Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

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To cite this article: Erol Bintaş, Mehmet Bozkurt, Kamil Küçükyılmaz, Ramazan Konak, Mustafa Çınar, Hasan Akşit, Kamil Seyrek & Abdullah Uğur Çatlı (2014) Efficacy of Supplemental Natural Zeolite in Broiler Chickens Subjected to Dietary Calcium Deficiency, Italian Journal of Animal Science, 13:2, 3141, DOI: 10.4081/ijas.2014.3141

To link to this article: https://doi.org/10.4081/ijas.2014.3141

Published online: 17 Feb 2016.

PAPER

Efficacy of supplemental natural zeolite in broiler chickens subjected to dietary calcium deficiency

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Abstract

Natural zeolite, or sodium aluminosilicate, influences calcium (Ca) and phosphorus (P) utilisation in chicks. A 2×2 factorial arrangement of treatments was used to investigate the effect of dietary Ca (recommended and below recommended levels) and zeolite (0 and 0.8%) on growth, plasma, tibia and faeces in chickens from 1 to 42 days of age. Zeolite supplementation did not affect overall body weight (BW) gain, feed intake (FI) or feed conversion ratio (FCR) of broiler chickens (P>0.05). Overall mortality of zeolite-fed chickens was lower than in untreated ones (P<0.01). Reduction of dietary Ca of approximately 10 to 18% decreased (P<0.05) BW at 14 and 42 days of age in association with reduced FI, but overall FCR was unchanged. Serum protein and sodium constituents were reduced in birds fed zeolite (P<0.05). Decreasing dietary Ca level increased (P<0.01) serum, total protein and glucose concentrations, but decreased Ca level. Zeolite decreased bone ash in birds fed a Ca-deficient diet while increased faecal excretion of ash, Ca, P and aluminum. However, zeolite increased tibia weight (P<0.05) and thickness (P<0.01). No significant response (P>0.05) in relative weight and gross lesion scores of liver or footpad lesion scores was found related to changes in dietary regimens.

The results of the present study do not corroborate the hypothesis that the effectiveness of zeolite may be improved in Ca-deficient diets in association with its ion exchange capability.

Introduction

Zeolites are crystalline, hydrated aluminotectosilicates of alkali and alkaline-earth cations, having infinite, three-dimensional structures of interconnecting channels and large pores, capable of trapping molecules in proper conditions (Mumpton and Fishman, 1977; Mumpton, 1999). Among many properties attributed to zeolites, most typically related to their effectiveness in animal nutrition is their ability to selectively exchange a variety of cations without much major changes in their structure (Waldroup *et al.*, 1984; Elliot and Edwards, 1991; Shariatmadari, 2008). Beneficial effects may also be attributed to the silicon (Si), aluminum (Al) or sodium (Na) content of zeolites because it has been established that these minerals can influence calcium (Ca)-metabolism, thus improving Ca and phosphorus (P) utilisation (Leach *et al.*, 1990; Watkins and Southern, 1991). While some of the experiments report beneficial effect due to the inclusion of zeolite to bird diets, there are still some results indicating toxic effects. The Al within the synthetic zeolite could be released and cause poisoning when fed to broilers and laying hens at the level of 1% (Edwards *et al*., 1992; Roland *et al*., 1993).

Studies have revealed that the zeolite clinoptilolite is able to adsorb damaging toxins that can potentially reduce the growth of animals (Oğuz and Kurtoğlu, 2000), affects gut morphology, decreases pH, and lowers pathogenic bacteria counts, which suggests that intestinal health can be improved by its use (Wu *et al.*, 2013; Khambualai *et al.*, 2009). However, despite the purported mechanisms mentioned above, few studies have demonstrated improvements in the growth of broiler chickens as result of including zeolite in their diet (Fethiere *et al.*, 1994; Karamanlis *et al.*, 2008). Indeed, the majority of studies have demonstrated no such beneficial effect (Watkins and Southern, 1991; Wu *et al.*, 2013), and some have even revealed adverse effects (Çabuk *et al.*, 2004). Reasons such as level of usage, type of zeolite (natural or synthetics) and the levels of impurities are to be blamed for discrepancies reported from the experiments (Shariatmadahari, 2008).

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) resulting participation of much Ca in bones (Ballard and Edwards, 1988). Natural zeolites have remarkable ion-exchange capability at around 2.5 meq/g and selectivity for Ca. Roland *et al.* (1985) hypothesised that the beneficial effect of zeolite on bone quality may be related to its high affinity for Ca and its ion-exchange capability. However, the mode of action of zeolites on performance, mineral utilisation by skeletal structure is not well characterised at the time of writing.

The low Ca, bone resorption hypothesis is on the belief that when bird becomes deficient in dietary Ca, this, in turn, stimulates the absorption and utilisation of ingested Ca (Ballard and Edwards, 1988). Even though the purported mechanism mentioned above, the evidence showing the beneficial attributes of natural zeolite on bone mineralisation and growth of broiler chickens fed diet deficient in Ca is much more limited, in most cases it is either preliminary or there is no evidence at all. Three available reports with synthetic zeolites dated back two decades ago (Leach *et al.*, 1990; Watkins and Southern, 1991, 1992) show that when dietary calcium was deficient or marginal, zeolite improves calcium utilisation in broiler chickens, as evidenced by improved growth rate, bone mineralisation and a reduction in rachitic lesions.

Therefore, the present study was conducted to further evaluate the effect of feeding natural zeolite when dietary Ca varies. The influence of dietary Ca and natural zeolite and their interactive effects on growth, mineral concentrations of plasma, tibia and faeces, and bone growth were evaluated. Hepatic lesion scores for aflotoxicosis, and footpad lesion scores derived from litter ammonia burn were also assessed.

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Key words: Natural zeolite, Broiler performance, Blood constituent, Bone mineralisation, Footpad lesion score.

Received for publication: 10 October 2013.
Accepted for publication: 30 January 2014.

Italian Journal of Animal Science 2014; 13:3141
doi:10.4081/ijas.2014.3141

Materials and methods

Birds and housing

A feeding experiment was performed using 1344 feather-sexed 1-day-old broiler chicks of a commercial strain (Ross-308), with post-hatch weights of 44.02±0.02 g. The treatments were based on a 2×2 factorial design, consisting of two levels of dietary Ca (the recommended level and a level lower than the recommendation) and two levels of supplemental zeolite (0 and 0.8%). The chickens were assigned to four dietary treatments with six replicates. Fifty-six chicks (28 males and 28 females) were randomly assigned to each replicate and placed in floor pens. The chicks were kept in 24 wire pens (2.4×1.6 m) on wood shavings as litter material. Bird density was 14 chicks per square meter. Each pen was equipped with two hanging feeders and one bell-type drinker. The birds were given *ad libitum* access to feed and water. Birds were reared in an environmentally controlled grower house with an automatic heating and ventilation system. The lighting cycle was 23 h/d maintained. The ambient temperature in the experimental house was thermostatically controlled by a heating system and wall fans. This temperature was set at 32°C on the first three days day of the experiment and gradually decreased 1°C every third day until 21 days of age and maintained at 22°C thereafter. On day 10 and 16, chicks were vaccinated against infectious bursal disease and Newcastle disease, respectively, via drinking water. The Ministry of Agriculture, General Directorate of Research Institutional Animal Care and Use Committee approved the techniques and procedures involved in the animal care and handling.

Experimental diets

The basal diet was a typical corn-wheat-soybean diet that was formulated to meet or exceed all nutrient recommendations published in the Ross rearing guideline (Aviagen, 2007). The experimental period was divided into 3 phases; a starter phase (1 to 14 d), a grower phase (15 to 28 d), and a finisher phase (29 to 42 d). The ingredient composition and nutrient content of the basal diets for three experimental phases are presented in Table 1. These diets contained no antibiotics, anticoccidials or growth enhancers and were isoenergetic and isonitrogenous. Dietary Ca level reduced by 10, 16 and 18% in starter, grower and finisher diet, respectively, to establish experimental deficiency, which were described as Ca-deficient diet. Whereas, those were adequate in Ca was stated as Ca-adequate diet, thereafter. The diets in mash form were prepared every 2 wk and were stored in sacks in a cool place. Chemical composition was determined according to AOAC (1990).

The zeolitic material used was clinoptilolite-rich tuff that was obtained from the Palaeogene-rich tuffs of Gördes area, western Turkey. From x-ray diffractometry of the powder, the sample was shown to consist of about 88% clinoptilolite, 5% Smeklit, 5% Opal-CT, 2% Quartz. The chemical composition of the zeolite mined from Gordes-manisa-Turkey was as follows: SiO_2 66.16%, Al_2O_3 12.07%, K_2O 3.78%, CaO 2.16%, Fe_2O_3 1.68%, MgO 0.89%, Na_2O 0.46%, TiO_2 0.07, P_2O_5 0.02%, MnO 0.03 and LOI 12.6%. The grain-size distributions for the samples studied were 0.20 to 0.50 mm after the tuff was crushed in an industrial crusher. Prior to the start of the experiment, the starter diet with no zeolite was analysed for mycotoxins. Zearalenone, deoxynivalenol and fumonisins were below detection limits as established by the techniques previously described (Dalcero *et al.*, 1997). The level of naturally occurring AFB_1, AFG_1 and AFG_2 were 9 to 18 and 2 μg/kg. Subsequent analysis of corresponding mycotoxins in grower and finisher diets showed close similarity to that of starter diet.

Performance

The growth performance of broilers was evaluated by recording body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and mortality. The body weight of broilers in each pen was measured individually on day 1, 14, 28 and 42. Based on feed refusals, the average feed intake and feed conversion ratio was measured per pen basis. On the same days, the feed conversion ratio was calculated as the amount of feed consumed per unit of body weight gain, adjusting for weight at hatch and bird mortality. Mortality in each pen was recorded daily.

Serum biochemical parameters

At the end of the experiment (42 days of age), two birds (one male and one female) per experimental unit (twelve birds per treatment), whose body weight were closer to group mean, were selected randomly and used for serum analysis and concomitant measurements indicated above. Blood samples were collected by cardiac puncture and placed into non-additive blood collection tubes in order to separate the serum. Sera were separated by centrifugation at 1800×g after 1 h of incubation at room temperature and stored at -20ºC until the analysis. Serum total protein (04657586190; Roche, Basel, Switzerland), glucose (04657527190; Roche), Ca (04718933190; Roche), inorganic P (04718984190; Roche), Na (S600-50; Teco, Anaheim, CA, USA), chlorine (Cl) (Teco) C501-480 and magnesium (Mg) (M527-100; Teco) concentrations were measured with a spectrophotometer (UV1601; Shimadzu, Kyoto, Japan) using commercial available kits.

Tibia measurements

The birds were killed by cervical dislocation and both tibias were removed for subsequent analysis. The excised tibias were cleaned of adherent tissues and all flesh, and proximal cartilages were removed. Bone measurements were performed on the right tibias. The measurements, including tibia length and thickness, were made using a micrometer (model IT-014UT; Mitutoyo, Kawasaki, Japan). Tibia weight was expressed as a proportion of live body weight.

Left tibias were used for measuring bone ash and mineral content. Bones were sealed individually in plastic bags and then stored at -20°C until analysis. The bones were thawed at room temperature for 6 h in an air conditioned room before the analysis began. Each tibia was broken into small pieces, weighed, oven-dried at 105°C for 24 h, cooled in a dessicator, weighed, and dry-ashed at 600°C for 12 h, cooled in a dessicator, and weighed (AOAC, 1990). The ash content was expressed as a percentage of dry bone weight.

Concomitantly, using corresponding ash samples, the concentrations of minerals (*i.e.* Ca, P, and Mg) were measured at element-specific wavelengths (Ca, 315.887 nm; P, 214.914 nm; Mg, 279.077 nm; Al, 309.27 nm) using an inductively coupled plasma (ICP) (Optima 2100 DV; PerkinElmer, Waltham, MA, USA). Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element (Merck, 170373 Calcium ICP Standard and Merck, 170340 Phosphorus ICP Standard, Mg 279.077, Al 396.153).

Liver and footpad histopathological measurements

The liver was excised and relative weight (%) was determined (n=12 per treatment). Histopathological changes were evaluated blindly in the liver of sampled birds and were scored based on descriptions of aflatoxin-induced hepatic pathology (Hoerr, 2003). Changes scored included vacuolar degeneration and fatty change in hepatocytes, both scored on a 0 to 3 scale with 0 indicating no change (0=no changes, liver unremarkable; 1=mild aflatoxicosis lesions; 2=moderate aflatoxicosis lesions; 3=severe aflatoxicosis

Table 1. Ingredient and nutrient composition of the starter, grower and finisher diets (as fed).

	Starter (1 to 14 days)				Grower (15 to 28 days)				Finisher (29 to 42 days)			
	Ca-adequate		Ca-deficient		Ca-adequate		Ca-deficient		Ca-adequate		Ca-deficient	
	Control	Zeolite	Control	Zeolite	Control	Zeolite	Control	Zeolite	Control	Zeolite	Control	Zeolite
Ingredients, g/kg												
Corn	368.6	352.6	370.9	355.9	397.0	382.6	402.0	390.7	427.8	452.8	435.0	424.3
Wheat	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0	160.0	200.0	196.8
Soybean meal (48% CP)	355.7	360.0	355.3	359.3	321.7	324.0	321.0	322.0	286.3	290.9	285.1	287.0
Soybean oil	35.04	38.80	34.33	37.59	45.89	50.59	45.49	48.27	52.89	55.55	50.57	55.14
DCP	17.58	17.66	17.57	17.64	16.17	16.19	16.16	16.22	15.29	15.52	15.30	15.35
Limestone	12.65	12.34	11.54	11.06	9.69	9.20	6.01	5.53	9.11	8.57	5.42	4.89
NaCl	2.39	2.33	2.38	2.30	2.42	2.34	2.46	2.34	2.44	2.37	2.44	2.35
Lysine HCl	1.00	1.12	0.99	1.08	-	-	-	-	-	-	-	-
DL-methionine (99%)	2.57	2.68	2.52	2.66	2.84	2.66	2.64	2.65	2.22	2.26	2.21	2.22
Threonine	0.65	0.65	0.65	0.65	0.49	0.49	0.49	0.49	0.15	0.14	0.15	0.15
Vitamin premix°	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Mineral premix#	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NaHCO3	0.32	0.32	0.32	0.32	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Anticoccidial§	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zeolite		8.00		8.00		8.00		8.00		8.00	8.00	
Analysed composition, %												
Dry matter	88.46	88.96	88.40	88.65	88.48	88.65	88.43	88.57	88.47	88.48	88.39	88.66
Crude protein (Nx6.25)	22.58	22.46	22.50	22.54	21.12	20.98	21.20	21.03	19.58	19.39	19.61	19.52
Ether extract	5.89	5.69	5.83	5.72	7.03	7.45	7.01	7.24	7.80	8.00	7.59	8.00
Crude fibre	3.28	3.35	3.29	3.43	3.24	3.22	3.24	3.23	3.19	2.94	3.20	3.16
Crude ash	6.4	7.14	6.29	7.03	5.83	6.57	5.50	6.21	5.52	6.16	5.16	5.80
Calcium	1.08	1.04	0.93	0.95	0.88	0.90	0.76	0.74	0.87	0.85	0.73	0.70
Total phosphorus	0.70	0.73	0.69	0.71	0.68	0.66	0.66	0.67	0.61	0.63	0.62	0.61
Calculated composition												
Calcium^,$, %	1.05^	1.05^	0.95$	0.95$	0.90^	0.90^	0.75$	0.75$	0.85^	0.85^	0.70$	0.70$
Lysine°°, %	1.26	1.26	1.26	1.26	1.07	1.07	1.07	1.07	0.97	1.08	0.97	0.98
Methionine°°, %	0.58	0.58	0.58	0.58	0.57	0.57	0.57	0.57	0.51	0.51	0.51	0.51
Methionine+cysteine°°, %	0.95	0.95	0.95	0.95	0.92	0.92	0.92	0.92	0.84	0.84	0.84	0.84
Threonine°°, %	0.89	0.89	0.89	0.89	0.82	0.82	0.82	0.92	0.73	0.73	0.73	0.73
ME°°, kcal/kg	3023	3002	2996	3018	3109	3125	3109	3115	3170	3153	3149	3168

Ca, calcium; CP, crude protein; DCP, dicalcium phosphate; ME, metabolisable energy. °Provided per kg of diet: trans-retinol 12,000 U; cholecalciferol, 1500 U; α-tocopherol acetate, 75 mg; vitamin K_3, 5 mg; vitamin B_1, 3 mg; vitamin B_2, 6 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.03 mg; nicotine amide, 40 mg; pantothenic acid, 10 mg; folic acid, 0.75 mg; D-biotin, 0.075 mg; choline, 375 mg. #Provided per kg of diet: Mn, 80 mg; Fe, 40 mg; Zn, 60 mg; Cu, 5 mg; I, 0.5 mg; Co, 0.2 mg; Se, 0.15 mg. §Provided per kg of diet: Narasin, 70 mg/kg diet. ^Values as recommended by the breeder for starter, grower and finisher periods (Aviagen, 2007). $Values calculated by discounting 10, 16 and 18%, respectively, from recommended values for corresponding growth phases. °°Calculated values.

lesions). While determining the final body weight at day 42, footpad lesion scores of all birds were concurrently evaluated. For the scoring, footpad lesions are assigned to one of these 4 classes: 0=no lesions, no discoloration or scars; 1=mild lesions, parts of footpad are discoloration to light brown; 2=discoloration of footpad to dark brown; 3=severe-deep lesions, ulcers, and scabs (Arno, 2008).

Faeces collection and analysis

Following the 36-day growing period, twelve birds per treatment (one male and one female bird per replicate) were selected randomly and transferred to an offsite cage facility. Birds were placed in colony cages as groups, allowing the collection of faeces. Birds were maintained in their respective experimental treatments and had *ad libitum* access to feed and water. Broilers were allowed to adjust cage management for 3 days, followed by a three-day total collection of faeces between days 39 and 42. Faecal samples were collected for three consecutive days (minimum 500 g/day/treatment), were homogenously mixed and stored at -20°C until analysis. Faecal samples were analysed for ash, Ca and P contents in order to determine the level of mineral excretion. For this purpose, samples of excrete were weighed, oven-dried at 105°C for 24 h, cooled in a dessicator, weighed, and dry-ashed at 600°C for 12 h (AOAC, 1990). And then, concentrations of minerals were measured at specific wavelengths for each element (Ca, 315.887 nm; P, 214.914 nm) by using an inductively coupled plasma (ICP) (Optima 2100 DV; PerkinElmer, Waltham, MA, USA). Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions (Merck, 170373 Calcium ICP Standard and Merck, 170340 Phosphorus ICP Standard) of each element. Percentage weight of faecal ash was calculated by dividing ash weight (dry matter basis) to initial faeces weight.

Statistical analysis

The experiment used a completely randomised design. Data on growth performance parameters (BWG, FI, FCR and mortality) were analysed on pen basis, whereas data on serum biochemical parameters, tibia measurements, bone ash and mineral constituents of bone and faeces, lesion scores related to liver and footpad were based on individual broilers. The data was analysed on a two-factorial ANOVA using the GLM procedure found in SAS software (SAS, 2001). The main effects of zeolite, Ca, and zelite by Ca level interaction were tested. Significant differences between treatment means were separated using the Duncan's multiple range test with a 5% probability. Arcsin transformation was applied to the percentage values (*i.e.* mortality and relative weights of liver and tibia, ash and mineral content tibia and faeces) before testing for differences.

Results and discussion

Growth performance

Performance traits of broilers including body weight gain BWG, FI, FCR, and mortality are depicted in Table 2. Inclusion of zeolite in diets had no effect on BWG, FI and FCR of broiler chickens fattened over 42 days (*i.e.*, intervals of 1 to 14, 14 to 28, and 1 to 42 days), (P>0.05). However, chickens fed zeolite had lower mortality during the study (1 to 42 days) than that in unsupplemented chicks, with a marked reduction (P<0.01) of 60% (1.62 *vs* 4.09%).

Lowering dietary Ca from 1.05 to 0.95% induced a significant (P<0.05) decrease in FI during days 1 to 14 and 1 to 28 (P<0.05). A similar pattern was observed with a reduction of 94 g during the entire experimental period. The reduction in FI of birds fed Ca-deficient diets was concomitant with slight decreases in BWG at 14 and 42 days of age. Feed conversion ratio and mortality were unaffected by the alteration in dietary Ca level. Exceptionally, FCR improved between day 1 and 28 in response to the feeding of a Ca-deficient diet. No significant zeolite-Ca interaction was found overall in the performance indices measured (P>0.05).

Serum biochemical parameters

Serum P, Mg, and Cl concentrations were unaffected by dietary modifications in zeolite

Table 2. The effect of dietary modifications with calcium and zeolite on body weight gain, feed intake, feed conversion ratio and mortality of chickens in the starter, overall growth phase.

	Starter (1 to 14 days)				Grower (1 to 28 days)				Overall (29 to 42 days)			
	BWG, g	FI, g	FCR, g feed/g gain	Mortality, %	BWG, g	FI, g	FCR, g feed/g gain	Mortality, %	BWG, g	FI, g	FCR, g feed/g gain	Mortality, %
Deficient dietary Ca level												
Zeolite-unsupplemented	347	528	1.52	3.22	1141	1881	1.64	4.10	2325	4277	1.83	4.10
Zeolite-supplemented	345	535	1.55	1.18	1145	1887	1.64	1.18	2345	4323	1.84	1.47
Adequate dietary Ca level												
Zeolite-unsupplemented	358	557	1.55	1.74	1142	1977	1.73	2.91	2364	4338	1.83	4.08
Zeolite-supplemented	364	555	1.52	1.18	1172	1987	1.69	1.47	2443	4449	1.82	1.77
SEM°	6.18	8.67	0.02	0.66	14.55	28.17	0.02	0.88	27.97	76.23	0.03	0.84
Probabilities												
Dietary Ca level	0.0233	0.0117	0.9751	0.2805	0.3474	0.0024	0.0066	0.6188	0.0234	0.2340	0.7674	0.8683
Zeolite	0.7526	0.8170	0.9253	0.0650	0.2638	0.7726	0.4157	0.0232	0.0901	0.3139	0.9365	0.0082
Dietary Ca level×zeolite	0.5662	0.6438	0.2524	0.2805	0.3961	0.9466	0.4280	0.4137	0.3012	0.6696	0.8535	0.8493
Main effects#												
Zeolite-unsupplemented	352	543	1.53	2.48	1142	1929	1.68	3.50[a]	2345	4307	1.83	4.09[a]
Zeolite-supplemented	354	545	1.53	1.18	1159	1937	1.67	1.32[b]	2394	4386	1.83	1.62[b]
Deficient dietary Ca level	346[b]	532[b]	1.53	2.20	1143	1884[b]	1.64[b]	2.64	2335	4300	1.84	2.78
Adequate dietary Ca level	361[a]	556[a]	1.53	1.46	1157	1982[a]	1.71[a]	2.19	2404	4394	1.82	2.92

BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; Ca, calcium.°Data are means of 6 replicate pens with SEM for each treatment; #data were analysed as a 2×2 arrangement. [a,b]Means within columns, within main effects, with different superscript differ at P<0.05.

and Ca (Table 3). Zeolite decreased serum Na and total protein levels (P<0.05). Chicks administered a diet deficient in Ca had lower serum Ca (P<0.05) but higher total protein (P<0.01) and glucose (P<0.05) levels compared with those in birds fed a Ca-adequate diet.

Bone measurements

Physical properties of tibia bone including length, thickness, and relative weight (%) are presented in Table 4. Significant zeolite-Ca interaction occurred related to the proportional weight of bone. Supplementation of zeolite in a Ca-deficient diet increased bone weight by approximately 7%, but when zeolite was included in the diet adequate in Ca, the increase was low (0.3%). Zeolite had a beneficial effect on tibia thickness, which increased by 0.56 mm (P<0.01), but not on bone length. Lowering the dietary Ca level influenced none of the bone measurements (P>0.05).

Tibia ash and mineral content

Dietary modifications with zeolite and Ca had no influence on tibia Ca, P, and Mg levels (P>0.05). Significant zeolite-Ca interaction occurred with tibia ash (P<0.01). Whereas zeolite decreased tibia ash when added to a diet deficient in Ca, a contrasting pattern was observed with addition to a feeding regimen with adequate Ca. Bone Al content was undetermined owing to its being below the detection limit.

Faecal excretion of ash, calcium, phosphorus, magnesium, and aluminum

Zeolite-fed chickens over-excreted ash with faeces (P<0.01) compared with excretion in untreated chicks (14.50 *vs* 12.56%). A concomitant increase (P<0.01) in faecal Ca, but not Mg, excretion was observed in response to zeolite. Zeolite decreased faecal P excretion in chicks fed a Ca-adequate diet, whereas a contrasting pattern was observed in chicks receiving a Ca-deficient diet (P<0.001).

Reduction in dietary Ca level evoked strongly significant decreases in faecal excretion of Ca, Mg, and Al (P<0.0001). Dietary provision with zeolite markedly increased Al excretion through faeces in both Ca-deficient and Ca-adequate diets (P<0.01), but the magnitude of response to zeolite was greatest when it was administered in the latter (P<0.01).

Lesion scores of footpad and liver

No significant response (P>0.05) in relative weight of liver [overall mean=3.03%; standard error of the mean (SEM)=0.12], gross lesion scores of liver (overall mean=1.09; SEM=0.14), or footpad lesion score (overall mean=0.27; SEM=0.02) were found related to changes in dietary Ca level with or without zeolite supplementation.

General remarks

Zeolite has displayed promise as a growth promoter in several initial evaluations (Leach *et al.*, 1990; Fethiere *et al.*, 1994; Karamanlis *et al.*, 2008), but some feeding studies have not supported this finding (Elliot and Edwards, 1991; Watkins and Southern, 1991; Wu *et al.*, 2013). Hence, no general consensus exists about whether zeolite enhances growth in broiler chickens. Furthermore, several experiments have even shown adverse effects (Çabuk *et al.*, 2004; Acosta *et al.*, 2005).

In this study, dietary inclusion of 8 g/kg zeolite provided no benefit for improving growth rate and efficiency of feed conversion. Several authors have associated the beneficial effect of zeolite on the growth performance of birds to the fact that zeolite improves ion exchange (Roland *et al.*, 1985; Elliot and Edwards, 1991), maintains efficient immobilisation of enzymes, and influences gut microflora (Khambualai *et al.*, 2009). Zeolite is thought to induce epithelial cell generation in broilers (Albengres *et al.*, 1985) and cause hypertrophy of intestinal villus and epithelial cell function in ducks (Khambualai *et al.*, 2009), which could in turn improve digestion and absorption of nutrients. Considering the purported mechanism mentioned above, we cannot assign an association to these changes, and the often reported lack of beneficial effect of zeolite on broiler growth and production was also evidenced in our study. Another theory on why this may occur is that broilers are not maximising growth but maximising their survival and bone quality.

A review of related experiments has sug-

Table 3. Effects of dietary modifications with calcium and zeolite on serum total protein, glucose, calcium, phosphorus, sodium, magnesium, and chlorine concentrations of chickens.

	Serum profile						
	Total protein, g/dL	Glucose, mg/dL	Ca, mg/dL	P, mg/dL	Na, mEq/L	Mg, mmol/L	Cl, mEq/L
Deficient dietary Ca level							
Zeolite-unsupplemented	2.93[a]	129	3.80	8.56	152	0.38	114[a]
Zeolite-supplemented	2.46[b]	137	4.15	8.92	145	0.37	98[b]
Adequate dietary Ca level							
Zeolite-unsupplemented	1.90[c]	113	4.43	10.27	152	0.44	92[b]
Zeolite-supplemented	1.72[c]	124	4.79	8.83	150	0.39	112[a]
SEM°	0.16	5.64	0.31	1.04	1.98	0.02	8.02
Probabilities							
Dietary Ca level	0.0001	0.0159	0.0498	0.4415	0.3721	0.1472	0.6423
Zeolite	0.0451	0.0929	0.2591	0.6064	0.0257	0.3079	0.7949
Dietary Ca level×zeolite	0.3702	0.8386	0.9883	0.3907	0.2220	0.4356	0.0272
Main effects[#]							
Zeolite-unsupplemented	2.42[a]	121	4.11	9.42	152[a]	0.41	103
Zeolite-supplemented	2.09[b]	131	4.47	8.88	148[b]	0.38	105
Deficient dietary Ca level	2.69[a]	133[a]	3.98[b]	8.47	149	0.37	106
Adequate dietary Ca level	1.81[b]	119[b]	4.61[a]	9.55	151	0.41	102

Ca, calcium; P, phosphorus; Na, sodium; Mg, magnesium; Cl, chlorine. °Data are means of 12 chickens (two chickens per replicate pen) with SEM for each treatment; [#]data were analysed as a 2×2 arrangement. [a-c]Means within columns, within main effects, with different superscript differ at P<0.05.

gested that the inconsistent responses to zeolite are probably due to imbalances in dietary nutrients (Shariatmadari, 2008; Karamanlis *et al.*, 2008). The adverse effect of remarkably higher ash level due to excessive higher dietary zeolite supplementation has been generally overlooked by researchers. Nutritional drawbacks did not occur in our study because the recommended nutrient specifications for the breed used were met precisely. Indeed, the relatively lower level of zeolite supplementation in the study (0.8%) made this condition easier to establish. Another explanation for such discrepancies among the studies is the variation in physical properties of zeolite such as particle size, mineralogical composition, chemical composition, purity, homogeneity of zeolite material, crystal size, and cation exchange properties (Pond *et al.*, 1988). Because natural zeolite is obtained from various mines, its content may vary greatly, which could in turn influence performance outcome.

The most pronounced implication of zeolite in this study was the marked reduction in overall bird mortality compared to that in untreated counterparts (1.62 *vs* 4.09%). The strength of the experimental evidence supporting claims of health benefits from zeolite has been evaluated in a comprehensive review by Evans and Farrell (1993). Eleven of 26 studies showed reduced mortality in response to dietary zeolite application, whereas others showed either no benefit or conflicting results. To date, no systematic investigation of the effect of zeolite on the control of mortality in poultry has been carried out.

Dietary addition of zeolite has also been shown to reduce the toxicity of litter ammonia and aflotoxins (Gupta *et al.*, 1997), which are contaminants in feedstuffs such as wheat, corn, and soybean. Zeolite has anti-microbial activity against *Salmonella* spp. and *Escherichia coli* through selective adsorption of pathogenic bacteria under in vitro conditions (Mavilia *et al.*, 1999), as shown in experimental studies with broiler chickens (Afaf *et al.*, 2011; Wu *et al.*, 2013). Mumpton and Fishman (1977) have also suggested that the presence of zeolite in the diet of broiler chicks could effectively prevent mortality. The question remains whether the benefit of zeolite on bird liveability is an expression of antimicrobial activity, toxin-binding efficacy, or both in our study.

An important effect worth considering when discussing potential improvement related to zeolite is increased bioavailability of minerals (Leach *et al.*, 1990; Mumpton, 1999). Sodium aluminosilicate influences the metabolism of elements, as evidenced by changes in serum and bone in chicks (Watkins and Southern, 1991; Evans and Farrell, 1993; Eleroğlu *et al.*, 2011). The beneficial effect of zeolite on Ca is likely related to its affinity for calcium and its high capability for ion exchange (Mumpton and Fishman, 1977; Elliot *et al.*, 1991). Owing to the capacity for exchange of ions such as Ca and Mg and the absorption of these ions, the use of dietary zeolite in broilers increases blood calcium, affecting the involvement of Ca in bones (Ballard and Edwards, 1988). Some earlier studies have shown benefits in terms of increased concentration of serum Ca (Hussein *et al.*, 1990; Roland *et al.*, 1993) and bone ash (Elliot *et al.*, 1991; Watkins and Southern, 1991; Rabon *et al.*, 1995), whereas others have reported no such effect (Elliot and Edwards, 1991; Keshavarz and McCormick, 1991; Eleroğlu *et al.*, 2011). However, in the present study, no beneficial effect was observed with regard to Ca, P, and Mg retention in the serum and bone of broiler chicks with respect to the feeding of a diet containing zeolite.

In addition to some beneficial attributes of zeolite in Ca utilisation in chicks, unwanted alterations in serum Ca and P balance have been noted by Watkins and Southern (1991). A depressive effect on serum P level caused by zeolite supplementation has been reported in several studies (Hussein *et al.*, 1990; Utlu *et al.*, 2007). Indeed, the beneficial effect of zeolite on absorption and retention of minerals has been inconsistent and largely dependent on the amount in diets.

According to Leach *et al.* (1990), zeolites are more effective in diets that are low in Ca. Thus, we believe that the lower the dietary intake of Ca, the more beneficial the effect of zeolite on absorption and utilisation of Ca and the greater the possibility that zeolite may positively affect Ca retention in bone. However, results regarding bone mineralisation do not confirm this hypothesis. By contrast, zeolite supplementation reduced bone ash ($P<0.01$) when chicks were fed a Ca-deficient diet (Table 5). In parallel with this, Leach *et al.* (1990) have reported that rather than increasing the utilisation of dietary Ca, zeolite may in fact decrease P availability.

Significantly, increased output of Ca and P and, eventually, ash in faeces but not their unchanged serum and bone augmentation under the conditions of this study indicated that zeolite did not enhance resorption of these minerals but exaggerated their faecal excretion when the birds were on a low-Ca diet. Despite the unchanged bone mineralisation, improvement in bone size (*i.e* weight and thickness) is noticeable. Zeolite may exert its effect through other mechanisms. For example, zeolite contains Na, Al, and Si, all of which influence mineral metabolism (Roland *et al.*, 1991), thus increasing the rate of bone and eggshell formation (Carlisle, 1982; Roland, 1990; Evans and Farrell, 1993). Increased tibia Mn and Cu (Watkins and Southern, 1991, 1992) and Zn (Ward *et al.*, 1990) have been

Table 4. Weight, length and thickness of tibia of chicks administered diet varying in calcium with and without zeolite.

	Tibia measurements		
	Weight, %	Length, cm	Thickness, mm
Deficient dietary Ca level			
Zeolite-unsupplemented	1.02[b]	11.15	9.55
Zeolite-supplemented	1.09[a]	11.07	10.22
Adequate dietary Ca level			
Zeolite-unsupplemented	1.05[ab]	11.01	9.52
Zeolite-supplemented	1.02[b]	11.21	9.98
SEM°	0.016	0.10	0.19
Probabilities			
Dietary Ca level	0.2587	0.9714	0.4910
Zeolite	0.3519	0.5480	0.0069
Dietary Ca level×zeolite	0.0031	0.1914	0.5846
Main effects[#]			
Zeolite-unsupplemented	1.03	11.08	9.54[b]
Zeolite-supplemented	1.05	11.14	10.10[a]
Deficient dietary Ca level	1.05	11.11	9.89
Adequate dietary Ca level	1.03	11.11	9.75

Ca, calcium. °Data are means of 12 chickens (two chickens per replicate pen) with SEM for each treatment; [#]data were analysed as a 2×2 arrangement. [a,b]Means within columns, within main effects, with different superscript differ at $P<0.05$.

observed previously and may have contributed to increases in bone thickness and bone weight in the present study.

The results of the present study showed that the magnitude of response to zeolite was greatest for faecal Al excretion compared with those of the other parameters measured. Birds administered a diet with zeolite excreted about 1-fold higher Al (531 *vs* 954 mg/kg faeces) via faeces compared with that in untreated chicks. This would suggest that the Al is remaining intact; Al is being held by zeolite structure, and is therefore not available for absorption. Natural zeolite contains considerable Al (Eleroğlu *et al.*, 2011; Wu *et al.*, 2013), which differs in a range between 12 and 16% based on the location of the mine. Hence, a mode of action that cannot be discounted as having important influence is the effect of zeolites on Al metabolism in birds. Al can complex with P in the digestive system, depleting P and ultimately impairing its availability (Stoker and Nelson, 1968; Leach and Burdette, 1987). Based on the well-documented antagonism between Al and P in zeolite-supplemented diets (Hussein *et al.*, 1990), more Al would be available to bind with P, possibly creating insoluble alumino-phosphate compounds less available for absorption but wasted via faeces. This effect, in turn, would increase skeletal resorption of Ca and increase the availability of Ca for eggshell and bone formation (Rao and Roland, 1989; Roland *et al.*, 1991).

However, we do not believe that Al is involved in this way because Ca retention in bone was not increased and P was not decreased. Contrarily, faecal excretion of Ca and ash increased in response to dietary zeolite application (Table 6). The lack of concrete evidence on the probable correlation of Al from zeolite with other minerals brings into question the extent to which Al is responsible for the improvement, inefficiency, or both in using zeolite in poultry nutrition.

The counteracting effect of zeolite against experimentally induced levels of aflotoxins (generally applied at 1000 to 2500 mg/kg feed) did occur in former studies (Miazzo *et al.*, 2000; Zhao *et al.*, 2010). The relative weight in an expected range and lower gross lesion score for aflatoxicosis in chick livers indicates that naturally occurring total aflotoxins (24 μg/kg feed) in the feed mixture were incapable of inducing clinical-type mycotoxicosis and its associated severe lesions in chicks. Hence, on the basis of the results of the present study, it is not possible to postulate whether zeolite can act as a mycotoxin adsorbent in the case of mild aflatoxicosis in chickens.

Dietary addition of zeolite reduces the toxicity of litter ammonia (Gupta *et al.*, 1997; Pond *et al.*, 1988) via an ammonia-binding effect; thus, it conveys benefits in reducing the severity of footpad lesions in chicks. The footpad lesion scores of chicks in our study were very low and resemble scores in very healthy legs. This result suggests that the optimal litter and management conditions in broiler houses may not allow zeolite to alleviate the ulceration of footpad skin.

Table 5. Bone ash and calcium, phosphorus and magnesium levels of chickens fed diets differing in calcium with and without zeolite.

	Bone mineralisation, %			
	Ash	Ca	P	Mg
Deficient dietary Ca level				
Zeolite-unsupplemented	36.94[a]	15.89	7.19	0.28
Zeolite-supplemented	35.66[b]	15.76	7.19	0.28
Adequate dietary Ca level				
Zeolite-unsupplemented	35.92[ab]	15.21	6.82	0.28
Zeolite-supplemented	36.80[a]	14.17	7.06	0.27
SEM°	0.37	0.66	0.183	0.005
Probabilities				
Dietary Ca level	0.8746	0.0959	0.1877	0.1879
Zeolite	0.5904	0.3834	0.5119	0.2377
Dietary Ca level×zeolite	0.0067	0.4956	0.5090	0.7265
Main effects[#]				
Zeolite-unsupplemented	36.43	15.55	7.00	0.28
Zeolite-supplemented	36.23	15.96	7.12	0.27
Deficient dietary Ca level	36.34	15.82	7.19	0.28
Adequate dietary Ca level	36.36	14.69	7.94	0.27

Ca, calcium; P, phosphorus; Mg, magnesium. °Data are means of 12 chickens (two chickens per replicate pen) with SEM for each treatment; [#]data were analysed as a 2×2 arrangement. [a,b]Means within columns, within main effects, with different superscript differ at P<0.05.

Table 6. Effects of dietary deficiency of calcium and supplemental zeolite on faecal excretion of ash, calcium, phosphorus, magnesium and aluminum levels in chickens.

	Faecal excretion of ash and minerals, %				
	Ash	Ca	P	Mg	Al
Deficient dietary Ca level					
Zeolite-unsupplemented	12.15[d]	2.08[c]	1.36[d]	0.51	552[c]
Zeolite-supplemented	14.04[b]	2.26[b]	1.53[c]	0.52	884[b]
Adequate dietary Ca level					
Zeolite-unsupplemented	12.97[c]	2.92[a]	1.73[a]	0.56	511d
Zeolite-supplemented	14.97[a]	2.99[a]	1.65[b]	0.56	1024[a]
SEM°	0.10	0.04	0.02	0.005	7.70
Probabilities					
Dietary Ca level	0.0001	0.0001	0.0001	0.0001	0.0001
Zeolite	0.0001	0.0062	0.0666	0.6040	0.0001
Dietary Ca level×zeolite	0.6151	0.1978	0.0001	0.2690	0.0001
Main effects[#]					
Zeolite-unsupplemented	12.56[b]	2.50[b]	1.52	0.54	531[b]
Zeolite-supplemented	14.50[a]	2.63[a]	1.59	0.54	954[a]
Deficient dietary Ca level	13.10[b]	2.17[b]	1.45	0.52[b]	718[b]
Adequate dietary Ca level	13.97[a]	2.96[a]	1.69	0.56[a]	767[a]

Ca, calcium; P, phosphorus; Mg, magnesium; Al, aluminum. °Data are means of 12 chickens (two chickens per replicate pen) with SEM for each treatment; [#]data were analysed as a 2×2 arrangement. [a-d]Means within columns, within main effects, with different superscript differ at P<0.05.

Conclusions

Overall, our results suggest that dietary zeolite supplementation in broilers provide no performance benefits while exaggerating faecal excretion of minerals. The assumption that zeolite has a larger positive effect when nutritional deficiency is present – the case of Ca in this study – was not verified by broiler growth performance indices and bone mineralisation. However, the thickness and weight of bone and the liveability of birds seem to benefit from zeolite, an outcome that merits further investigation. Noticeably, markedly increased faecal excretion of Al despite the moderate inclusion rate (8 g/kg diet) of zeolite may introduce doubt about its usefulness in broiler nutrition when applied at higher inclusion rates. The results also suggest that moderate reductions in dietary Ca intake retard growth but do not adversely affect bone growth in modern broiler hybrids.

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