoting effect, evident in mineral utilization and metabolism has been reported. The beneficial effects may be related to the Al, Si, Zn, Na or K concentrations of zeolite, because these minerals have been shown to influence mineral metabolism and electrolyte balance, leading to an increased formation of bone (Roland *et al.*, 1993; Utlu *et al.*, 2007). Mineral metabolism and electrolyte balance largely regulate bone formation that would be linked to their primary action on mineral absorption, tissue distribution and excretion (Watkins & Southern, 1991). On the other hand, it has also been reported that zeolites suppressed P utilization by forming an indigestible compound with P through its aluminosilicate component, increased Ca utilization (Elliot & Edwards, 1991) and had an indirect effect on P absorption and metabolism (Leach *et al.*, 1990). Although several studies claimed that the addition of zeolites increased Ca utilization and the rate of bone ash deposition during growth (Watkins & Southern, 1991; 1992; Ballards & Edwards, 1988; Debeic, 1994; Zhang & Hung 1992; Rabon *et al.*, 1995), other researchers reported no zeolite effect (Altan *et al.*, 1998; Elliot & Edwards, 1991; Gezen & Eren, 2002; Keshavarz & McCormick, 1991). Moreover, the extent of performance-enhancement effects has been attributed to the type of zeolite used, its purity and physicochemical adsorption properties as well as the supplementation level used in the diet (Papaioannou *et al.*, 2005; Tiwari, 2007).

It is well established that the health and performance of birds are influenced by the nutrient and metabolites of blood that can be estimated by understanding the relationships between bone characteristics and blood biochemical parameters. The purpose of this study was, therefore, to evaluate the changes of some blood serum biochemical and bone characteristics of broilers with different levels of zeolite in their diet.

Materials and Methods

The zeolite used in this study was collected from well-defined zeolitic stratigraphic units in the Sivas-Yavu region of Turkey (Yalçın, 1997). Mineralogical associations were carried out on bulk samples by means of a Rigaku DMAX IIIC automated diffractometer at the Cumhuriyet University, Sivas. The material added to the basal diet during this investigation comprised mainly of clinoptilolite (50%), mordenite (40%), quartz (5%), feldspar (5%) and trace amounts of smectitic clay. The X-ray diffraction pattern and morphologies of the zeolite were fully explained in another study (Eleroglu & Yalçın, 2005). The samples were analyzed at the Activation Laboratories Ltd. (Actlabs, Ancaster, Canada) for major oxides and trace element content, using an inductively coupled plasma mass spectrometer (ICP-MS).

The SiO₂, Al₂O₃, H₂O related to loss on ignition, and CaO are the essential components of the zeolitic material and Fe₂O₃ and MgO are represented in minor amounts (Table 1). Heulandite and mordenite-bearing tuffs are richer in alkaline-earth elements such as Ca, negligible Sr and Ba rather than alkali ones such as Na and K. The ratios of SiO₂/(Al₂O₃+Fe₂O₃), (Na₂O+K₂O)/(CaO+BaO+SrO) and Na₂O/K₂O are 4.68, 0.35 and

Table 1 Chemical composition of the natural zeolitic volcanic tuff

Major oxides (wt. %)		Trace Elements (mg/kg)			
SiO ₂	63.82	Cr	60	Sb	1
TiO ₂	0.297	Ni	20	Rb	17
Al ₂ O ₃	11.72	Co	3	Ba	1255
ΣFe ₂ O ₃	1.92	Sc	7	Sr	3571
MnO	0.022	V	31	Ga	13
MgO	1.04	Cu	10	Nb	10
CaO	4.11	Pb	22	Hf	5
Na ₂ O	0.95	Zn	40	Zr	194
K ₂ O	0.67	Sn	4	Y	30
P ₂ O ₅	0.07	W	1	Th	13
LOI	14.84	As	9	U	2

ΣFe₂O₃ = Total iron, LOI = Loss on ignition at 1000 °C.

Table 2 Ingredients and composition of experimental diets (%)

Feed ingredients	0–11 days	11–21 days	21–35 days	35–42 days
Maize	54.1	55.3	57.7	61.7
Soybean meal	16.2	11.5	8.2	4.2
Full-fat soybean	16	20	21	21
Chicken meal	3.9	3.9	3.9	3.9
Sunflower meal	3	3	3	3
Meat-bone meal	2.6	2.6	2.6	2.6
Vegetable oil	1.11	2.18	2.30	2.48
Fish meal	1	-	-	-
Marble powder	0.60	0.52	0.43	0.25
Vitamin-mineral premix*	0.30	0.25	0.25	0.25
DL-Methionine	0.27	0.21	0.15	0.15
Lysine	0.240	0.110	0.070	0.075
Salt	0.10	0.10	0.12	0.11
Vitamin D ₃	0.100	0.075	0.040	-
Vitamin E	0.05	-	-	0.10
Enzyme	0.10	0.10	0.10	0.10
Sodium bicarbonate	0.075	0.075	0.050	0.075
Choline chloride	0.07	0.07	0.05	0.05
Anticoccidiostat	0.05	0.05	0.05	-
Toxin binder	0.05	-	-	-
Calculated nutrients composition (g/kg)				
ME (MJ/kg)	12.73	13.15	13.31	13.48
Crude protein	230	215	205	190
Crude cellulose	44.4	45.2	44.9	44.8
Crude ash	60.7	57.1	54.3	51.0
Ether extract	89.1	106.1	109.3	113.1
Lysine	15	13	12	11
Methionine	6.1	5.3	4.6	4.4
Methionine + Cystine	10.8	9.8	9.0	8.6
Threonine	9.8	8.8	8.4	7.8
Calcium	10.5	9.5	9.0	8.0
Total phosphorus	7.6	7.2	7.1	6.7
Available phosphorus	5.0	4.6	4.6	4.3

*Each kg of vitamin-mineral premix contained: vitamin A, 4.400.000 IU; vitamin D₃, 1.600.000 IU; vitamin E, 20.000 mg; vitamin K₃, 1.600 mg; vitamin B₁, 1.200 mg; vitamin B₂, 3.200 mg; vitamin B₃, 20.000 mg; vitamin B₅, 6.000 mg; vitamin B₆, 1.600 mg; vitamin B₉, 800 mg; vitamin B₁₂, 8 mg; biotin, 80 mg; antioxidant dry, 50.000 mg; Cu, 6.000 mg; Fe, 20.000 mg; Mn, 48.000 mg; Se, 80 mg; Zn, 40.000 mg; Co, 80 mg; I, 500 mg.

1.42, respectively, and can be classified as Ca-zeolite by extruding very small impurities. Besides, transition metals and other trace elements are present in negligible amounts in zeolites.

Two hundred and forty day-old sexed broiler chicks (Ross 308 strain) were obtained from a commercial hatchery (Kayseri Yemsel Company, Turkey). The birds were randomly allocated to 12 pens, each with 10 males and 10 females. There were four dietary treatments, each with three replicates. The

experiment was conducted as a completely randomized design. Three maize-soybean meal basal diets (starter 0 - 11 days, grower 11 - 21 - 35 days and finisher 35 - 42 days) were formulated to provide adequate levels of all nutrients for broilers (NRC, 1994; Table 2). The diets of the starter phase (0 - 11 days) were formulated to contain 230 g crude protein (CP)/kg and 12.73 MJ of metabolizable energy (ME)/kg of diet. The diet for grower, phase 1, contained 215 g CP and 13.15 MJ ME/kg and for grower, phase 2, 205 g CP and 13.31 MJ ME/kg of diet while the diet for the finisher phase contained 190 g CP and 13.48 MJ ME/kg diet. The basal diets (control) were supplemented with four levels of zeolite (0, 1, 3 and 5%) to provide 0, 10, 30 and 50 g/kg of total Ca-zeolite in the diet. Feed and water were provided *ad libitum*.

A broiler house was divided into 12 sections with 2 x 1 x 1 m dimensions (length x width x height) and separated by mesh wire fences that prevented air exchange between sections and stocked with 14 birds/m². Its preparation was done, as specified by Türkoğlu *et al.* (1997), prior to the introduction of the chicks. The interior of the broiler house was naturally ventilated. The treatment groups were randomly distributed in the houses and the same airflow was provided. The temperature was maintained at 32 °C during the first week and was then reduced by 3 °C per week until 20 °C was reached. This temperature was maintained until the end of the experiment. The birds were exposed to light for 24 h during the first three days, and then to 23.5 h light and 0.5 h dark daily until slaughter.

At two stages of the study about 5 mL blood samples were collected from the experimental birds. At the ages of 21 days (48 birds) and 42 days (48 birds) blood was collected by venipuncture of the wing vein, kept on ice and transferred to the laboratory. Serum was separated and used for biochemical assays. The concentration of serum glucose, cholesterol, total protein, uric acid, Ca, P, Na, K and Cl were measured, using commercial kits on an auto-analyzer (Technicon RA-1000).

One set of six males and six females per treatment, 48 birds in total, was selected randomly, and slaughtered at the age of 21 days while another set was slaughtered at the age of 42 days. At slaughter the right tibia bone of each bird was removed as a drumstick with flesh intact. The drumsticks were labelled and immersed in boiling water (100 °C) for 10 min. After cooling to room temperature, the drumstick was defleshed manually and the patella was removed. The bone was then air-dried for 24 h at room temperature and tibia bone weight was determined. Fat from the tibia was extracted for 16 h with ethanol, followed by ethyl ether extraction for 16 h in a Soxhlet apparatus. They were then dried in an oven at 100 °C, and ashed for 6 h at 600 °C to measure the fat-free tibia ash content (AOAC, 1995).

Data was analyzed by a completely randomized design within blood and bone groups using the GLM procedure of MINITAB software (Minitab, 2000). Results were presented as mean $\pm$ SEM and differences among treatment means were compared, using the Duncan's multiple-range test.

Results and Discussion

The data collected at the end of the third and sixth week was evaluated statistically on the serum biochemical parameters (Tables 3 and 4). Serum concentrations of glucose, cholesterol, uric acid, total protein, and some element contents (Ca, P, Na, K and Cl) did not differ significantly ($P > 0.05$) between the dietary treatments and the control. It was also evident that the tibia bone parameters (weight, volume, specific gravity, N, ash, Ca, and P) in male, female and mixed-sex broilers, were not affected by dietary Ca-zeolite (Tables 5 and 6; $P > 0.05$). However, there have been several reports in the literature indicating a response to zeolites in blood serum and tibia bone parameters of poultry (Elliot *et al.*, 1990; Leach *et al.*, 1990; Park *et al.*, 2002). Our findings were similar to those of Oğuz *et al.* (2000), who reported that the addition of natural zeolites (clinoptilolite at 1.5% or 2.5%) in the aflatoxin-free diets did not significantly alter the serum biochemical parameters, total protein, glucose, cholesterol, uric acid, Ca and P.

The level of cholesterol in the serum was not affected by dietary treatments (Tables 3 and 4; $P > 0.05$). The results were in agreement with those in the literature (Dwyer *et al.*, 1997; Keçeci *et al.*, 1998; Curtui, 2000; Lotfollahian *et al.*, 2004; Miles & Henry, 2007; Safaeikatouli *et al.*, 2011). Altner *et al.* (2010) found that the total cholesterol levels of serum in laying hens fed rations with added microbial phytase and supplemented zeolites, were not considerably different. Conversely, Park *et al.* (2002) indicated that blood cholesterol concentration was significantly lower in 3.0% natural zeolite treatments than in the control. Curtui (2000) also reported that zeolite supplementation in the diet (0.5%) caused a significant decrease in total protein in serum, and an increase in uric acid concentration, whereas some researchers (Lotfollahian *et al.*, 2004; Safaeikatouli *et al.*, 2011) observed increases in the total serum protein concentration by 3% zeolite supplementation. On the other hand, Pond & Yen (1983) and Ward *et al.* (1991) found no effect of

Na-zeolite A or clinoptilolite on blood urea nitrogen. Our findings are similar to work those of Keçeci *et al.* (1998), Curtui (2000) and Safaeikatouli *et al.* (2011), who concluded that glucose levels were not affected by zeolite supplementation. Ledoux *et al.* (1999) and Miles & Henry (2007) who used hydrated NaCa-aluminosilicate in broiler diets, noted that there was no difference in the glucose components due to dietary treatments. In contrast, Lotfollahian *et al.* (2004) observed a significant increase in serum glucose concentration at elevated levels of zeolite.

Calcium, P, Na, K and chloride concentrations were within the reference ranges of 9.6 - 10.5 mg/dL, 7.4 - 8.9 mg/dL, 145.7 - 154, 5.8 - 9.2 and 111.7 - 121.3 mmol/L, respectively, and did not differ between the Ca-zeolite supplemented and control groups (Tables 3 and 4; $P > 0.05$). Therefore, it could be argued that there was no synergetic or antagonistic relationship between Ca-zeolite levels and the macro-mineral content in broiler feed. Although zeolites could induce alterations in element absorption, such as Ca and P, and electrolyte balance (Watkins & Southern, 1991), Ca-zeolite used in this investigation did not negatively affect the balance of serum Ca and P concentrations in both sexes and the mixed sex, which is in agreement with the results of Ward *et al.* (1993) and Frost *et al.* (1992).

Table 3 The effect of dietary supplementation of different levels of Ca-zeolite on serum biochemical parameters and blood mineral content at three weeks of age

Parameters	Groups				
	Sex	Control	1% Zeolite	3% Zeolite	5% Zeolite
Glucose (mg/dL)	M	236.3 ± 5.53	244.7 ± 18.43	249.7 ± 2.75	265.5 ± 16.82
	F	255.0 ± 15.26	237.2 ± 14.22	248.3 ± 9.29	257.0 ± 16.93
	Mixed	245.7 ± 7.69	240.9 ± 5.05	249.0 ± 3.31	261.3 ± 14.74
Cholesterol (mg/dL)	M	118.7 ± 5.97	147.5 ± 9.66	135.0 ± 10.15	120.2 ± 19.98
	F	109.0 ± 10.04	123.3 ± 14.01	123.3 ± 0.29	122.0 ± 29.28
	Mixed	113.8 ± 7.88	135.4 ± 11.54	129.2 ± 4.96	121.1 ± 23.06
Uric acid (mg/dL)	M	5.12 ± 1.01	5.80 ± 1.03	6.03 ± 1.51	5.10 ± 0.80
	F	4.17 ± 0.46	4.68 ± 0.03	4.95 ± 0.33	4.78 ± 0.30
	Mixed	4.64 ± 0.52	5.24 ± 0.51	5.49 ± 0.82	4.94 ± 0.36
Total Protein (g/dL)	M	2.23 ± 0.19	2.45 ± 0.15	2.20 ± 0.15	2.18 ± 0.28
	F	2.32 ± 0.16	2.38 ± 0.14	2.35 ± 0.13	2.37 ± 0.28
	Mixed	2.28 ± 0.17	2.42 ± 0.14	2.28 ± 0.11	2.28 ± 0.26
Calcium (mg/dL)	M	9.97 ± 0.10	10.08 ± 0.23	10.15 ± 0.56	10.45 ± 0.44
	F	9.63 ± 0.23	10.02 ± 0.42	9.88 ± 0.26	10.28 ± 0.34
	Mixed	9.80 ± 0.14	10.05 ± 0.32	10.02 ± 0.36	10.37 ± 0.10
Phosphorus (mg/dL)	M	7.82 ± 0.75	8.38 ± 0.86	8.57 ± 0.54	7.58 ± 0.84
	F	8.00 ± 0.23	8.63 ± 0.44	8.43 ± 0.28	8.88 ± 0.43
	Mixed	7.91 ± 0.45	8.51 ± 0.51	8.50 ± 0.34	8.24 ± 0.61
Sodium (mmol/L)	M	147.7 ± 1.04	147.7 ± 0.76	148.2 ± 3.75	153.7 ± 10.32
	F	147.2 ± 1.53	147.2 ± 1.04	148.0 ± 1.73	154.0 ± 9.18
	Mixed	147.4 ± 0.38	147.4 ± 0.63	148.1 ± 2.74	153.8 ± 9.75
Potassium (mmol/L)	M	6.63 ± 1.01	6.57 ± 0.93	7.00 ± 0.40	7.78 ± 0.55
	F	7.55 ± 0.44	7.63 ± 0.91	7.13 ± 0.63	8.38 ± 0.73
	Mixed	7.09 ± 0.66	7.10 ± 0.90	7.07 ± 0.48	8.09 ± 0.09
Chlorine (mmol/L)	M	114.0 ± 2.18	113.2 ± 1.44	113.5 ± 2.50	118.7 ± 11.56
	F	112.5 ± 2.60	111.7 ± 2.08	115.2 ± 2.25	121.3 ± 8.81
	Mixed	113.3 ± 0.25	112.4 ± 1.42	114.3 ± 2.10	120.0 ± 10.18

In another study it has been reported that serum Ca concentrations were not affected by dietary zeolites (Alçiçek *et al.*, 1998). Other researchers also demonstrated that using aluminosilicates in dietary rations, had no effect on serum P levels of broiler chicks (Roland *et al.*, 1990; Scheideler, 1993; Dwyer *et al.*, 1997; Ledoux *et al.*, 1999; Lotfollahian *et al.*, 2004). It has been stated that zeolites did not affect serum K and Na levels, but increased serum Ca levels (Pond & Yen, 1983; Roland *et al.*, 1993). Azar *et al.* (2011) showed that the perlite (aluminosilicate) levels and particle sizes did not affect serum Ca, P, Cl and Na concentrations. However, they influenced the serum Mg and K concentrations appreciably. Similarly, Nazifi *et al.* (2008) determined that supplementing broiler diets with natural zeolites (1.2%), had no significant effect on the levels of serum Na, K, Cl, and Mg, but that concentrations of serum Ca and P showed important changes. On the other hand, Utlu *et al.* (2007) reported that zeolite supplementation did not affect serum Ca, but P concentrations decreased significantly in supplemented birds compared to the control. Similarly, Watkins & Southern (1992) showed large decreases of P concentrations in zeolite-supplemented hens that could be related to the increase of serum Al concentrations. It has been reported that high levels of dietary Al

Table 4 The effect of dietary supplementation of different levels of Ca-zeolite on serum biochemical parameters and blood mineral content at six weeks of age

Parameters	Groups				
	Sex	Control	1% Zeolite	3% Zeolite	5% Zeolite
Glucose (mg/dL)	M	243.2 ± 3.25	236.7 ± 22.77	240.8 ± 16.59	242.3 ± 14.01
	F	244.7 ± 33.65	229.7 ± 29.19	228.0 ± 14.80	230.2 ± 8.43
	Mixed	243.9 ± 18.31	233.2 ± 25.34	234.4 ± 14.38	236.3 ± 8.71
Cholesterol (mg/dL)	M	127.2 ± 16.92	125.7 ± 28.01	113.0 ± 9.00	99.0 ± 6.26
	F	132.2 ± 12.85	118.5 ± 12.01	115.2 ± 11.73	121.8 ± 19.05
	Mixed	129.7 ± 14.87	122.1 ± 19.91	114.1 ± 10.26	110.4 ± 12.66
Uric acid (mg/dL)	M	5.42 ± 1.47	4.87 ± 1.08	4.22 ± 1.68	5.12 ± 0.08
	F	5.88 ± 1.08	4.90 ± 1.48	4.78 ± 1.46	5.68 ± 0.38
	Mixed	5.65 ± 1.27	4.89 ± 1.10	4.50 ± 1.56	5.40 ± 0.23
Total Protein (g/dL)	M	2.83 ± 0.67	2.60 ± 0.35	2.33 ± 0.06	2.17 ± 0.25
	F	2.65 ± 0.33	2.58 ± 0.13	2.63 ± 0.15	2.53 ± 0.12
	Mixed	2.75 ± 0.46	2.59 ± 0.22	2.48 ± 0.10	2.35 ± 0.18
Calcium (mg/dL)	M	10.30 ± 0.30	10.35 ± 0.68	9.95 ± 0.48	10.02 ± 0.40
	F	10.18 ± 0.20	10.35 ± 0.28	10.22 ± 0.38	10.02 ± 0.13
	Mixed	10.25 ± 0.23	10.35 ± 0.43	10.09 ± 0.29	10.02 ± 0.27
Phosphorus (mg/dL)	M	7.80 ± 0.71	8.18 ± 1.35	8.03 ± 0.70	7.50 ± 0.66
	F	7.78 ± 0.18	8.85 ± 0.58	7.45 ± 0.94	7.42 ± 0.38
	Mixed	7.79 ± 0.39	8.52 ± 0.97	7.75 ± 0.23	7.46 ± 0.45
Sodium (mmol/L)	M	149.8 ± 3.82	149.8 ± 4.16	145.7 ± 3.21	146.7 ± 2.08
	F	148.3 ± 2.47	150.5 ± 2.65	146.2 ± 3.69	146.5 ± 3.77
	Mixed	149.1 ± 2.90	150.2 ± 3.40	145.9 ± 3.45	146.6 ± 2.92
Potassium (mmol/L)	M	7.55 ± 0.13	5.80 ± 0.85	9.17 ± 2.52	7.70 ± 1.65
	F	7.12 ± 1.13	6.77 ± 1.15	7.02 ± 1.73	8.00 ± 1.65
	Mixed	7.34 ± 0.58	6.29 ± 0.99	8.10 ± 1.98	7.85 ± 1.46
Chlorine (mmol/L)	M	114.7 ± 3.40	113.5 ± 1.73	113.3 ± 1.53	113.5 ± 0.50
	F	114.7 ± 1.53	114.7 ± 1.89	113.5 ± 1.32	113.8 ± 2.75
	Mixed	114.7 ± 2.13	114.1 ± 1.81	113.4 ± 1.01	113.7 ± 1.28

depressed the concentrations of P and increased the concentration of Ca in the plasma (Hussein *et al.*, 1990). The expected effects of zeolites might exhibit variation due to factors such as the Al and P content of the zeolite, and the level of Ca and P in the broiler diets. It could be said that zeolite could not affect the serum Ca and P levels in both sex and mixed sex, because the Ca-zeolite used in our study contained high Al levels and the Si-Al frame structure of this mineral did not collapse during digestion in the broiler. Apparently, the effects of zeolite supplementation to diets broiler on the blood parameters generally depend on the balance of element content in the diets of broilers.

Table 5 The effect of dietary supplementation of different levels Ca-zeolite on some tibia bone parameters and bone mineral contents at three weeks of age

Parameters	Groups				
	Sex	Control	1% Zeolite	3% Zeolite	5% Zeolite
Calcium (g/kg)	M	175.8 ± 4.7	174.5 ± 7.8	171.1 ± 8.3	164.9 ± 7.4
	F	168.5 ± 4.9	171.3 ± 0.9	172.9 ± 3.0	164.5 ± 5.8
	Mixed	172.1 ± 1.0	172.9 ± 4.0	172.0 ± 5.6	164.7 ± 1.5
Phosphorus (g/kg)	M	86.0 ± 1.0	83.9 ± 3.3	84.3 ± 3.8	78.9 ± 4.9
	F	83.1 ± 3.0	80.3 ± 5.6	84.7 ± 0.9	80.0 ± 2.8
	Mixed	84.5 ± 1.1	82.1 ± 4.3	84.5 ± 2.2	79.4 ± 3.6
Ash (g/kg)	M	451.4 ± 10.1	451.8 ± 22.1	447.2 ± 5.9	458.5 ± 7.5
	F	441.8 ± 19.0	448.6 ± 7.3	454.3 ± 7.8	435.8 ± 11.1
	Mixed	446.5 ± 4.6	450.3 ± 7.6	450.8 ± 6.1	447.2 ± 7.6
Nitrogen (g/kg)	M	50.0 ± 2.6	48.7 ± 2.2	50.2 ± 3.1	49.1 ± 1.4
	F	49.3 ± 1.9	47.2 ± 2.5	48.1 ± 3.1	46.4 ± 2.1
	Mixed	49.7 ± 2.3	47.9 ± 2.4	49.1 ± 3.1	47.7 ± 0.7
Weight (g)	M	2.28 ± 0.10	2.26 ± 0.21	2.44 ± 0.23	2.35 ± 0.09
	F	2.20 ± 0.32	1.98 ± 0.21	2.09 ± 0.38	2.18 ± 0.15
	Mixed	2.24 ± 0.19	2.12 ± 0.20	2.27 ± 0.08	2.26 ± 0.09
Volume (cm ³)	M	2.42 ± 0.14	2.67 ± 0.14	2.58 ± 0.38	2.58 ± 0.14
	F	2.25 ± 0.50	2.08 ± 0.29	2.42 ± 0.38	2.42 ± 0.14
	Mixed	2.34 ± 0.19	2.38 ± 0.21	2.50 ± 0.00	2.50 ± 0.13
Specific gravity (g/cm ³)	M	0.95 ± 0.04	0.85 ± 0.03	0.96 ± 0.07	0.91 ± 0.02
	F	0.92 ± 0.14	0.97 ± 0.07	0.86 ± 0.04	0.90 ± 0.01
	Mixed	0.94 ± 0.08	0.91 ± 0.02	0.91 ± 0.04	0.90 ± 0.01

There was no significant effect on tibia bone parameters, tibia bone weight, ash and Ca concentrations due to the various types and levels of Ca-zeolite used during both periods. It has been reported that an increase in the tibia ash content was a useful indicator in the evaluation of bone mineralization in poultry (Ahmad *et al.*, 2000; Abas *et al.*, 2011). Similarly, Moghadam *et al.* (2005) claimed that the use of zeolites in diets did not have any considerable effect on the apparent digestibility of Ca and tibia ash content. Our findings were contrary to those of Elliot *et al.* (1990) and Leach *et al.* (1990) who mentioned that zeolites had beneficial effects on bone ash and strength, but no effect on tibial dyschondroplasia. Ballard & Edwards (1988) reported that 1% zeolite supplementation in broiler diets containing 0.65% Ca, increased tibia ash content significantly. Yalçın *et al.* (1995) observed a similar influence of added zeolites on the bone ash of broilers. Although tibial dyschondroplasia is a metabolic cartilage disease representing the endpoint of several mechanisms, the incidence seemed to increase when high dietary levels of P were fed (Edwards, 1984) or when dietary Ca was lower than 0.85% (Edwards, 1988; Leach *et al.*, 1990; Ledwaba & Roberson,

2003). Furthermore, high levels of Ca in broiler diets were proven to decrease feed consumption and the toxic effects (Shafey *et al.*, 2011).

It has been signified that the addition of zeolites to broiler diets increased the level of the tibia bone ash (Watkins & Southern, 1991). As a matter of fact, the beneficial effect of zeolite A has been inconsistent and largely dependent on the dietary level of Ca. The dietary inclusion of synthetic zeolite A (at 0.75% or 1.5%), when broilers were on a diet with inadequate or marginal levels of Ca resulted in an increase in bone ash content along with a reduction of rachitic lesions (Leach *et al.*, 1990). According to Watkins & Southern (1991), the dietary use of 0.75% zeolite A in broilers is accompanied by alterations in mineral absorption and tissue distribution, resulting in an increased tibia ash content and density and improved fresh tibia shearing force scores, but only when dietary calcium ranged from 0.6% to 0.8% (Papaioannou *et al.*, 2005).

Table 6 The effect of dietary supplementation of different levels of Ca-zeolite on some tibia bone parameters and bone mineral content at six weeks of age

Parameters	Groups				
	Sex	Control	1% Zeolite	3% Zeolite	5% Zeolite
Calcium (g/kg)	M	139.9 ± 7.7	136.3 ± 8.2	148.0 ± 10.1	148.7 ± 17.6
	F	150.6 ± 11.4	146.9 ± 6.0	139.0 ± 6.8	149.6 ± 15.8
	Mixed	145.2 ± 9.6	141.6 ± 7.0	143.5 ± 8.2	149.2 ± 16.5
Phosphorus (g/kg)	M	63.9 ± 2.8	67.5 ± 3.7	72.6 ± 4.7	72.4 ± 8.4
	F	74.0 ± 4.9	71.3 ± 2.7	68.2 ± 3.5	73.3 ± 8.2
	Mixed	68.9 ± 1.1	69.3 ± 3.2	70.5 ± 3.9	72.8 ± 8.3
Ash (g/kg)	M	380.3 ± 12.8	360.4 ± 16.1	380.1 ± 18.3	392.1 ± 35.6
	F	401.5 ± 27.6	390.1 ± 8.4	366.5 ± 27.5	404.2 ± 24.8
	Mixed	390.9 ± 19.0	375.2 ± 12.1	373.3 ± 21.0	398.1 ± 28.3
Nitrogen (g/kg)	M	39.5 ± 2.0	40.7 ± 0.7	42.5 ± 5.0	41.8 ± 5.6
	F	40.6 ± 4.2	39.7 ± 3.1	43.1 ± 6.5	42.1 ± 1.9
	Mixed	40.1 ± 1.8	40.1 ± 1.8	42.8 ± 4.4	41.9 ± 3.8
Weight (g)	M	7.43 ± 0.33	7.18 ± 0.33	7.03 ± 0.37	7.36 ± 0.16
	F	6.22 ± 0.20	6.29 ± 0.41	6.63 ± 0.26	6.41 ± 0.17
	Mixed	6.83 ± 0.15	6.74 ± 0.32	6.83 ± 0.19	6.89 ± 0.17
Volume (cm ³)	M	7.92 ± 0.38	7.67 ± 0.14	7.50 ± 0.87	7.67 ± 0.72
	F	6.08 ± 0.38	6.33 ± 0.63	5.75 ± 0.43	5.58 ± 0.88
	Mixed	7.00 ± 0.38	7.00 ± 0.33	6.63 ± 0.57	6.63 ± 0.25
Specific Gravity (g/cm ³)	M	0.94 ± 0.04	0.94 ± 0.05	0.95 ± 0.13	0.97 ± 0.10
	F	1.00 ± 0.10	1.01 ± 0.17	1.16 ± 0.09	1.17 ± 0.15
	Mixed	0.97 ± 0.07	0.98 ± 0.10	1.06 ± 0.09	1.07 ± 0.03

In another study it has been claimed that the addition of zeolites increased Ca utilization and the content of bone ash during growth (Watkins & Southern, 1992). Gezen & Eren (2002) pointed out that zeolite supplementation (2%) to broiler diets resulted in a reduction of incidence and severity of tibial dyschondroplasia at 21 days of age. On the other hand, Rabon *et al.* (1995) claimed that using zeolites in the diet had some effect on tibia ash, while others reported no such effect (Elliot & Edwards, 1991; Keshavarz & McCormick, 1991; Altan *et al.*, 1998). A few writers (Pond *et al.*, 1988; Safaeikatouli *et al.*, 2010) suggested that the structure of the mineral, the geographical source of the involved zeolite, or its unique crystal structure, size, shape of cavities, porosity and the metal oxide content as well as environmental conditions and animal species, could be responsible for these inconsistent findings.

Conclusion

The results of this experiment suggested that additional Ca-zeolites (1, 3, 5%) in diets did not have any adverse effects on blood and bone characteristics of broiler males and females. Furthermore, at 5% inclusion of Ca-zeolite, broilers did not exhibit any negative response in terms of health status or cost of feeding. In conclusion, the findings showed that the addition of Ca-zeolites to broiler diets was generally acceptable and had no detrimental effects on the parameters monitored.

Additional experiments are needed to determine the effect of zeolites at different testing conditions, on natural zeolite types with different elemental ratios (Si/Al+Fe, alkali/alkaline-earth, Na/K) and various adsorptive abilities of zeolites in broilers.

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