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Effects of Physical Size of Clinoptilolite on Growth Performance, Serum Biochemical Parameters and Litter Quality of Broiler Chickens in the Growing Phase

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Abstract

A total of 448 Ross 308 male broiler chickens were assigned to seven treatments with four replicates, each containing 16 chicks. The birds were reared from d 21 to 42 and effects of physical sizes (<0.25 mm, 0.4- 0.8 mm and 1- 2 mm) and levels of clinoptilolite (1.5 and 3%) were examined. They were fed *ad libitum* by a basal commercial broiler diet with 3050 Kcal/Kg ME and 19.06% CP/Kg from 21 to 42 d. The chickens which received clinoptilolite (1.5% with particle size of 0.4- 0.8 mm) showed a significant increase in body weight gain compared to the control group birds receiving no clinoptilolite (P<0.05). Adding clinoptilolite to the diet caused a significant improvement in feed conversion ratio in days 21-28, 28-35, 35-42 and 21-42 (P<0.05). Broilers fed 1.5% clinoptilolite with particle size of 0.4- 0.8 mm had lower feed conversion ratio compared to the other groups. The highest level of blood glucose, total protein and albumin were found in birds receiving 1.5% clinoptilolite with the particle size of 0.4- 0.8 mm, while the lowest level of blood glucose, total protein and albumin were obtained in the control groups (P<0.05). The lowest litter moisture was observed in the groups fed with 3% clinoptilolite with a particle size of 1-2 mm. Therefore, it can be concluded that supplementation of the diet with clinoptilolite has positive effects on performance and litter moisture in broilers.

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Introduction

The use of natural zeolites has recently developed as a feed additive in poultry diets (Olver, 1989). Clinoptilolite is probably the most common zeolite in volcanic sediments (Bernal and Lopez-Real, 1993). Zeolites are porous materials, characterized by the ability to lose and gain water reversibly and to exchange their constituent cations without major change of their structure (Mumpton and Fishman, 1977). Over 45 distinct natural kinds of zeolite are known and more than 100 species having no natural counterparts have been synthesized. The chemical formula of pure clinoptilolite is CaNa $(AlO_2)6$ $(SiO_2)30$-24 H_2O (Olver, 1997).

Zeolites are used for various applications including adsorbents, ion exchangers and catalysts in industry, agriculture, veterinary medicine, sanitation and environmental protection (Martin-Kleiner *et al*., 2001). Biological applications include the removal of ammonia from waste water, air filtration and deodoration, soil amelioration and fertilization (Bernal and Lopez-Real, 1993).

Many experiments showed that the dietary inclusion of zeolites improves average daily gain and feed conversion ratio in pigs (Papaioannou *et al*., 2004), calves (Mumpton and Fishman, 1977), sheep (Pond and Lee, 1984), and broilers (Suchy *et al*., 2006; Evans *et al*., 2005). The wide ranges of applications of zeolites are based on their physicochemical properties. Type of the zeolite, purity and the supplementation level used in the diets are the important factors that affect its effectiveness (Utlu *et al*., 2007). Besides, the particle size of the zeolitic material, crystallite size and the degree of aggregation, as well as the porosity of individual particles determine the access of ingesta fluids to the zeolitic surface during passage across gastrointestinal tract and strongly affect its ion exchange, absorption and catalytic properties (Papaioannou *et al*., 2005).

During the application of clinoptilolite as a feed supplement, the utilization of feed nutrients increases (Olver, 1997). Clinoptilolite positively affects the digestive mechanism and is involved in the elimination of heavy metals (Tepe *et al*., 2004). Mumpton and Fishman, (1977) reported that both the ion exchange and absorption properties of natural zeolites make far more efficient use of dietary nitrogen by animals, reduce intestinal disease in pigs and ruminants and control the moisture content of manure.

Unfortunately, there is no comprehensive information on the effect of physical size of clinoptilolite on boiler performance. Therefore, the aim of this study was to investigate the impact of physical size and the level of clinoptilolite on growth performance, serum biochemical parameters and litter quality of broiler chickens in the growing phase.

Materials and Methods

Bird husbandry and diets

This experiment was conducted in a completely randomized design. Four hundred frothy eight broiler chickens were randomly allocated to seven dietary treatments. The treatments included: control group (no clinoptilolite) besides various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4- 0.8 mm and 1- 2 mm) of clinoptilolite. Clinoptiolite treatments consist of: A) 1.5% clinoptilolite with the particle size of <0.25 mm, B) 1.5% clinoptilolite with the particle size of 0.4- 0.8 mm, C) 1.5% clinoptilolite with the particle size of 1-2 mm, D) 3% clinoptilolite with the particle size of <0.25 mm, E) 3% clinoptilolite with the particle size of 0.4- 0.8 mm, F) 3% clinoptilolite with the particle size of 1-2 mm. Dietary treatments offered to broiler chickens in the growing phase from d 21 to 42. Each treatment had four replicates with 16 birds per pens. The size of each pen was 120×150 cm.

Each pen was equipped with bell-drinker and a feeder. The experimental diets were formulated to meet the minimum nutrient requirements of broilers, as established by the National Research Council (NRC, 1994). The composition and the calculated nutrient content of the experimental diets are presented in Table 1. Experimental diets (in mash form) and water were provided ad libitum. A continuous lighting program was provided during the experimental period.

Table 1. Ingredients and main nutrients composition of basal diet for growing periods (as fed basis)

Ingredients	Amount (%)
Corn	61.56
Soybean meal (44% CP)	32.06
Soybean oil	3.03
Dicalcium phosphate	1.04
Limestone	1.38
Salt	0.32
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
DL-methionine	0.06
Salinomycin	0.05
Calculated composition	
Metabolizable energy (Kcal/Kg)	3050
Crude protein (%)	19.06
Lysine (%)	1.02
Methionine (%)	0.37
Methionine+ cystine (%)	0.69
Calcium (%)	0.86
Available phosphorus (%)	0.33
Sodium (%)	0.14

[1]Each kg of vitamin premix contained: Vitamin A, 3,500,000 IU; Vitamin D_3, 1,000,000 IU; Vitamin E, 9000 IU; Vitamin K_3, 1000 mg; Vitamin B_1, 900 mg; Vitamin B_2, 3,300 mg; Vitamin B_3, 5,000 mg; Vitamin B_5, 15,000 mg; Vitamin B_6, 150 mg; Vitamin B_9, 500 mg; Vitamin B_{12}, 7.5 mg; Biotin, 500 mg; Choline chloride, 250,000 mg.

[2]Each kg of mineral premix contained: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg; Se, 100 mg.

Growth performance measurements

Performance data were recorded in the periods of d 21 to 28, 28 to 35, 35 to 42 and 21 to 42. Feed intake (FI) was determined for each repetition as the difference between the amount of feed supplied and the remaining feed at the end of each experimental period, and body weight gain (BWG) was calculated as the difference between the final and initial bird weights. Feed conversion ratio (FCR) was determined as the ratio between feed intake and weight gain at each phase of the experimental period.

Serum biochemical parameters

In d 42, 2mL of blood was collected via the wing vein from 2 birds in each treatment, blood samples were centrifuged (2,000 ×g for 10 min) and serum was separated and then stored at -20°C until assayed for measuring biochemical parameters (glucose, cholesterol, triglyceride, total protein, albumin, alkaline phosphatase and aspartate amino transferase) using appropriate laboratory kits (Pars Azmoon, Tehran, Iran) (Gowenlock *et al.*, 1988).

Litter quality

The litter samples were collected in nylon bags and refrigerated. Moisture content was determined by weighing samples before and after placing in an oven at 103°C for 24 hrs (AOAC, 1990). The pH was measured by using a digital pH-meter (Elster Microcomputer, Germany) in aqueous extract, obtained by mechanically shaking the samples with distilled water at a solid:water ratio of 1:5 for 1 h (FCQAO, 1994). Total nitrogen content of litter was analyzed following the Kjeldhal method (APHA, 2005).

Statistical analysis

The data obtained from the experiment were analyzed using SAS (SAS Institute, 1999) statistical programs with the ANOVA. Significant differences among treatment means were separated using Duncan's multiple range test in a 5% probability level (Duncan, 1955).

Results

Growth performance

Data presented in Table 2 show the effects of clinoptilolite on body weight gain, feed intake and feed conversion ratio of broilers. Treatments affected body weight gain in days of 21-28, 28-35, 35-42 and 21-42, so that, chickens given clinoptilolite (1.5% with particle size of 0.4- 0.8 mm) showed a significant increase in body weight gain compared to control groups ($P<0.05$). No significant differences were observed among treatments with regard to feed intake in different periods ($P>0.05$). Adding clinoptilolite to the diet caused a significant improvement in feed conversion ratio in days of 21-28, 28-35, 35-42 and 21-42 ($P<0.05$). Broiler chickens fed by 1.5% clinoptilolite with the particle size of 0.4-0.8 mm had lower feed conversion ratio compared to the other groups.

Serum biochemical parameters

The effect of clinoptilolite on serum biochemical parameters are shown in Table 3. The effect of treatments on bood parameters such as glucose, total protein and albumin were significant (P<0.05). The level of blood glucose, total protein and albumin were high in birds receiving 1.5% clinoptilolite with particle size of 0.4-0.8 mm compared to the control group (P<0.05). The effect of treatments on other serum biochemical parameters including, cholesterol, triglyceride, alkaline phosphatase and aspartate amino transferase were not significant (P>0.05).

Litter quality

The litter quality in different treatments is presented in Table 4. The litter moisture in the groups fed 3% clinoptilolite with the 0.4 to 0.8 mm particle size was significantly lower than the control groups (P<0.05), but pH level and the amount of nitrogen were not significantly influenced by the treatments (P>0.05).

Table 2. Effect of clinoptilolite on broiler chickens performance in growing phase

Treatments	BWG(g)				FI (g)				FCR			
	21-28d	28-35d	35-42d	21-42d	21-28d	28-35d	35-42d	21-42d	21-28d	28-35d	35-42d	21-42d
Control	515.00^{d}	603.75^{c}	581.72^{b}	1700.48^{d}	1007.44	1314.46	1462.64	3784.53	1.95^{a}	2.17^{a}	2.51^{a}	2.22^{a}
A	539.30^{cd}	623.52^{bc}	589.25^{ab}	1752.08^{cd}	985.75	1240.75	1308.03	3534.53	1.82^{a}	1.99^{b}	2.22^{bc}	2.01^{bcd}
B	624.38^{a}	688.25^{a}	641.30^{a}	1953.94^{a}	981.82	1290.50	1334.37	3606.69	1.57^{b}	1.87^{b}	2.08^{c}	1.84^{e}
C	545.25^{cd}	644.05^{abc}	626.26^{ab}	1815.56^{bc}	977.90	1216.53	1438.16	3632.60	1.79^{a}	1.88^{b}	2.29^{b}	1.99^{cd}
D	559.50^{bc}	616.75^{bc}	609.00^{ab}	1785.25^{cd}	1038.84	1240.87	1509.43	3789.14	1.85^{a}	2.01^{b}	2.47^{a}	2.11^{b}
E	593.02^{ab}	657.26^{ab}	639.78^{ab}	1890.07^{ab}	1063.37	1227.94	1353.43	3644.73	1.79^{a}	1.86^{b}	2.11^{c}	1.92^{ed}
F	529.27^{cd}	619.85^{bc}	598.58^{ab}	1747.72^{cd}	978.03	1247.42	1417.27	3642.70	1.84^{a}	2.01^{b}	2.36^{ab}	2.08^{bc}
SEM	22.05	33.81	18.17	33.75	54.14	38.30	48.22	53.85	0.08	0.04	0.08	0.02
P value	0.04	0.03	0.04	0.03	NS	NS	NS	NS	0.02	0.02	0.04	0.04

Mean values in the same column with different superscript letters were significantly different ($P<0.05$).
NS, non-significant ($P>0.05$).
Control (no clinoptilolite), A) 1.5% clinoptilolite with particle size of <0.25 mm, B) 1.5% clinoptilolite with particle size of 0.4- 0.8 mm, C) 1.5% clinoptilolite with particle size of 1-2 mm, D) 3% clinoptilolite with particle size of <0.25 mm, E) 3% clinoptilolite with particle size of 0.4- 0.8 mm, F) 3% clinoptilolite with particle size of 1-2 mm.

Table 3. Effect of clinoptilolite on serum biochemical parameters of broiler chickens at d 42

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Alkaline phosphatase (U/L)	Aspartate amino transferase (U/L)
Control	207.96[b]	135.45	64.48	3.44[b]	1.93[c]	3336.80	295.73
A	223.39[b]	138.32	71.56	4.08[a]	2.23[abc]	3241.30	284.62
B	269.27[a]	148.82	79.21	4.47[a]	2.63[a]	3140.20	255.59
C	240.47[ab]	133.63	76.15	4.04[a]	2.43[ab]	3211.10	258.12
D	214.10[b]	145.17	70.67	3.46[b]	2.02[bc]	3302.70	259.77
E	226.38[b]	135.25	78.26	4.25[a]	2.38[ab]	3185.53	276.15
F	227.62[b]	139.95	72.16	3.96[ab]	2.19[bc]	3172.75	274.75
SEM	9.47	10.04	5.00	0.2	0.16	59.86	19.54
P value	0.04	NS	NS	0.03	0.04	NS	NS

Mean values in the same column with different superscript letters were significantly different (P<0.05). NS, non-significant (P>0.05).

Control (no clinoptilolite), A) 1.5% clinoptilolite with particle size of <0.25 mm, B) 1.5% clinoptilolite with the particle size of 0.4- 0.8 mm, C) 1.5% clinoptilolite with theparticle size of 1-2 mm, D) 3% clinoptilolite with the particle size of <0.25 mm, E) 3% clinoptilolite with the particle size of 0.4- 0.8 mm, F) 3% clinoptilolite with the particle size of 1-2 mm.

Table 4. Effect of clinoptilolite on litter quality of broiler chickens

Treatments	pH	Nitrogen (%)	Moisture (%)
Control	7.95	4.58	38.50[a]
A	7.75	4.48	34.25[bc]
B	7.56	4.20	35.50[c]
C	7.88	4.56	37.75[ab]
D	7.70	4.49	34.50[bc]
E	7.85	4.35	37.00[abc]
F	7.66	4.45	33.75[c]
SEM	0.12	0.15	0.19
P value	NS	NS	0.04

Mean values in the same column with different superscript letters were significantly different (P<0.05). NS, non-significant (P>0.05).

Control (no clinoptilolite), A) 1.5% clinoptilolite with the particle size of <0.25 mm, B) 1.5% clinoptilolite with the particle size of 0.4- 0.8 mm, C) 1.5% clinoptilolite with the particle size of 1-2 mm, D) 3% clinoptilolite with the particle size of <0.25 mm, E) 3% clinoptilolite with the particle size of 0.4- 0.8 mm, F) 3% clinoptilolite with the particle size of 1-2 mm.

Discussion

Traditionally, zeolites have been incorporated in animal diets as a technological additive to improve growth and feed efficiency (Angulo *et al.*, 1995). The present study found that supplementing diet with clinoptilolite significantly improves body weight gain and decreased feed conversion ratio compared to the control groups. Numerous reports indicated that zeolite improves the production characteristics of layers and broilers (Elliot and Edwards, 1991; Evans *et al.*, 2005; Herzig *et al.*, 2008). The expected effects of zeolites may exhibit variation due to factors including nature, concentration, the aluminum content of the zeolite and the level of calcium and phosphorus in the diet (Ozturk *et al.*, 1998).

The best attribute given to zeolite is its beneficial effect on feed efficiency in both laying hens and broiler chickens. There seems to be a general agreement on this issue (Olver, 1997), although a few reports suggested that zeolite had no beneficial effect (Amon *et al.*, 1997) or even had a negative effect on this parameter (Nakaue and Koellikker, 1981).

Different mechanisms have been proposed to explain the effect of zeolite on the digestive and productive results of the birds. It has been reported that the addition of clay to the feedstuffs improved the nutrient digestibility and the enzymatic activity of gastrointestinal secretions (Ouhida *et al.*, 2000; Alzueta *et al.*, 2002). The ion exchange properties of the zeolite could alter the pH and the ionic composition (including trace elements) of gastrointestinal fluids, thereby changing the enzymatic activity of gastrointestinal secretions (Martin-Kleiner *et al.*, 2001). Ouhida *et al.* (2000) reported that sepiolite significantly increases organic matter digestibility, with decreases in the water-relative viscosity of jejunal digesta in broiler chickens, improving the action of digestive enzymes.

The current findings imply that using clinoptilolite with the physical size of 0.4-0.8 mm produces more beneficial performance effects compared to the other particle sizes. There is evidence that coarser grinding to a more uniform particle size improves the performance of birds maintained on mash diets (Amerah *et al.*, 2007). This effect may result from the positive effect of feed particle size on gizzard development. A more developed gizzard is associated with increased grinding activity, resulting in increased gut motility and greater digestion of nutrients (Amerah *et al.*, 2007). A large, well-developed gizzard improves gut motility through increasing the levels of cholecystokinin release (Svihus *et al.*, 2004), which stimulates the secretion of pancreatic enzymes and the gastro-duodenal refluxes (Li and Owyang, 1993). Larger feed particles may have been retained in the gastrointestinal tract for a longer time which in turn, may improve energy utilization and nutrient digestibility (Nir *et al.*, 1994).

Demirel *et al.* (2011) observed that, dietary clinoptilolite increases serum albumin, triglyceride and VLDL levels in Rats. Lotfollahian *et al.* (2004) used two types of aluminosilicates and different levels of dietary calcium and phosphorus and obtained a significant increase in serum total protein of broilers. In accordance with our results, no significant changes in serum cholesterol, triglyceride, alkaline phosphatase and aspartate amino transferase by supplementation of clinoptilolite in boilers have been found. But, the broilers given clinoptilolite showed a significant increase in serum glucose, total protein and albumin.

Proper environmental conditions are crucial for the performance of broiler chickens. Wet litter is an environment that supports bacterial and molds growth (Pappas *et al*., 2010). It is also the primary cause of ammonia emissions, one of the most serious environmental factors affecting broiler production. Controlling litter moisture is the most important step in avoiding ammonia problems. Clays and clay products, used as bedding material provide control over ammonia generation (McWard and Taylor, 2000; Tasistro *et al*., 2007). A study conducted by Olver (1997) showed that using zeolite causes a lower manure and litter moisture content. The adsorption property of zeolite has been widely used to combat litter problems. Kithome *et al*. (1999) indicated that zeolites were even suitable for reducing nitrogen losses during composting of poultry manure. In the present study, no significant differences were noted between treatments regarding the nitrogen content. But, there was a tendency to have drier litter conditions in the treatment with added clinoptilolite compared to the control diet. It is concluded that the supplementation of diet with clinoptilolite has positive effects on growth performance and litter moisture of broilers, but more research is required for the effect of physical size of clinoptilolite on boiler performance.

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