Effect of azomite on growth performance, nutrient utilization, serum biochemical index and bone mineralization of broilers fed low protein diet

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ABSTRACT
The present study investigated the effect of Azomite (AZO) on the growth performance, nutrient utilisation, and bone mineralisation of broilers fed low protein diet. A total number of 180 one-day-old male chicks were randomly distributed to three treatment groups having six replicates of 10 birds each. Experimental diets included; positive control (PC) with 21.5% CP (starter) and 19.0% CP (finisher), negative control (NC) with 2% lower CP than PC in each period and treatment group (AZO-0.25) in which NC was supplemented with 0.25% AZO. Azomite significantly (p < 0.05) improved live body weight, average daily gain and feed conversion ratio, but not the feed intake, as compared to NC. Eviscerated, breast muscle and leg muscle percentages were significantly (p < 0.05) higher in the birds fed AZO-0.25 than NC, while abdominal fat (AF) percentage was significantly (p > 0.05) higher in NC. Azomite improved the digestibility of dry matter (DM), crude protein (CP), apparent metabolisable energy (AME), calcium (Ca) and phosphorus (P) (p < 0.05) compared to the NC. Trypsin activity was increased (p < 0.05) in birds fed diet with AZO-0.25 compared with NC. Blood creatinine, growth hormone (GH) and calcitonin (CT) was also higher (p < 0.05) in birds supplemented with AZO-0.25. Treatment also affected (p < 0.05) the tibia breaking strength (TBS), ash, Ca and P contents. Overall, AZO improved the growth performance, nutrient utilisation and tibia mineralisation in broilers fed with low CP diet, which looks an economically promising approach.

HIGHLIGHTS
- The dietary supplementation of Azomite improved the growth and nutrient utilisation in broiler chicken.
- The efficiency of digestive enzymes and digestibility was enhanced by dietary Azomite.
- Dietary Azomite improved the tibia breaking strength and bone mineralisation in broiler chicken.

Introduction
In poultry nutrition, much attention is paid to protein sources, owing to importance of protein as a major constituent of biologically active compounds in the body. It is also principally involved in the synthesis of body tissues, repair and growth of the body. Furthermore, all enzymes and hormones which play key roles in the physiology of any living organisms are protein in nature (Beski et al. 2015). The reduction of protein content in the poultry diet not only reduces the feed price for economical poultry production but also minimises the potential for environmental pollution from nitrogen waste. Numbers of research studies have been conducted to establish the minimum level of crude protein (CP) that would support the optimum performance. However, conflicting results from these studies do not allow a clear conclusion on the effects of low protein diets in practical broiler production (Moran and Stilborn 1996), (Kamran et al. 2008; Hernandez et al. 2013; Yang et al. 2015). Reduction of 2 to 3% CP in the broiler diet resulted in decreased rate and efficiency of growth and poor quality of carcase traits even when diets fulfilled with all other nutrient requirements (Bregendahl et al.
acronym which stands for trace elements (Awad et al. 2017) and nitrogen retention on the digestibility (Temim et al. 1999), morphology of intestinal villi (Laudadio et al. 2012), protein metabolism (Awad et al. 2017) and nitrogen retention (Nahm 2002).

The large portion of dietary nitrogen intake is not retained by the animal body but excreted in the environment which causes the environmental pollution. Reducing the protein content of diet could therefore be utilised as a tool to minimise the excretion of nitrogen and ammonia from poultry houses (Ferguson et al. 1998; Khajali and Moghaddam 2006; Namroud et al. 2008). Therefore, scientists are paying more attention to reduce the environmental pollution without compromising the growth performance of broiler. In recent years, poultry industry has focussed on the use of synthetic amino acids (Nukreaw and Bunchasak 2015) and several other feed additives like exogenous enzymes (Nabizadeh et al. 2017) phytogenic substances (Paraskeuas et al. 2016; Arain et al. 2018; Saeed et al. 2018; Arif et al. 2019) and mineral supplementation (Nabi, Arain, et al. 2020) to improve the production performance of poultry birds. Although, fast growth rate may cause metabolic disorders such as high incidence of skeletal disorders and increased fat deposition (Yagoub and Babiker 2008; Nabi et al. 2018) which raises major concern for both producers and consumers. Excessive body fat deposition in broiler results in poor energy metabolism and overall feed utilisation (Pasternak and Shalev 1983; Saeed et al. 2017) which results in the economic losses for producers. Therefore, low protein diet can be supplemented with suitable natural feed additives to reduce the economic losses without compromising the performance of broilers.

Recent research developments have expanded the physiological and economic benefits of dietary inclusion of aluminosilicates, particularly with respect to hydrated sodium calcium aluminosilicates (HSCAS). Azomite® (AZO) is a product marketed as a hydrated sodium calcium aluminosilicate that is comprised of trace minerals and rare earth elements. Azomite® is an acronym which stands for ‘A to Z of minerals including trace elements’. Although, AZO has been used in agriculture for over 70 years, there are limited scientific studies on the use of this product in animal nutrition. Recently, AZO has got attention as a mineral booster and natural feed additive in poultry and aquaculture to improve the growth performance and digestibility of nutrients. It is a mixture of residues from animals and plants along with minerals, containing more than 70 trace and other minerals (Fodge and Fodge 2014). Some previous studies reported that dietary supplementation of AZO in tilapia (Oreochromis niloticus X Oreochromis aureus) increased the growth performance, feed efficiency, nutrient digestibility, and digestive enzyme activity (Liu et al. 2009; Batool et al. 2018). In addition, efficiency of nutrient utilisation, digestive enzymes activity and immunity was improved in white shrimp (Tan et al. 2014) and grass carp (Ctenopharyngodon idellus) supplemented with AZO (Liu et al. 2011). According to a recent study (Pirzado et al. 2020), AZO in conjunction with a low-energy diet exhibited positive effects on the growth performance, bone parameters, and nutrient digestibility in broilers. Furthermore, supplementation of AZO at 0.25 and 0.50% in the diet of broiler improved the growth performance, immune functions and tibia breaking strength (Pirzado et al. 2021).

Azomite possesses a number of potential benefits like improving the growth performance, feed utilisation while reducing the environmental pollution. Although, the dietary inclusion of AZO may provide potential benefits as observed previously with the individual addition of aluminosilicate clays or REE’s in poultry production, there is limited research on exploring the utilisation of AZO in poultry. Keeping in view of potential advantages of AZO, this study was aimed to evaluate the effect of AZO on the growth performance, nutrient utilisation and bone mineralisation in broilers fed with a low protein diet.

Materials and methods

**Birds, treatments and management conditions**

A total of 180 one-day-old broiler chicks (Ross 308) were purchased from a commercial hatchery (Beijing Huatu Broiler Company Limited) and randomly allocated to three treatment groups having six replicates of 10 birds each. The birds were kept in solid floor pens covered with wood shavings and managed under recommended temperature (32°C during 1st week and the gradual decrease by 2°C each week until it reached the 22°C) and relative humidity (55 to 65%). Up to day 7, the broilers were subjected to light schedule of 23 h light and one hour darkness. From day 7, the birds were subjected to gradually decline in light schedule (one hour/week) and fixed at 18 h light and six hours darkness following the European Union standards on the protection of animals used for scientific purposes. Fresh drinking water and feed were provided ad libitum to all birds during the entire experimental period. The experiment was conducted...
in two phases: starter (1–21) and finisher phase (22–42). Three basal diets were prepared for the study, positive control comprising 21.5% CP (starter) and 19.0% CP (finisher), negative control with 2% age unit CP lower in each phase and in AZO-0.25 than PC, (Table 1). Azomite was purchased from a commercial company (Taiwan Lytton Company, New Taipei City Taiwan) and was mixed in feed mill at a concentration of 0.25%. The detailed composition of AZO is presented in Table 2. The basal diet was in mash form and formulated to meet the nutritional requirements of 1 to 6 week-old broiler chicks.

Table 1. Ingredient composition of experimental basal diet.

| Ingredients% | Positive control (Starter) | Finisher | Negative control (Starter) | Finisher | AZO-0.25 (Starter) | Finisher |
|--------------|----------------------------|----------|----------------------------|----------|--------------------|----------|
| Corn         | 57.47                      | 59.47    | 63.94                      | 66.24    | 63.94              | 66.24    |
| Soy bean Oil | 1.50                       | 4.32     | 0.52                       | 3.45     | 0.52               | 3.45     |
| Soy bean     | 30.96                      | 25.05    | 25.51                      | 19.1     | 25.51              | 19.1     |
| CSM          | 5.00                       | 7.00     | 5.00                       | 7.00     | 5.00               | 7.00     |
| Salt         | 0.35                       | 0.35     | 0.35                       | 0.35     | 0.30               | 0.30     |
| CaHPO4       | 1.53                       | 1.39     | 1.50                       | 1.40     | 1.50               | 1.40     |
| Lime stone   | 1.54                       | 1.40     | 1.61                       | 1.44     | 1.51               | 1.34     |
| Lys          | 0.24                       | 0.22     | 0.21                       | 0.26     | 0.21               | 0.26     |
| Met          | 0.14                       | 0.15     | 0.11                       | 0.13     | 0.11               | 0.13     |
| Cys          | 0.07                       | 0.04     | 0.05                       | 0.02     | 0.05               | 0.02     |
| Chol         | 0.20                       | 0.01     | 0.20                       | 0.01     | 0.20               | 0.01     |
| Premix*      | 0.50                       | 0.10     | 0.50                       | 0.10     | 0.50               | 0.10     |
| Zeolite      | 0.50                       | 0.50     | 0.50                       | 0.50     | 0.40               | 0.40     |
| Azomite      | 0.25                       | 0.25     | 0.25                       | 0.25     |                    |          |
| Total        | 100                        | 100      | 100                        | 100      | 100                | 100      |
| Nutritional value of diet | | | | | | |
| ME (kJ/kg)   | 12342.8                    | 12761.2  | 12342.8                    | 12761.2  | 12342.8            | 12761.2  |
| Protein (%)  | 21.50                      | 19.00    | 19.50                      | 17.00    | 19.50             | 17.00    |
| Lys (%)      | 1.200                      | 1.050    | 1.050                      | 0.950    | 1.050             | 0.950    |
| Met (%)      | 0.450                      | 0.440    | 0.400                      | 0.400    | 0.400             | 0.400    |
| Met + Cys (%)| 0.900                      | 0.800    | 0.800                      | 0.710    | 0.800             | 0.710    |
| Thr (%)      | 0.866                      | 0.724    | 0.778                      | 0.640    | 0.778             | 0.640    |
| Trp (%)      | 0.311                      | 0.248    | 0.279                      | 0.212    | 0.279             | 0.212    |
| Ca (%)       | 0.990                      | 0.904    | 0.990                      | 0.906    | 0.987             | 0.898    |
| Total P (%)  | 0.679                      | 0.669    | 0.661                      | 0.61     | 0.661             | 0.61     |
| Avail P (%)  | 0.456                      | 0.552    | 0.451                      | 0.549    | 0.451             | 0.549    |

*The premix provided (for 1 kg of diets) VA 10000 IU, 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) 0.0.35 mg and Se (as sodium selenite) 0.15 mg.

Recording of data

On days 21 and 42 of the experiment, live body weight (LBW) and feed consumption were recorded for each replicate of the treatment groups. The average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) was calculated. At the end of experimental trial (on day 42) two birds from each replicate were slaughtered to measure the eviscerated, breast muscle, leg muscle, abdominal fat and immune organ weights.

Nutrient digestibility

Titanium Oxide was used as an indigestible marker for determining the digestibility of nutrients. Faeces were collected from days 39–41 and composited to get a representative sample. The faeces were dried at 65°C for 72 hrs, ground and then passed through 0.40 mm screen. Nutrient analysis of faeces and basal diet was conducted. Dry matter and ash were determined according to the methods described by AOAC (2000). Crude protein (Dumatherm, Gerhardt, Germany), Gross Energy (GE) through calorimeter (Oxygen Bomb

Table 2. Mineral analysis of Azomite (provided by the Taiwan Lytton Company China).

| Mineral                  | g/100g/mg/kg |
|--------------------------|-------------|
| Calcium oxide            | 5.17 g/100g |
| Potassium oxide          | 5.18 g/100g |
| Sodium oxide             | 2 g/100g    |
| Magnesium oxide          | 0.78 g/100g |
| Sulfur trioxide          | 0.21 g/100g |
| Ferric oxide / iron      | 1.37 g/100g |
| Manganese oxide          | 0.02 g/100g |
| Zinc                     | 64.3 mg/kg  |
| Cobalt                   | 22.3 mg/kg  |
| Copper                   | 13.5 mg/kg  |
| Molybdenum               | 12.6 mg/kg  |
| Fluorine                 | 320 mg/kg   |
| Lithium                  | 859 mg/kg   |
| Boron                    | 29 mg/kg    |
| Chromium                 | 7.85 mg/kg  |
| Lanthanum                | 257 mg/kg   |
| Cerium                   | 360 mg/kg   |
| Cerium                   | 360 mg/kg   |
| Praseodymium             | 26.75 mg/kg |
| Tungsten                 | 31.00 mg/kg |
| Vanadium                 | 9.35 mg/kg  |
| Nickel                   | 2.27 mg/kg  |
| Tin                      | 2.90 mg/kg  |
Calorimeter, C 2000 Version, IKA, Germany) while Ca and P were analysed by using atomic absorption spectrometer (novAA 400 P, analytikjena, Germany) and UV-VIS Spectrophotometer (Model: 1780, Shimadzu, Japan), respectively. The contents of TiO₂ in diets and faeces were determined according to the method described previously (Short et al. 1996). The apparent digestibility of nutrients was calculated using following formula:

\[
\text{Apparent digestibility of nutrients} = 1 - \left( \frac{\text{Titanium g/g in faeces}}{\text{Titanium g/g in feed}} \right) \times \left( \frac{\text{Nutrient g/g in faeces}}{\text{Nutrient g/g in feed}} \right) \times 100
\]

**Intestinal enzymes activity**

The digesta was collected from jejunum and stored in liquid nitrogen. The enzymatic activities of lipase, amylase and trypsin were analysed using the commercial kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**Blood biochemical indices**

At the end of the experimental trial blood samples (5 ml each) from subcutaneous veins were collected in anticoagulant labelled vacutainers from two birds per replicate for serum biochemical analysis. The serum was harvested and centrifuged at 3000 rpm for 15 min at 4°C and stored at −20°C until analysis. The contents of total cholesterol (TC), creatinine (CREAT), uric acid (UA), glucose (GLU) and total protein (TP), were analysed by using an automated IDEEX Vet Test Chemistry Analyser (IDEEX Laboratories, Inc). The concentrations of immunoglobulins, IgA, IgG and IgM in plasma were analysed using specific ELISA kits instructions (Shang Hai Lengton Biosciences Co., LTD, Shang Hai, China). The serum concentration of growth hormone (GH), Parathyroid Hormone (PTH) and calcitonin (CT) were determined by immunoradiometric assay method, and gamma counter (Bio Source International, Camarillo, CA), by using commercial kits as per manufacturer instructions (Immutopics, Inc., San Clemente, CA).

**Tibia sampling**

On day 42, tibial bones from two birds per replicate were isolated from the skin, muscle, and soft tissues of slaughtered birds, dried and then stored at 4°C. The tibial biomorphometric measurement including weight, length and diameter were done using weighing balance, ruler, and Vernier calliper, respectively. The diameter was measured at the narrowest and widest points and then averaged. The bone breaking strength was determined using TA xT2i Texture Analyser (Stable Micro Systems, London, UK). After the measurement of bone strength, the broken tibia was placed in plastic bags for the determination of ash, Ca and P. Tibia bone samples were defatted with ethanol and diethyl ether for 48 hrs. The samples were dried in the oven at 100°C for 24 hr then weighed and burnt in muffle furnace at 600°C for 16 hrs. Ash was dissolved in 10 ml of hydrochloric acid (HCl) and 5 ml of nitric acid (HNO₃). Digested samples were filtered and diluted with deionised water to the required volume and analysed for Ca and P, using Atomic Absorption Spectrometer (novAA 400 P, analytikjena, Germany) and UV-VIS Spectrophotometer (Model: 1780, Shimadzu, Japan) respectively.

**Statistical analysis**

Data for different parameters were tested for homogeneity of the variances using Levene’s test (Levene, 1960) before statistical analysis. After that data were statistically analysed using two-way analysis of variance (ANOVA) using SPSS software (SPSS, 19.0). The significant differences among means of treatments were compared by Tukey’s test. The results were considered significant at \( p < .05 \).

**Results**

**Growth performance**

Dietary supplementation with AZO exhibited a positive effect on growth performance in broilers (Table 3). Birds fed diet supplemented with AZO had higher \((p < .05)\) LBW and ADG compared with NC diet in both starter and finisher phases, whereas, no significant difference was observed between PC and AZO. The ADFI was not affected by the treatment in the starter phase; however, it was lowest \((p < .05)\) in PC treatment in finisher phase and overall periods. Moreover, FCR was higher \((p < .05)\) in birds on NC and the lowest on AZO diet.

**Carcass traits**

Birds given AZO increased \((p < .05)\) the eviscerated yield, breast muscle, leg muscle and abdominal fat
Table 3. Effect of Azomite on growth performance in broiler chickens fed low protein diet.

| Parameters                  | PC              | NC              | AZO - 0.25 | SEM  | P Value |
|-----------------------------|-----------------|-----------------|------------|------|---------|
| **Starter 1–21 day**        |                 |                 |            |      |         |
| One-day-old Weight          | 41.45 ±         | 41.44 ±         | 41.40 ±    | 0.02 | 0.989   |
| LBW (g)                     | 938 ±a          | 906 ±b          | 957 ±      | 1.29 | 0.043   |
| ADG (g)                     | 44.69 ±a,b      | 43.16 ±b        | 45.57 ±    | 0.62 | 0.046   |
| ADFI (g)                    | 58.62 ±         | 56.75 ±         | 59.36 ±    | 0.58 | 0.463   |
| FCR                         | 1.31 ±b         | 1.36 ±          | 1.30 ±     | 0.09 | 0.006   |
| **Finisher 1–42 day**       |                 |                 |            |      |         |
| LBW (g)                     | 2735 ±a         | 2565 ±b         | 2790 ±     | 12.9 | 0.001   |
| ADG (g)                     | 65.11 ±         | 61.07 ±b        | 66.44 ±    | 0.87 | 0.008   |
| ADFI (g)                    | 97.77 ±a,b      | 101.61 ±a       | 101.86 ±   | 0.84 | 0.006   |
| FCR                         | 1.54 ±          | 1.66 ±b         | 1.53 ±     | 0.01 | 0.002   |

a,b,c Means in same row with no common superscript differ significantly (p < .05).

PC: Positive Control; NC: Negative Control; AZO-0.25: Azomite 0.25%; LBW: live body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio.

Nutrient digestibility

The dietary supplementation of AZO in low CP diet significantly increased the digestibility of DM, Ca (p < .05), CP, AME and P (p < .01) compared to NC treatment, but no difference was observed in comparison with PC diet (Table 5). However, no effect of treatment was observed on the digestibility of ash.

Enzymatic activity

No effect of AZO supplementation was observed on the activities of lipase and amylase enzymes (Table 6). However, AZO enhanced (p < .05) the activity of trypsin enzyme as compared to NC but no difference was observed when compared with PC diet.

Blood biochemical indices

Azomite supplementation had no effect (p > .05) on total cholesterol (TC), uric acid (UA), glucose (Gl), immunoglobulin G, A, and M (IgG IgA, IgM) however, AZO supplementation enhanced (p < .05) the serum creatinine (CREAT), growth hormone (GH) and calcitonin (CT) contents compared to NC (Table 7).

Tibia parameters

Azomite supplementation had no effect (p > .05) on tibia weight (TWT), tibia length (TL) and tibia diameter (TD). Dietary supplementation of AZO resulted in an increase in tibia breaking strength (TBS) and Ca content (p < .05) compared to NC, but no difference was noted in comparison with PC. However, ash and P...
content were higher ($p < .05$) in the AZO than other two groups (Table 8).

**Discussion**

Azomite has been recognised as a mineral booster and feed additive for promoting the growth performance and nutrient utilisation by improving the digestive functions in aquaculture farming (Liu et al. 2011; Batool et al. 2018). Azomite may be acting via multiple mechanisms including toxin binding, nutrient allowance, or the provision of some other benefit due to its distinct composition of hydrated sodium calcium aluminosilicate, rare earth minerals, and other trace elements. The current study provides new insights into the use of AZO with low protein diet in broiler chicken. To the best of our knowledge, there is limited information available regarding the potential use of AZO in broiler diet and its effects on the growth performance and nutrient utilisation. In the present study, reduction of dietary CP in broiler diet showed negative effects on LBW, ADG and FCR, however, Azomite supplementation ameliorated these negative effects and exhibited similar or even better growth performance compared to standard diets (PC). It is well established that reduction of dietary CP levels in broiler diet decreases the growth performance (Awad et al. 2015; Rehman et al. 2018). Our findings are in agreement with earlier studies as NC in our study (low CP diet) resulted in poor growth performance as compared to PC (diet with recommended CP levels). Another study demonstrated that the broilers fed with low protein diets (containing 1% low CP than the recommended level), at constant ME, with the same amino acids levels did not adversely affect growth performance, carcass parameters and liver functions, while dietary crude protein and crude fibre utilisation were improved significantly (Salah 2016). Moreover, our findings revealed that AZO supplementation can increase the growth performance. Earlier study (Batool et al. 2018) has reported that supplementation of AZO improved the weight gain and FCR in catfish (*Pangasius hypophthalmus*). Previous studies also indicated improved growth performance and FCR that was attributed to the enhanced digestibility and metabolism of nutrients in response to AZO supplementation in the diet (Fodge et al., 2011; Azam et al., 2016).

Broiler fed low protein diet had lower eviscerated yield, breast muscle and leg muscle percentage, while abdominal fat was higher in birds fed with low CP diet in the present study. Similar findings have been reported earlier in broilers fed with low CP diet had higher fat percentage (Namroud et al. 2008; Abudabos 2012). This may be due to the higher energy ratio with low protein diet that results in insufficient energy metabolism and overall nutrient utilisation. Interestingly, our study indicated that Azomite supplementation significantly increased the percentage of eviscerated yield, breast muscle and leg muscle. These findings verified that AZO supplementation can significantly increase the breast muscle percentage in broiler chicken (Emerson and Hooge 2008). Moreover, AZO has also shown to stimulate the muscle mass of catfish (*Pangasius hypophthalmus*) previously (Batool et al. 2018). The improvement in carcase performance observed with AZO supplementation is mainly attributed to the increased digestibility, absorption and utilisation of nutrients as revealed by higher digestibility of DM, CP and ME in supplemented birds as compared to birds fed low protein diet. Moreover, supplementation of AZO in a low CP diet also enhanced the digestibility of Ca and P in the present study. These findings agree with previous study which reported that dietary AZO increased the digestibility of DM and CP in tilapia fish (Fodge and Fodge 2011).

It is suggested that improvements in nutrient digestibility may be attributed to the increased activity of digestive enzymes. Very few studies have evaluated the effect of AZO on the activity of digestive enzymes. In the present study, AZO supplementation in a low CP diet improved the trypsin activity in broiler. Our findings are in agreement with earlier studies which reported an improvement in the trypsin and amylase activity in shrimp and Tilapia fish in response to AZO supplementation (Fodge and Fodge 2014; Tan et al. 2014). These findings collectively suggest that the use of AZO in diet can enhance the activity of digestive enzymes and nutrient uptake in the small intestine. The increased secretion of digestive enzymes (mainly trypsin) seemed to be one of the primary mechanisms

### Table 8. Effect of Azomite on tibia breaking strength, Ash, P and Ca contents% in broiler chickens fed low protein diet.

| Parameter | PC | NC | AZO - 0.25 | SEM | $P$ Value |
|-----------|----|----|------------|-----|----------|
| TWT (g)   | 7.51 | 6.73 | 7.76      | 0.20 | 0.082    |
| TL (cm)   | 8.77 | 7.68 | 9.02      | 0.26 | 0.091    |
| TD (cm)   | 0.86 | 0.79 | 0.87      | 0.01 | 0.106    |
| TBS (kg)  | 22.45 | 19.57 | 24.65 | 1.17 | 0.033    |
| Ash%      | 47.88 | 46.65 | 50.18 | 0.04 | 0.003    |
| P%        | 7.72 | 7.41 | 8.56      | 0.17 | 0.007    |
| Ca%       | 17.22 | 13.75 | 18.23 | 0.78 | 0.031    |

$a,b,c$Means in same row with no common superscript differ significantly ($p < .05$). PC: Positive Control; NC: Negative Control; AZO-0.25: Azomite 0.25%; TWT: tibia weight; TL: tibia length; TD: tibia diameter; TBS: tibia breaking strength; Ca: calcium; P: phosphorus.
by which AZO enhances the nutrient digestion and absorption in the present study.

Serum indices are critical indicators to monitor the health, diagnosis and treatment of disease and also determine the nutritional status of birds (Schmidt et al. 2007). Serum biochemical indicators of birds in the present study were within the normal physiological range for chicken. However, CREAT concentration was decreased in birds fed low CP diet. These results are in agreement with previous study reporting significant decrease in the CREAT level in broilers fed with a low CP diet (Arczewska-Włosek et al. 2018). However, CREAT level was increased in response to AZO supplementation. It is well established that CREAT is a product of CREAT phosphate in the muscles tissues and its production is associated with muscle mass (Wyss and Kaddurah-Daouk 2000; Rajman et al. 2006). Hence, increased muscle mass observed in the present study may be attributed to the enhanced level of CREAT in broiler.

Moreover, increase observed in the serum TP in response to the AZO supplementation is also in agreement with earlier study reported higher serum TP in AZO supplemented koi carp fingerlings (Jaleel et al., 2015). These findings may be attributed to enhanced digestion, absorption, and utilisation of CP in the gastrointestinal tract (GIT) of broiler chicken. In addition, serum GH and CT levels were increased by AZO supplementation in the present study. The enhanced secretion of GH in the blood is directly associated with the accelerated protein synthesis and higher fat breakdown to release the dietary energy necessary for the tissue growth. Growth hormone is considered as an important regulator of the bone size, muscle mass and body growth (Ohlsson et al. 1998). The improvement in CT level is generally indicated as an available uptake and storage of dietary calcium, which positively influences the metabolic pathway of the bone formation (Talmage and Grubb 1977). The potential effect of AZO on serum CT concentration observed in the present study might enhance the Ca metabolism subsequently leading to inhibit the osteoclast (bone resorption) while activating the bone forming osteoblasts that ultimately improves the Ca deposition in bones. As CT improves the Ca and P deposition in the bone but stops bone resorption, the net effect of CT is the enhanced level of Ca and P in the bone with a concomitant increase of these minerals by absorption in the blood (Frandson and Spurgeon 1992).

Effect of AZO on bone mineralisation is not reported yet. In the present study, AZO increased the tibia breaking strength, ash, P and Ca percentage in the tibia of birds as compared to NC. The positive effect of AZO might probably be attributed to an increased digestion and absorption of Ca and P in the bone, which led to the increased tibia breaking strength and mineralisation. These findings are in agreement earlier studies reporting that breaking strength and ash content of bones are associated with bone mineralisation in chicken (Onyango et al. 2003). Moreover, the bone mineralisation makes bones harder which enables the skeleton to withstand the gravity, additional loading and prevent the leg deformities in broilers (Shim et al., 2012). Another study demonstrated that inclusion of organic mineral mixture (Mn, Zn, and Cu) enhanced the reproductive performance, egg shell quality, plasma profile, yolk mineral concentration, yolk lipid oxidation, and immune response in laying hens under high ambient temperature (Saleh et al. 2020). In the present study AZO seems to possess sufficient potential for dietary supplementation in low CP diets in broiler chicken to address problems associated with low CP rations. Overall, our findings indicated an improvement in the growth performance, nutrient utilisation, and bone mineralisation in response to AZO supplementation of a low protein diet, which is similar or even better in comparison with standard diet.

Conclusion

Azomite supplementation in a low protein diet significantly enhanced the growth performance, nutrient utilisation and trypsin activity in broilers. Azomite also improved the tibia breaking strength, Ca and P contents of the tibia bone. Therefore, it is suggested that AZO supplementation at 0.25% of the diet is enough to entirely alleviate the negative impact of low protein diet in broilers. Reducing the CP contents of the diet with AZO supplementation seems a promising approach to lower feed cost while improving the growth performance and nutrient utilisation in broilers diet to harvest more economic returns.

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Ethical approval

This animal study (short title: Azomite in poultry nutrition) was carried out in strict accordance with the
recommen-dation of the ethical committee of Graduate School of Chinese Academy of Agricultural Sciences, Beijing P.R. China, with the approval number (IACUC#AECAAS-FRI-CAAS20191029). All procedures and experiments comply with the guideline and were approved by the local ethic committee of the Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing China with respect to animal experimentation and care of animals under study, and all efforts were made to minimise suffering.

**Disclosure statement**

The authors of this manuscript declare that there is no conflict of interests that could possibly arise.

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