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Full Length Article

Effect of Azomite with Low Energy Diet on Growth Performance, Nutrient Digestibility and Bone Mineralization of Broiler Chickens

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Abstract

The present research was examined to determine the influence of azomite (AZO) with low energy diet on growth performance, nutrient digestibility, and bone parameters in broilers chickens. A total of 180 one day old broiler chicks Arbor Acers (AA) randomly assigned into three treatments with six replicates and 10 birds per/replicate. Treatments were (1) Control as a basal diet, (2) LME (basal diet - 100 kcal ME kg^{-1}) and (3) AZO-0.25 (LME + AZO-0.25%). The results of this study showed that growth performance in terms of live body weight (LBW) and average daily gain (ADG) significantly improved with AZO-0.25 supplementation ($P < 0.05$), while feed conversion ratio (FCR) was lower ($P < 0.05$) in group fed with AZO-0.25 than LME. Average daily feed intake (ADFI) was significantly ($P < 0.05$) lower in CONT compared to LME and AZO-0.25 treatments. Broilers fed diet with AZO-0.25 increased ($P < 0.05$) the dry matter (DM), crude protein (CP), apparent metabolizable energy (AME), phosphorus (P) and calcium (Ca) digestibility compared to LME. Furthermore, tibia length (TL), tibia breaking strength (TBS), ash, P and Ca percentage significantly ($P < 0.05$) increased in birds fed AZO-0.25 compared with LME. Overall, azomite could be considered as a feed additive to reduce the energy in order to enhance the growth performance, nutrient digestibility and bone parameters without negative effect on intestinal morphology.

Keywords: Growth performance; Nutrient digestibility; Bone strength; Broiler chickens

Introduction

The current scenario is very critical for poultry industry due to increasing the feed cost in worldwide, generally 60-70% energy ingredients cost spent in broiler diet. Therefore, numerous studies were conducted to reduce the production cost of some energy ingredients, through improved feed efficiency in broiler chickens by management of nutrient supply (Donohue and Cunningham 2009). While, plenty of reports indicated that reduction of nutrient supply could decrease the growth performance, FCR and leading to skeletal disorders and also increases the fat accumulation in carcass (Thomas *et al*. 1978; Butzen *et al*. 2015; Mohammadigheisar *et al*. 2018; Saleh *et al*. 2018; Boontiam *et al*. 2019; Saleh *et al*. 2019). Therefore, poultry researchers and scientists feel immense pressure to find the natural feed additives which may increases the growth performance and optimize nutrient utilization. In addition, the impact of certain mineral supplements on broiler performance, health status has been attracting current research interest. In this regard combination of rare earth elements (REE) and trace minerals are being actively researched for their effects of growth performance, FCR and nutrient digestibility in broiler chickens (He *et al*. 2010; Adu *et al*. 2011; Cai *et al*. 2014).

Azomite is considered a very useful natural mineral product in Utah (USA) mined from volcanic eruption into a seabed and is a distinction from any other mineral deposit in the world. It is the mixture of animal, plants residues and minerals. It contains more than 70 trace minerals, especially rich in rare earth elements (REE). The trace and REE are most important in diet because they play key role in physiological process, proper growth, health status and bone development (Lei and Liu 1997; Ladipo *et al*. 2015). Interestingly, azomite is widely used as a natural mineral booster in aqua feed industry as well as in livestock which improve the feed quality that can facilitates the utilization of nutrients which are not available to the animals (Fodge *et al.* 2014). Azomite is certified by OMRI (Organic Material Review Institute, U.S.A.) and AAFCO (Association of American Feed control Officials) as kind of natural mineral booster that can be used in livestock, aquatic diet and

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organic agriculture. In particular, several studies have been reported that dietary azomite increases the weight gain and lower the FCR by improving the digestibility of protein, enzymes activity and nutrient utilization in GMT tilapia and white shrimp (Liu *et al*. 2011; Tan *et al*. 2014; Azam *et al*. 2016), thereby reducing the amount of nutrient excreted in feces which may also decreases the environmental pollution. Moreover, the beneficial effect of azomite may be result of the improved dry matter, protein digestibility and capacity for energy utilization (Fodge *et al.* 2014). Furthermore, Lumpkin *et al*. (2014) and McNaugton (2011), documented that addition of azomite in diet improved the growth performance and intestinal morphology in broilers and pigs. These literatures confirmed that azomite could improve the digestion and utilization of nutrients by increasing digestive enzymes activity or other mechanism. Thus, there is possibility to compensate for potential negative effect of low energy or low other nutrient diet via the application of azomite. Broilers require high dietary energy to meet their vigorous metabolism and rapid growth, thus energy account for most of cost of diet. It is possible to lower dietary cost of broilers by lowering dietary energy and meanwhile to alleviate negative effect on growth performance by azomite addition.

Therefore, the main purpose of current study was to examine the supplementation of azomite with low energy diet on growth performance, nutrient digestibility, and bone development of broiler chickens, and tried to lower feed cost of broilers.

Materials and Methods

Dietary treatments and bird's management

All experimental procedures, protocols and animal care for this study were approved by Feed Research Institute, Graduate School of Chinese Academy of Agricultural Sciences, Beijing China. A total number of 180 one-day old male chicks were purchased from Beijing Huadu Broiler Company. Chicks were weighed and randomly allocated into three treatments with six replicates of 10 chickens per replicate. The experiment was conducted in two phases, starter (1–21) and finisher phase (22–42). The three dietary treatments for this experiment consisted of control (CONT) containing 2950 kcal kg^{-1} starter phase and 3050 kcal ME kg^{-1} finisher phase; low energy (LME) was containing2850 kcal ME kg^{-1} starter phase and 2950 kcal ME kg^{-1} in finisher phase and LME with 0.25% Azomite addition (AZO-0.25). The ingredient composition and calculated nutrient analysis showed in Table 1. 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 23 h lighting: 1 h darkness was provided. The *adlibitum* access of feed and water provided to the broilers.

Growth performance and carcass traits

Live body weight (LBW) and feed intake (FI) of broilers were recorded and the average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were determined. ADG and FCR were calculated by using following formula.

ADG = Final Body Weight-Initial Body Weight / Age in days, FCR= FI / ADG

Apparent digestibility of nutrients

Before the one week of feces collection 0.4% titanium Oxide (TiO_2) was added in diets as indigestible marker to determine the digestibility of nutrients. Feces were collected continuously three days from 39–41d from each replicate. After dried at 65°C for 72 h, feces were ground and passed through 0.40 mm sieve. Diet and feces were analyzed for dry matter and ash (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 TiO_2 in diets and feces were determined according to (Sort *et al*. 1996).

The digestibility of nutrient was calculated according to the indicator method. Following formula was used for calculation of nutrient digestibility.

$$\text{Digestibility (\%)} = 1 - \left[\left(\frac{\text{Nutrient in Feces}}{\text{Titanium in Diet}}\right) \times \left(\frac{\text{Nutrient in Diet}}{\text{Titanium in Feces}}\right)\right] \times 100$$

Intestinal enzymes activity

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

Morphology of small Intestine

Whole small intestine was dissected from the slaughtered birds carefully and cut into 3 segments. The duodenum was divided from gizzard to pancreo-biliary-duct, the jejunum from pancreo-biliary-duct to Meckel's diverticulum and ileum from Meckel's diverticulum to ileo-ceacal junction. The intestinal segments were flushed with 10% formalin to remove the content and one centimeter long of each segment were excised, then fixed in 10% formalin for minimum 48 h. The sections 5-μm thick were prepared and dyed in hematoxylin-eosin solution. The structure of mucosa was observed at 40× magnification using an

Table 1: Ingredient composition and nutrient content for basal diet

	Control		LME	
Ingredients	Starter	Finisher	Starter	Finisher
Corn	57.47	58.98	58.53	63.60
Soya bean Oil	1.50	4.32	0	0.58
Soy Bean	30.96	25.05	30.76	24.16
CSM	5.00	7.00	5.00	7.00
Salt	0.35	0.35	0.35	0.35
CaPo4	1.53	1.39	1.52	1.36
Lime stone	1.54	1.40	2.18	1.42
Lys	0.24	0.22	0.25	0.24
Meth	0.14	0.15	0.14	0.15
Cyst	0.07	0.04	0.07	0.04
Chol	0.20	0	0.20	0.00
Premix	0.50	0.10	0.50	0.10
Zeolite	0.50	0.50	0.50	0.50
Total	100	100	100	100
Calculated nutritional Levels				
ME (kcal/kg)	2950	3050	2850	2950
Protein (%)	21.50	19.00	21.50	19.00
Lys (%)	1.200	1.050	1.200	1.050
Meth (%)	0.450	0.440	0.450	0.440
TSAA (%)	0.900	0.800	0.900	0.800
Thr (%)	0.866	0.724	0.866	0.724
Trypt (%)	0.311	0.248	0.310	0.245
CF (%)	3.471	3.027	3.485	3.054
EE (%)	4.769	7.023	3.331	3.473
Ca (%)	1.000	0.902	1.005	0.906
P (%)	0.679	0.689	0.679	0.692
Avail P (%)	0.450	0.552	0.450	0.555

The premix provided (for 1 kg of diets) VA 10000IU, VB1 1.8 mg, VB2 40 mg, VB12 0.71 mg, VD3 2000 IU, VE 10 IU, VK3 2.5 mg, biotin 0.12 mg, folic acid 0.5 mg, D-pantothenic acid 11 mg, Cu (as copper sulfate) 8 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 60 mg, Zn (as zinc sulfate) 40 mg, I (as potassium iodide) 0.35 mg and Se (as sodium selenite) 0.15 mg

Table 2: Effect of azomite supplementation to low energy diet on growth performance in broiler chickens

Parameters	Control	LME	AZO-0.25	*P*. Value
LBW (g)	2705 ± 0.08^{b}	2641 ± 0.07^{b}	2894 ± 0.08a	$P < 0.05$
ADG (g)	64.4 ± 1.95^{b}	62.9 ± 1.90^{b}	67.8 ± 1.38^{a}	$P < 0.05$
ADFI (g)	97.8 ± 3.66^{b}	103.1 ± 2.21^{a}	103.9 ± 2.44^{a}	$P < 0.05$
FCR	1.54 ± 0.02^{b}	1.63 ± 0.06^{a}	1.52 ± 0.03^{b}	$P < 0.05$

^{abc} Means in same row with no common superscript differ significantly ($P < 0.05$). CONT=Control; LME= Low energy; AZO-025= LME+ 0.25% Azomite. LBW= live body weight; ADG= average daily gain; ADFI= average daily feed intake; FCR; feed conversion ratio

Table 3: Effect of Azomite supplementation to low energy diet on nutrient digestibility in broiler chickens

Parameter	Control	LME	AZO - 0.25	*P*. Value
DM%	72.91 ± 0.23^{ab}	71.42 ± 0.09^{b}	74.40 ± 0.12^{a}	$P < 0.05$
CP%	66.66 ± 0.14^{ab}	64.86 ± 0.06^{b}	67.60 ± 0.14^{a}	$P < 0.05$
ME%	76.98 ± 0.20^{ab}	75.21 ± 0.11^{b}	78.25 ± 0.07^{a}	$P < 0.05$
Ash%	65.20 ± 0.04^{a}	62.40 ± 0.03^{a}	64.14 ± 0.03^{a}	$P > 0.05$
P%	45.55 ± 0.39^{a}	41.17 ± 0.25^{b}	49.75 ± 0.06^{a}	$P < 0.05$
Ca%	51.49 ± 0.39^{a}	47.78 ± 0.17^{b}	55.21 ± 0.05^{a}	$P < 0.05$

^{a,b,c} Means in same row with no common superscript differ significantly ($P < 0.05$). CONT= Control; LME= Low energy; AZO-025= LME+ 0.25% Azomite. DM= dry matter; CP= crude protein; ME= metabolizable energy; P= phosphorus; Ca= calcium

Olympus BX 43 digital microscope (Olympus Tokyo, Japan) and photographed using digital camera (eXcope T500). At least 10 intact well oriented crypts and villi were measured per section and used to calculate villus height (VH), crypt depth (CD).

Tibia bone analysis

Tibia were dissected from slaughtered birds at 42 days. 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 550°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 S.P.S.S. Statistics 19.0 Significant differences among means of treatments were compared with Tukey's test. The means and standard error of means are presented. The significant level is set at 5%.

Results

Growth performance

The results of growth performance in broilers fed low energy diet supplemented with AZO-0.25 are presented in Table 2. The LBW and ADG significantly ($P < 0.05$) higher in AZO-0.25 compare to CONT and LME treatment. Moreover, AZO-0.25 found significantly lower ($P < 0.05$) FCR than LME treatment, while lower ADFI recorded in CONT diet.

Nutrient digestibility

Table 3 shows that effect of supplementation of AZO-0.25 in low energy diets on digestibility of nutrients. Data revealed that digestibility of DM, CP, ME, P and Ca was significantly ($P < 0.05$) higher in AZO-0.25 treatment compared to LME. However, digestibility of ash (%) was showed non-significant ($P > 0.05$) difference among treatments.

Enzymes activity

The activity of intestinal enzymes supplemented with AZO-0.25 diet with low protein is presented in Table 4. There were no significant ($P > 0.05$) difference were observed among all treatments on activity of lipase, amylase and trypsin enzymes. However, activity of enzymes was

Table 4: Effect of Azomite on digestive enzymes activity in jejunum of broiler

Parameter	Control	LME	AZO-0.25	*P*. Value
Lipase U/mg	267.66 ± 63.92[a]	213.03 ± 6.33[a]	246.05 ± 61.44[a]	*P* > 0.05
Amylase U/mg	3.28 ± 1.19[a]	3.27 ± 0.68[a]	3.66 ± 0.22[a]	*P* > 0.05
Trypsin U/mg	168.38 ± 41.99[a]	133.11 ± 27.96[a]	175.13 ± 18.68[a]	*P* > 0.05

[abc] Means in same row with no common superscript differ significantly ($P < 0.05$). CONT=Control; LME= Low energy; AZO-025= LME+ 0.25% Azomite

Table 5: Effect of Azomite on intestinal morphology of broiler

Parameter	Control	LME	AZO - 0.25	*P*. Value
		Villus Height μm (VH)		
Duodenum	1594.4 ± 260.5[a]	1557 ± 171.4[a]	1682 ± 275.0[a]	*P* > 0.05
Jejunum	1423.0 ± 230.3[a]	1250 ± 250[a]	1288 ± 86.9[a]	*P* > 0.05
Ileum	1033 ± 125.3[a]	999 ± 181.0[a]	1092 ± 69.3[a]	*P* > 0.05
		Crypt Depth μm (CD)		
Duodenum	217 ± 18.7[a]	201 ± 23.8[a]	216 ± 23.3[a]	*P* > 0.05
Jejunum	186 ± 12.3[a]	157 ± 15.0[a]	176 ± 21.0[a]	*P* > 0.05
Ileum	173 ± 26.8[a]	149 ± 29.2[a]	167 ± 18.9[a]	*P* > 0.05
		Villus Height :Crypt Depth µm		
Duodenum	7.86 ± 0.28[a]	7.58 ± 0.79[a]	7.32 ± 0.53[a]	*P* > 0.05
Jejunum	7.911 ± 1.15[a]	7.63 ± 0.32[a]	7.82 ± 0.85[a]	*P* > 0.05
Ileum	6.20 ± 1.05[a]	6.05 ± 0.58[a]	6.30 ± 0.72[a]	*P* > 0.05

[a,b,c]Means in same row with no common superscript differ significantly ($P < 0.05$). CONT=Control; LME= Low energy; AZO-025= LME+ 0.25% Azomite.

Table 6: Effect of Azomite on bone mineralization of broiler

Parameter	CONT	LME	AZO-0.25	*P*. Value
TWT (g)	7.19 ± 0.82[a]	6.73 ± 0.64[a]	7.48 ± 0.71[a]	*P* > 0.05
TL (cm)	8.53 ± 1.02[ab]	7.52 ± 0.37[b]	8.83 ± 0.64[a]	*P* < 0.05
TD (cm)	0.84 ± 0.42[a]	0.78 ± 0.03[a]	0.84 ± 0.05[a]	*P* > 0.05
TBS (kg)	22.35 ± 32[ab]	19.41 ± 2.7[b]	25.94 ± 4.45[a]	*P* < 0.05
Ash %	48.5 ± 0.01[a]	45.26 ± 0.01[b]	49.21 ± 0.01[a]	*P* < 0.05
P %	7.64 ± 03[a]	6.82 ± 0.2[b]	8.23 ± 0.7[a]	*P* < 0.05
Ca %	16.8 ± 1.24[ab]	14.62 ± 1.13[b]	18.5 ± 3.15[a]	*P* < 0.05

[a,b,c] Means in same row with no common superscript differ significantly ($P < 0.05$). CONT=Control; LME= Low energy; AZO-025= LME+ 0.25% Azomite. TWT=tibia weight; TL=tibia length; TD=tibia diameter; TBS=tibia breaking strength; Ca=calcium; P=phosphorus

numerical higher in AZO-0.25 than that of birds fed with LME.

Intestinal morphology

As shown in Table 5, no significant ($P > 0.05$) difference observed on villus height and crypt depth of intestine among all treatments. However, villus height and crypt depth were numerically higher in AZO-0.25 treatment. No significant difference observed on VH/CD ratio among all treatments.

Bone parameters

The results presented in Table 6 noted that TL and TBS were significantly ($P < 0.05$) higher in birds fed AZO-0.25 compare LME diet, while TWT and TD found no significant difference among all treatments. Birds fed the AZO-0.25 diet had higher ($P < 0.05$) percentage of Ash, P and Ca compare to those fed the LME diet.

Discussion

The current study was designed to examine the dietary azomite added into low energy diet improves the growth performance of broiler chickens. To the best of our knowledge limited literature is currently available on the use of azomite in aqua culture species. However, only few academic reports are available on broiler chickens. The research findings of present study demonstrated that reduction of energy in broiler diet decreased the LBW, ADG and increased the FCR. However, supplementation of azomite with low energy in broiler diet enhanced LBW, ADG and lowered FCR, indicating the efficient utilization of feed. Batool *et al.* (2018) reported that supplementation of azomite in catfish diet increases the ADG and lower the FCR significantly. Similarly, Azam *et al*. (2016) found that significant improvement in BWG, ADG and FCR was reported when tilapia was fed a diet supplemented with azomite. Tan *et al*. (2014) also suggested that effect of the supplementing 0.2% azomite to the diet have great impact on growth performance and FCR in white shrimp due to the improvement in nutrient digestibility. These findings are agreed with results of current study. It has been suggested that azomite can improve the digestibility and nutrient utilization which can elevate the growth performance with low energy diets in broiler chickens. It seems that azomite might exert their action locally within the gastrointestinal tract, including effect on enterocyte, bacterial microflora as well as nutrient uptake, absorption and utilization, which may alleviate the negative effect of low energy on broiler growth performance Like growth performance, the better improvement in nutrient digestibility of DM, CP, ME, P and Ca was observed in broiler fed diets with azomite. Our research findings are well recognized in aquatic species. Fodge *et al.* (2011) reported that a supplementation level of azomite 0.25% significantly improved the digestibility of DM, and CP of tilapia fish. Remarkably, the positive impact of azomite on improvement in DM, CP, AME, P and Ca digestibility of nutrients in broiler chickens could be speculated by the improved activity of digestive enzymes. Azam *et al*. (2016) reported that supplementation of Azomite in tilapia fish improved the digestives enzymes activity. Tan *et al*. (2014) also revealed that supplementation of azomite improved the enzymes activities in stomach protease. The better absorption of nutrients depends upon the better morphology of intestine. Similarly, intestinal barrier integrity may improve the digestion and absorption ability in animals (Schmidt *et al*. 2007). Lumpkin *et al*. (2014) reported that Azomite supplementation in diet improves the villus height of broilers. Improving the VH may give verification for improvement in gut health and absorption of nutrients. Unfortunately, our study witnessed only numerical increase of VH and CD in birds fed with azomite supplementation.

The bone indices such as, bone weight, length, diameter, bone strength and ash content analyze the mineralization of bone in chickens (Onyango *et al*. 2003). Furthermore, the bone mineralization makes bone stronger which empowers the skeleton to withstand the gravity, addition loading and avert the leg abnormalities in broiler chickens (Shim *et al*. 2012). However, no study of azomite on bone parameters reported yet. The research findings of current study show that the supplementation of azomite with low energy to the broiler diet improved the tibia length, strength, ash, P and Ca percentage in broilers. Subsequently, azomite increased the utilization of minerals such as P and Ca which might led improving in tibia breaking strength and bone mineralization. From the above research findings, it is verified that azomite enhance the bone strength and mineralization. It linked obviously with significant increasing of availability of P and Ca by azomite addition.

Interestingly, the outcomes of present study showed that azomite with low energy diet have unique characteristics in terms of improving the growth performance in broilers and decreases the cost of feed. In general, improvement in growth performance and bone mineralization by enhancing the nutrient digestibility in GI tract of broiler chickens induced by dietary supplementation of azomite in a comparison with more or same beneficial effect of control diet. Azomite also numerical enhances the activity of enzymes without adverse effect on intestinal morphology in broiler chickens. Azomite had a positive effect on strength and mineralization of tibia bone was attributed to increase the retention of Ca and P in the tibia bones.

Conclusion

The low energy diet can negatively effect on growth performance, carcass, nutrient digestibility and bone development in broiler chickens, while supplemental azomite 0.25% with low energy diet had remarkably positive effect on growth performance and nutrient digestibility in broiler chickens. Inclusions of azomite with low energy diet improved the bone indicators in broilers. Therefore, azomite could be an effective supplement to reduce the energy level in broiler diet and the feed cost.

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