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Effect of Azomite on Growth Performance, Nutrient Retention, Immunity and Bone Mineralization of Broiler Chickens

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Key words
Azomite, Growth performance, Nutrient retention, Immunity, Bone mineralization, Broiler chickens

ABSTRACT

Present study was carried out to investigate the effect of azomite (AZO) on the growth performance, nutrient availability, intestinal enzymes activity, immune function, and bone mineralization of broiler chickens. A total of 240-day old male chicks were randomly assigned into comprising four treatments with six replicates (n=10), which included control (basal diet), control +0.25% AZO, control + 0.50% AZO and control + kitasamycin (as AGPs). The results indicate that LBGW, ADG and FCR was significantly ($P<0.05$) improved in AZO 0.25% and 0.50% than the control. Eviscerated (EV), breast muscle (BM) and leg muscle (LM) were significantly ($P<0.05$) higher in the AZO 0.50% treated groups than control. Digestibility of DM, CP, AME, Ca and P digestibility significant ($P<0.05$) higher in AZO 0.25% and 0.50% dietary treatments. Moreover, jejunal amylase and trypsin activity were improved in AZO 0.25% and 0.50% supplemented groups. CREAT was significantly increased in AZO 0.50% treatment, while IgG was significantly improved in AZO 0.25% treatment. Tibia diameter (TD), tibia breaking strength (TBS) were numerical higher in AZO 0.25%, while Ca and P % was significantly ($P<0.05$) increased in Azomite 0.25% treatment. The results indicated that addition of azomite at the dose of 0.25% and 0.50% in the diet had beneficial effect on growth performance, retention of nutrients, digestive enzymes and bone mineralization.

INTRODUCTION

Poultry is one of the most important source of animal protein for humans. In the past decades, antibiotics growth promotors (AGPs) played a crucial role to upgrade the poultry meat industry. However, the impulsive use of AGPs in animal production causes the antibiotic resistance, accumulation of antibiotic residues in meat and environmental contamination related to antibiotics, which may affect the health of human beings. Therefore, AGP's have been banned almost across the globe in the poultry industry. Researchers and scientists feel immense pressure to find the substitutes of AGPs which can increase the growth efficiency, digestibility, immunity and improve the general health of chickens (Tang *et al*., 2017; Jharomi *et al*., 2016; Alloui *et al*., 2014; Petracci *et al*., 2013). Fortunately, many feed additives have been developed, such as prebiotics, probiotics, organic acids and immune enhancers, to improve the efficiency of chickens, as substitutes of AGPs. Some researchers have also reported the growth promising effects of rare earth elements (REE) in some animal species (Lei and Liu, 1997; Ladipo *et al*., 2015).

Azomite is a 100% natural mineral product form an ancient deposit in Utah (USA) that contains approximately 70 minerals, trace and rare earth elements. These elements are essential in animal diet because they play major role in physiological processes which require the proper growth, general health, immunity and bone mineralization (Richards *et al*., 2010). Over last two decades, azomite widely used as a feed additive in aquaculture as well as in livestock and organic agriculture. Some research findings reported that azomite improves the feed quality, weight gain, nutrient digestibility, feed conversion ratio and immunity in shrimp, tilapia and catfish (Liu *et al*., 2009, 2011; Musthafa *et al*., 2016; Tan *et al*., 2014). Azomite also increases the activity of digestive enzymes in tilapia *Ctenopharyngodon idellus* (Liu *et al*., 2009; Tan *et al*., 2014). In addition, Emerson and Hooge (2008) summarized 13 field and commercial experiments concerning using azomite in chicken production and found that adding azomite to the diet improved breast meat yield. These findings suggest

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azomite could be an alternative option to the use of AGPs in broiler chickens based on its capability to improve the growth performance, digestibility of nutrients and immune functions in aquatic species. However, there are very few formal academic studies reported on broiler chickens with supplementation of azomite in diet. Therefore, the purpose of this study was chosen to investigate the effect of azomite on growth performance, nutrient retention, immunity and bone mineralization in broiler chickens.

MATERIALS AND METHODS

Dietary treatments and bird's management

All experimental procedures, protocols and animal care for this study was approved by Feed Research Institute, Graduate School of Chinese Academy of Agricultural Sciences, Beijing China. A total number of 240 one-day old male chicks were purchased from Beijing Huadu Broiler Company. Chicks were weighed and randomly allocated into four groups with six replicates of 10 chickens per replicate. The experiment was conducted in two phases, starter (1-21) and finisher phase (22-42). Four diets were prepared for the experimental trial, which included control (without any antibiotics), control +0.25% AZO, control + 0.50% AZO and control + kitasamycin (as AGP- antibiotics growth promotor). The ingredient composition and calculated nutrient analysis showed in Table I. The azomite sample was provided by Lytone Company, Taiwan. Before arrival of broiler chicks, the house was cleaned and disinfested. The experiment was conducted in stainless steel wired battery cages, the house temperature maintained during 1st week at 32°C and the gradually decrease 2°C each until it reached the 22°C at the last week. Relative humidity was maintained at 55% to 65%, and lighting procedure of 23h lighting:1h darkness was provided. The adlibitum access of feed and water provided to the broilers.

Growth performance and carcass parameters

At the 21d and 42d of the experiment, live body weight (LBW) and feed consumption were recorded on cage basis. The average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) was calculated. The FCR were corrected by dead birds. Two birds aged 42 days were selected from each replicate and slaughtered to measure the percentage of carcass, breast, leg and abdominal fat. Thymus, spleen and bursa of Fabricius were removed and weighed individually.

Table I. Ingredient composition and nutrient content for basal diet.

Ingredients (%)	1-21 days	22-42 days
Corn	57.47	58.98
Soybean oil	1.50	4.32
Soyabean meal	30.96	25.05
Cotton seeds meal	5.00	7.00
Table salt	0.35	0.35
Dicalcium phosphate	1.53	1.39
Limestone	1.54	1.40
L-Lysine chloride	0.24	0.22
DL-Methionine	0.14	0.15
Cysteine	0.07	0.04
L-Threonine	0.00	0.00
Choline chloride	0.20	0.10
Premix	0.50	0.50
Zeolite (stuffing)	0.50	0.50
Total	100	100
Nutrition value of diet		
AME (kcal/kg)	2950	3050
Crude protein (%)	22.00	19.50
Lysine (%)	1.200	1.050
Methionine (%)	0.450	0.400
TSAA (%)	0.900	0.800
Ca (%)	0.99	0.900
Total P (%)	0.679	0.552
Avail P(%)	0.450	0.420

The premix provided (for 1 kg of diets) VA 10000IU, VB1 1.8mg, VB2 40mg, VB12 0.71mg, VD3 2000IU, VE 10IU, VK3 2.5 mg, biotin 0.12mg, folic acid 0.5mg, D-pantothenic acid 11mg, Cu (as copper sulfate) 8mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 60 mg, Zn (as zinc sulfate) 40mg, I (as potassium iodide) 0.0.35 mg and Se (as sodium selenite) 0.15 mg.

Apparent nutrients retention

Before the one week of excreta collection 0.4% titanium Oxide (Tio2) was added in diets as indigestible marker to determine the digestibility of nutrients. Excreta were collected continuously three days from 39d-41d from each replicate. After dried at 65C° for 72 h, excreta were ground and passed through 0.40 mm sieve. Diet and excreta were analyzed for dry matter (method 930.15) and ash (method 942.05) AOAC (2000), crude protein by Dumatherm (Gerhardt company, Germany), gross energy (GE) by calorimeter (C2000, IKA, Germany), Ca by atomic absorption spectrometer (novAA 400P, analytikjena, Germany) and phosphorus (P) by ammonium molybdate

calorimetry. The content of TiO2 in diets and feces were determined according to the method described by (Sort *et al*., 1996). The availability of nutrient was calculated according to the indicator method. Following formula was used for calculation of nutrient retention.

Digestibility of Nutrients =1- [(TiO2 in diet/Tio2 in feces) X (Nutrient in diet / Nutrient in feces)] X 100

Intestinal enzymes activity

The digesta was collected from jejunum and store in liquid nitrogen container. The enzymic activity of lipase, amylase and trypsin were analysed according to the commercial kit's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Serum biochemical indices

Two birds were selected randomly from each replicate and take blood samples from wing vein. Ten milliliters of blood was collected into sterilized tubes and centrifuged at 3000 rpm for 15 minutes at 4°C to harvest serum. Serum samples were stored at -20°C for biochemical analysis. The content of total protein (TP), glucose (GLU), total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), creatinine (CREAT), uric acid (UA) was analyzed by using an automated IDEEX Vet Test Chemistry Analyzer (IDEEX Laboratories, Inc). The blood concentration of immunoglobulins (IgA, IgG and IgM) were analyzed following chickens specific ELISA kits instructions (Shanghai Lengton Biosciences Co., LTD, Shanghai, China).

Tibia bone analysis

Tibia were dissected from slaughtered birds at day 42. The skin, muscle and other soft tissues were removed carefully. After air-dried, the weight and length of tibia bones were measured. The diameter was measured at the narrowest and widest points using Vernier caliper, and then averaged. The bone breaking strength was determined using texture analyzer. After measurement of bone breaking strength, the broken bones were place in plastic bags for determining the content of ash, Ca and P. All tibia bone samples defatted with ethanol and diethyl ether for 48 h. The defatted samples were dried in oven at 100°C for 24 h, then weighed and ashed in muffle furnace at 600°C for 16 h. Ash was weighted and then dissolved in 10 ml of HCl and 5 ml of HNO_3. Digested samples were filtered and diluted with deionized water to the required volume and analyzed for Ca by atomic absorption spectrometer and P by ammonium molybdate calorimetry.

Statistical analysis

The differences among treatments were statistically analyzed by one-way ANOVA using SPSS Statistics 19.0 The significant differences among means of treatments were compared by Tukey test. The means and standard error of means are presented. The significant level is set at 5%.

RESULTS

The effects of dietary treatment on growth performance of the broiler chickens are presented in Table II. In starter feeding phase, the birds fed dietary AZO 0.25% and 0.50% had higher LBW and ADG ($P<0.05$) than the control and AGP, while lower FCR ($P<0.05$) recorded than control. But ADFI had no significant difference among all treatments. In finisher phase, birds fed with AZO 0.25% or 0.50% had higher ADG than the control ($P<0.05$), while 0.50% AZO was higher than other treatments ($P<0.05$). Birds fed 0.50% AZO had optimal FCR which lower than other treatments ($P<0.05$), among whom did not show significant difference. ADFI was higher in AZO 0.25% than the control significantly ($P<0.05$), but non-significant difference was recorded among other treatments. In overall phases, the birds fed dietary AZO 0.25% and 0.50% had higher LBW and ADG, and ADG of AZO 0.50% was more than AGP group ($P<0.05$). The lowest FCR was observed in AZO 0.50% group, which less than the control significantly ($P<0.05$), but there was no difference with AGP and AZO 0.25% group. ADFI was noted higher in Azomite 0.25% than the control, but non-significant difference was recorded in other treatments.

Carcass performance and immune organ ratio are presented in Table III. The results showed that percentage of eviscerated yield (EV), percentage of breast muscle (BM) yield and percentage of leg muscle (LM) yield were significantly higher in AZO 0.25%, AZO 0.50% and AGP groups than control ($P<0.05$), but non-significant difference between AZO and AGP treatment though AZO 0.5% has the highest percentage numerically. Moreover, addition of AZO and AGP trended to decrease percentage of abdominal fat (AF) ($P=0.099$), and AZO 0.5% has the lowest value. However, percentage of immune organs, including thymus, bursa and spleen, were not affected by dietary treatment.

The results about nutrient retention of broiler chickens are shown in Table IV. Compared with the control, addition of 0.25% or 0.5% azomite and AGP improved apparent digestibility of DM, CP, ME, Ca and P ($P<0.05$) except ash. Thereinto, the digestibility of DM in azomite treatments is higher than AGP ($P>0.05$). For other nutrients, the effects of azomite is similar to AGP. No significant difference was found between azomite treatments.

Table II. Effect of dietary treatment on growth performance of broiler chickens.

Treatment	Control	AZO 0.25 %	AZO 0.50 %	AGP	*P.* value
Starter (1-21 Day)					
LBW(g)	961 ± 0.03[b]	1021 ± 0.02[a]	1020 ± 0.2[a]	966 ± 0.02[b]	0.004
ADG(g)	43.70 ± 1.9[b]	46.11 ± 1.29[a]	46.48 ± 1.2[a]	43.91 ± 1.3[b]	0.005
ADFI(g)	58.62 ± 2.4	59.52 ± 1.61	59.77 ± 1.3	56.71 ± 3.1	0.111
FCR	1.34 ± 0.02[a]	1.29 ± 0.012[b]	1.28 ± 0.01[b]	1.29 ± 0.03[b]	0.002
Finisher (22-42 Day)					
LBW(g)	1498 ± 0.13	1748 ± 0.218	1715 ± 0.25	1741 ± 0.09	0.098
ADG(g)	77.66 ± 4.0[c]	87.85 ± 7.96[a]	89.42 ± 6.9[a]	85.96 ± 6.2[b]	0.024
ADFI(g)	142.5 ± 4.9[b]	157.3 ± 14.6[a]	152.5 ± 4.8[ab]	153.9 ± 9.0[ab]	0.067
FCR	1.83 ± .054[b]	1.80 ± 0.181[b]	1.71 ± 0.15[a]	1.80 ± 0.04[b]	0.039
Overall (1-42 Day)					
LBW(g)	2705 ± 0.08[b]	2952 ± 0.08[a]	3019 ± 0.12[a]	2881 ± 0.14[ab]	0.002
ADG(g)	63.37 ± 1.9[c]	69.23 ± 3.3[a]	70.83 ± 3.07[a]	67.56 ± 3.3[b]	0.002
ADFI(g)	97.8 ± 3.6[b]	103.9 ± 4.07[a]	102.7 ± 1.00[ab]	101.3 ± 4.4[ab]	0.038
FCR	1.54 ± 0.02[b]	1.50 ± 0.06[ab]	1.45 ± 0.059[a]	1.50 ± 0.42[ab]	0.037

[a,b,c] super scripts with different letters in a row showed significant (P<0.05) difference. LBW, live weight gain; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Table III. Effect of dietary treatment on carcass performance of broiler chickens.

Parameters	Control	AZO 0.25 %	AZO 0.50 %	AGP	*P.* value
EV (%)	70.74±0.83[b]	72.77±1.25[a]	74.03±1.77[a]	72.48±1.79[a]	0.004
BM (%)	15.15±0.41[b]	15.93±0.46[a]	16.44±0.29[a]	15.59±0.92[ab]	0.006
LM (%)	10.71±0.19[b]	11.32±0.20[a]	11.50±0.63[a]	11.09±0.65[a]	0.049
AF (%)	1.95±0.28[a]	1.69±0.27[ab]	1.59±0.23[b]	1.62±0.22[b]	0.099
Thymus (%)	1.24±0.41	1.31±0.26	1.43±0.42	1.67±0.38	0.534
Bursa (%)	0.32±0.10	0.40±0.13	0.41±0.20	0.43±0.13	0.597
Spleen (%)	0.84±0.17	0.98±0.23	1.06±0.32	1.04±0.44	0.634

[a,b,c] super scripts with different letters in a row showed significant (P<0.05) difference. EV, eviscerated; BM, breast muscle; LM, leg muscle; AF, abdominal fat.

Table IV. Effect of dietary treatment on apparent retention of nutrients.

Parameters	Control	AZO 0.25%	AZO 0.50%	AGP	*P.* value
DM (%)	72.91±0.2[b]	77.20±0.19[a]	77.14±0.19[a]	75.72±0.25[a]	0.001
CP (%)	66.86±0.1[b]	69.39±0.05[a]	69.74±0.10[a]	69.23±0.12[a]	0.000
ME (%)	76.96±0.2[a]	79.02±0.11[a]	79.13±0.08[a]	79.21±0.11[a]	0.028
Ash (%)	62.40±0.41	65.20±0.30	63.30±0.41	64.03±0.23	0.400
Ca (%)	51.31±0.3[b]	58.16±0.22[a]	56.02±0.36[a]	55.21±0.32[a]	0.006
P (%)	45.55±0.5[b]	50.64±0.04[a]	49.76±0.14[a]	49.57±0.18[a]	0.024

a,b,c super scripts with different letters in a row showed significant (P<0.05) difference. DM, dry matter; CP, crude protein; ME, metabolizable energy; Ca, calcium; P, phosphorus.

Table V. Effect of dietary treatment on enzymes in broiler chickens.

Enzyme	Control	AZO 0.25 %	AZO 0.50%	AGP	*P.* value
Lipase (U/mg)	253.64±60.28	258.86±54.26	263.93±37.47	252.35±35.562	0.977
Amylase (U/mg)	2.96±1.01^{b}	3.89±0.44^{a}	4.30±0.81^{a}	3.98±0.33^{a}	0.035
Trypsin (U/mg)	124.46±49.13^{b}	184.52±23.47^{a}	188.52±21.68^{a}	174.53±23.47^{a}	0.029

^{a,b,c} super scripts with different letters in a row showed significant (P<0.05) difference.

Table VI. Effect of dietary treatment on serum biochemical indexes of broiler chickens.

Parameters	Control	AZO 0.25 %	AZO 0.50 %	AGP	*P.* value
TC (mmol/L)	2.91±0.23	2.92±0.18	2.73±0.35	2.90±0.09	0.365
TG (mmol/L)	0.49±0.4	0.44±0.07	0.47±0.09	0.47±0.1	0.859
HDL (mmol/L)	1.49±0.23	1.31±0.21	1.15±0.25	1.42±0.9	0.166
LDL (mmol/L)	0.69±0.08	0.70±0.13	0.61±0.24	0.80±0.05	0.414
CREAT (mmol/L)	13.25±0.61^{b}	14.48±1.03^{ab}	15.95±1.84^{a}	13.98±0.6^{b}	0.004
UA (mmol/L)	283.34±51.9	319.75±23.7	291.50±58.5	300.25±57.5	0.832
GLU (mmol/L)	10.66±0.87	9.69±3.4	9.69±2.5	10.67±0.47	0.809
TP (g/L)	28.7±3.44	33.80±2.05	35.50±6.05	33.16±5.1	0.141
ALB (g/L)	10.55±1.65	12.17±0.97	12.45±1.38	12.65±0.76	0.121
GLB (g/L)	17.86±2.44	22.10±2.07	22.72±6.00	22.48±5.09	0.262
IgA (g/L)	0.87±0.19	1.01±0.13	1.12±0.19	1.12±0.2	0.117
IgG (g/L)	0.66±0.07^{b}	0.79±0.04^{a}	0.75±0.75^{ab}	0.73±0.07^{ab}	0.039
IgM (g/L)	5.13±0.95	6.64±1.46	6.95±1.1	6.59±1.53	0.141

^{a,b,c} super scripts with different letters in a row showed significant (P<0.05) difference. TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein; CREAT, creatinine; UA, uric acid; GLU, glucose, Ig, immunoglobulins; GH, growth hormone; CT, calcitonin; PTH, parathyroid hormone.

Table VII. Effect of dietary treatment on tibia parameters in broiler chickens.

Parameters	Control	AZO 0.25%	AZO 0.50%	AGP	*P.* value
TWT (g)	7.01±0.8	8.11±0.4	7.88±0.2	7.61±0.7	0.096
TL (cm)	8.14±0.6	9.39±0.7	8.96±1.0	8.62±1.0	0.250
TD (cm)	0.83±0.07^{b}	0.94±0.03^{a}	0.91±0.07^{a}	0.89±0.03^{ab}	0.021
TBS (kg)	20.26±2.0	27.54±5.9	24.20±2.2	23.23±9.5	0.366
Ash (%)	47.38±0.01^{b}	49.79±0.01^{a}	49.03±0.01^{a}	48.68±0.01^{ab}	0.080
P (%)	7.64±03^{b}	8.64±0.3^{a}	8.21±0.8^{ab}	8.17±0.43^{ab}	0.047
Ca (%)	16.8±1.2^{c}	19.8±1.03^{a}	19.2±1.04^{b}	18.3±1.6^{bc}	0.002

^{a,b,c} super scripts with different letters in a row showed significant (P<0.05) difference. TWT, tibia weight; TL, tibia length; TD, tibia diameter; TBS, tibia breaking strength; P, phosphorus; Ca, calcium.

Effect of dietary treatment on digestive enzymes activity is illustrated in Table V. Supplementation of azomite and antibiotic enhanced the activity of amylase and trypsin significantly (P<0.05). Whereas, non-significant difference was showed between azomite and antibiotic treatment. There was no significant dietary effect on the lipase activity (P>0.05).

Effect of azomite on the serum biochemical parameters of broilers are given in Table VI. The feeding of diet supplemented with azomite observed same effect on serum biochemical indexes. Results indicated that TC, TG, HDL, LDL, UA, GLU, TP, ALB and GLB level had

no significant difference among all treatments. However, creatinine level was higher in AZO 0.50% treatment than the control and AGP group significantly (P<0.05). The data also showed that IgG was higher in Azomite 0.25% than the control significantly (P < 0.05), but no significant difference was noticed between Azomite 0.50% supplemented and AGP treatments. IgA and IgM were not affected by the dietary treatments, but numerically higher values were recorded in azomite and AGP treatments. Dietary treatment did not influence the GH, PTH and CT level (P>0.05).

Effect of dietary treatments on the tibia bone is illustrated in Table VII. The results showed addition of azomite increased the tibia diameter and the content of Ca significantly (P<0.05), and also tended to increase tibia weight and the content of ash and P (P<0.10). The multiple comparison showed that the tibia bone from birds fed 0.25% AZO had more ash and P than the control (P<0.05), and more Ca than the control and AGP (P<0.05).

DISCUSSION

The incorporation of rare earth elements with animal feed significantly enhances the growth performance, innate immune response, and disease resistance in several animal species (Wan *et al*., 1998). Few studies have reported the application of azomite as feed additives for aquatic species, but limited literature is available on broiler chickens. Tan *et al*. (2014) found significant increase in weight gain and lower FCR in white shrimp by adding 2-4 kg azomite to the diet. McNaughton (2011) reported that dietary inclusion of 0.5% azomite improved body weight and FCR significantly in pigs. In our study, addition of azomite from 0.25% to 0.50% in broiler diet resulted in significant improvement in live weight gain and feed conversion, which supported the reports above despite the difference of animal species. In addition, our results along with the available literature confirmed the possibility of azomite being an alternative of AGPs based on its excellent growth promotion which is no less than antibiotics (kitasamycin) in our research trial.

As we expected, the positive effects of azomite in growth promotion were exhibited in the carcass improvement. In our study, percentages of eviscerated yield, breast muscle and leg muscle were significantly increased with addition of azomite 0.25% and 0.50%. Emerson and Hooge (2008), thought that dietary azomite could increase breast meat yield of broiler chickens based on meta-analyses of data from 13 unpublished commercial trials and 10 integrator field trials. These results implied that azomite could enhance the anabolism of nutrients.

Similar to the growth and carcass performance, the improvement in retention of nutrients by azomite was well reported in aquaculture. Fodge *et al*. (2011), reported that supplementation of azomite 0.25% to 0.50% in fish diet improve the DM and CP digestibility significantly. Our study verified the finding above, in which 0.25% or 0.5% azomite increased the retention of DM, CP, ME, Ca and P in broiler chickens. Fortunately, the positive effect of azomite on retention of nutrients could be speculated by the increased activity of digestive enzymes. Tan *et al*. (2014), found that 0.4% of azomite addition increased significantly the activities of stomach protease, hepatopancreas lipase. The significant increase of activity of lipase in the intestine when 0.25% to 0.75% of azomite was added in tilapia. In the present experiment, adding 0.25% and 0.5% of azomite increased the activity of amylase and trypsin in jejunum of broilers without influencing activity of lipase. It is clear from our study that azomite enhances the activity of proteolytic enzymes, and this result helps to explain about weight gain and FCR improvement.

Serum indices are critical indicators to monitor the health, diagnosis and treatment of disease and these also indicate the nutritional status of chickens (Schidmit *et al*., 2007). In the current study, dietary treatments did not influence the serum indices related to fat and protein metabolism except creatinine which is higher significantly in 0.5% azomite addition than the control and AGP groups. Creatinine is an important indicator of protein metabolism, and its level in serum is positive related with muscle mass (Wyss and Kaddurah-Daouk, 2000; Rajman *et al*., 2006). It is obviously coincident with the highest body weight and the highest percentage of carcass and muscle in birds fed 0.50% of azomite. However, the content of immunoglobulins in serum was influenced by azomite addition. The concentration of blood immunoglobulins is an important indicator associated with humoral immunity, because these immunoglobulins defend against pathogenic microorganisms and maintain good health of birds (Herich, 2016). The present data shows significant higher IgG was witnessed in 0.25% azomite treatment than the control, and numerical increasing of IgA and IgM could be found in 0.25% and 0.5% azomite addition. Fodge *et al*. (2014) reported that 0.50% azomite addition in broiler diet increased the content of IgG in blood. Jaleel *et al*. (2015) also found dietary addition of 4g/kg azomite significantly increased the immunoglobulin level in koe carp fingerlings. These reports verify that azomite could improve the immunity in broiler chickens.

Bone weight, length, diameter, breaking strength and ash content determine the bone mineralization in chickens (Onyango *et al*., 2003). Moreover, the bone mineralization makes bones harder which enables the skeleton to withstand the gravity, addition loading and prevent the leg deformities in broilers (Shim *et al*., 2012). However, the

effect of azomite on the bone parameters have not been reported. The findings of our study show that broiler fed diet with azomite 0.25% or 0.50% improved the tibia strength, Ca, P contents and ash percentage in tibia. On other hand tibia weight and tibia length were numerically higher in azomite supplemented treatments. Accordingly, azomite improved the availability of Ca and P which might led improvement in breaking strength and the bone mineralization due to Ca and P deposition in tibia. From the findings of previous studies, it is confirmed that azomite accelerates the bone mineralization.

CONCLUSION

The present study clearly showed that supplementation of 0.25 and 0.50% azomite improved the growth performance, nutrient retention, intestinal enzymes activity and bone mineralization of broiler chicken. Moreover, Azomite supplementation had positive effect IgG level in broiler chickens. Therefore, the addition of Azomite could replace the AGPs in poultry industry.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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