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Effects of Physical Sizes of Clinoptilolite on Protein Efficiency Ratio, Intestinal Morphology and Growth Indices of Broilers

Research Article

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ABSTRACT

A total of 448 Ross 308 seven-day old male broiler chicks were assigned to seven treatments with four replicates, each containing 16 chicks, to determine effects of physical sizes and rate of dietary inclusion of clinoptilolite. The chicks fed diet containing clinoptilolite at 1.5% and particle size of 0.4-0.8 mm showed significantly higher body weight gain than the control group that did not receive clinoptilolite ($P<0.05$). Adding clinoptilolite to the diet caused a significant improvement in feed conversion ratio during 22-42 and 7-42 days of age ($P<0.05$). Broilers fed diet containing clinoptilolite at 1.5% and particle size of 0.4-0.8 mm had a lower feed conversion ratio than other groups. Addition of clinoptilolite at 1.5% and particle size of 0.4-0.8 mm improved protein efficiency ratio than the control group in periods 22-42 and 7-42 days ($P<0.05$). Broilers fed diet containing clinoptilolite at 3% and particle size of 0.4-0.8 mm had significantly longer villus height in the duodenum and those fed clinoptilolite at 1.5% and particle size of 0.4-0.8 mm had longer villus height in the jejunum than the control group ($P<0.05$). Among different dietary treatments, the group receiving 1.5% clinoptilolite and particle size of 0.4-0.8 mm had the greatest ratio of villus height to crypt depth. In conclusion supplementation of diet with clinoptilolite with a particle size of 0.4-0.8 mm had positive effects on performance and intestinal morphology in broilers.

KEY WORDS broiler, clinoptilolite, crypt depth, performance, villus height.

INTRODUCTION

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations which have infinite, three dimensional structures. Natural zeolites are minerals formed at low temperature. Clinoptilolite is one of the most common zeolites in volcanic sediments (Bernal and Lopez-Real, 1993). Two of the most important characteristics which are related to its effectiveness in poultry feeding include the ability to lose and gain water reversibly and being capable of selectively exchanging a variety of cations in its structure without major changes (Shariatmadari, 2008). Over 45 distinct natural types of zeolite are known and more than 100 types have no natural counterparts have been synthesized. The chemical formula of pure clinoptilolite is CaNa $(AlO_2)6\ (SiO_2)30\ 24H_2O$ (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; Pappas *et al.* 2010). 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 have shown that the dietary inclusion of zeolites improves average daily gain and feed conversion 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 range of applications of zeolites is 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).

In addition, zeolitic material particle size, crystallite size and the degree of aggregation, as well as the porosity of individual particle determine the access of ingesta fluids to the zeolitic surface during passage through the gastrointestinal tract and strongly affect its ion exchange, adsorption and catalytic properties (Papaioannou *et al*. 2005). When clinoptilolite is used as a feed supplement, the utilization of feed nutrients increased (Olver, 1997).

Clinoptilolite positively affects the digestive mechanism and activity of digestive enzymes (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 and reduce intestinal disease in animals. Limited research is available on the impact of different sizes of clinoptilolite on broiler performance. The objective this study was to determine the impact of physical size of clinoptilolite on protein efficiency ratio, intestinal morphology and meat quality and growth indices of broilers.

MATERIALS AND METHODS

Bird husbandry and diets

This experiment was conducted in a completely randomized design. A total of 448 Ross 308 broilers were randomly allocated to seven dietary treatments.

The treatments were two levels (1.5 and 3%) and three sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F 3% clinoptilolite with particle size of 1-2 mm. Birds were reared from 7 to 42 days. Each treatment had four replicates with 16 birds per replicate. Each cage (120×150 cm) was equipped with a bell-drinker and a feeder. The experimental diets were formulated to meet minimum nutrient requirements of broilers (NRC, 1994). The composition and the calculated nutrient content of the experimental diets are presented in Table 1. Experimental diets and water were provided ad libitum. House temperature was maintained at 33 ˚C for the first week and reduced 2 ˚C weekly, thereafter. A continuous lighting program was provided during the experiment. The chemical composition of clinoptilolite used in the study is presented in Table 2.

Table 1 Ingredients (%) and main nutrients composition of basal diets

Ingredients	Starter (7-21 d)	Grower (22-42 d)
Corn	55.45	61.56
Soybean meal	38.02	32.06
Soybean oil	2.72	3.03
Dicalcium phosphate	1.41	1.04
Limestone	1.28	1.38
Salt	0.42	0.32
Vitamin premix	0.25	0.25
Mineral premix	0.25	0.25
DL-methionine	0.15	0.06
Salinomycin	0.05	0.05
Calculated composition		
Metabolizable energy (kcal/kg)	2950	3050
Crude protein (%)	21.22	19.06
Lysine (%)	1.17	1.02
Methionine (%)	0.48	0.37
Methionine + cystine (%)	0.83	0.69
Calcium (%)	0.92	0.86
Available phosphorus (%)	0.41	0.33
Sodium (%)	0.18	0.14

Each kg of vitamin premix contained: vitamin A: 3,500000 IU; vitamin D_3: 1000000 IU; vitamin E: 9000 IU; vitamin K_3: 1000 mg; vitamin B_1: 900 mg; Vitamin B_2: 3300 mg; vitamin B_3: 5000 mg; vitamin B_5: 15000 mg; vitamin B_6: 150 mg; vitamin B_9: 500 mg; vitamin B_{12}: 7.5 mg; Biotin: 500 mg and Choline chloride: 250000 mg.

Each kg of mineral premix contained: Mn: 50000 mg; Fe: 25000 mg; Zn: 50000 mg; Cu: 5000 mg; I: 500 mg and Se: 100 mg.

Table 2 Components of clinoptilolite

Components	(%)	Elements	mg/kg
SiO_2	67.83	As	43
Al_2O_3	11.64	Ba	309
Fe_2O_3	0.54	Cd	3
CaO	0.84	Cu	6
SO_3	0.20	Mn	278
Na_2O	4.50	Cr	12
K_2O	4.32	Hg	0.2
Cl^-	0.98	Zn	47

Growth performance measurements

Performance data were recorded in the periods from 7 to 21, 22 to 42 and 7 to 42 days of age. Feed intake (FI) was determined for each replication 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 weight. Feed conversion ratio (FCR) was determined as the ratio between feed intake and weight gain at each phase of the experimental period. Protein efficiency ratio (PER) was calculated at the end of different periods as weight gain divided by protein intake (Buamah and Singsen, 1975).

Intestinal morphology

At the conclusion of the study, birds were euthanized and the small intestine opened and weighed. For morphometric analysis, 3 cm tissue samples from the middle part of duodenum, jejunum and ileum were obtained, washed with 10% formalin and fixed in phosphate buffered saline (40 g NaH_2PO_4, 65 g Na_2HPO_4 and 1000 mL of 10% formalin) for 24-48 h. Tissues were dehydrated with an automatic tissue processor. The process consisted of a series of increased concentration of alcohols, infiltrated with xylene and embedded in paraffin. A microtome was used to make 3 tissue slices with 5 µm width. Slices were stained with hematoxylin-eosin. For each treatment, 12 glass slides were prepared and the mean of 10 villi per slide was used as the average value for further analysis. Villus height (VH), villus width (VW), crypt depth (CD), ratios of villus height: villus width (VH:VW) and villus height:crypt depth (VH:CD) were determined at a magnification of 10 × using a light microscope (Pelicano *et al.* 2005).

Meat quality measurements

Sample collection

On day 42, two broilers from each pen, with body weights similar to the pen average body weight were selected and slaughtered to determine meat quality. The tissue was separated manually from the bone and then homogenized using a blender with horizontal blades. Samples were frozen and stored in a freezer at -20 ˚C until further analysis. The samples were analyzed for moisture, Ph and water holding capacity (WHC), crude fat and 2-thiobarbituric acid reactive substances (TBARS). Crude fat was calculated using standard methods outlined by AOAC (1990). Fat was extracted with diethyl ether using a soxhlet apparatus (AOAC, 1990).

Measurement of lipid oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid distillation method of Tarladgis *et al.* (1960) and results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malonaldehyde (MDA) kg^{-1} meat. TBARS value was measured using raw broiler breast meat.

Measurement of the water holding capacity

Water holding capacity was estimated by centrifuging 1 g of muscle placed on tissue paper inside a tube for 4 min at 1500 g. The water remaining after centrifugation was quantified by drying the samples at 70 ˚C overnight. Water holding capacity (WHC) was calculated according to Castellini *et al.* (2002) as:

WHC= (weight after centrifugation (g)-weight after drying (g)) / (initial weight (g))

Measurement of moisture and pH

Moisture was determined after drying in a 100 ˚C oven for 16-18 h (AOAC, 1990). Meat pH was determined by blending a 10 g sample in 100 mL distilled water for one minute, and the pH was measured using a pH meter (Kim *et al.* 2010).

Statistical analysis

The data obtained from the experiment were analyzed using SAS (SAS, 1994) statistical programs with ANOVA procedure. Significant differences among treatment means were separated using Duncan' s multiple range test with a 5% probability (Duncan, 1955).

RESULTS AND DISCUSSION

Growth performance

The effects of clinoptilolite on body weight gain, feed intake and feed conversion ratio of broilers are shown in Table 3.

Clinoptilolite did not impact overall feed intake through 42 d, but did result in improved weight gain and resulting feed conversion ratio.

Specifically, chicks given 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 that did not receive clinoptilolite for periods 22-42 d and 7 to 42 d (P<0.05).

No significant differences were observed between treatments with regard to body weight gain in the period of 7-21 days (P>0.05).

Using clinoptilolite (1.5% with particle size of <0.25 mm) significantly decreased feed intake in periods 22-42 and 7-42 days compared to the control group (P<0.05). Adding clinoptilolite to the diet caused a significant improvement in feed conversion ratio during 22-42 and 7-42 days of age (P<0.05). Broilers fed by clinoptilolite (1.5% with particle size of 0.4-0.8 mm) had a lower feed conversion ratio than other groups.

Protein efficiency ratio

There was a significant effect of clinoptilolite on protein efficiency ratio (Table 4). Broilers fed clinoptilolite (1.5% with particle size of 0.4-0.8 mm) had better protein efficiency ratio than the control group in periods 22-42 and 7-42 days (P<0.05). No significant differences were observed between treatments with regard to protein efficiency ratio in the period of 7-21 days (P>0.05).

Breast meat quality

Table 5 shows the effect of clinoptilolite on breast meat quality. The effect of various treatments on breast meat quality indexes such as water holding capacity (WHC), pH, moisture and 2-thiobarbituric acid reactive substances (TBARS) were not significant (P>0.05).

Table 3 Effect of clinoptilolite on broiler performance

Treatments	BWG (g)			FI (g)			FCR		
	7-21 d	22-42 d	7-42 d	7-21 d	22-42 d	7-42 d	7-21 d	22-42 d	d 7-42
Control	574.50	1700.50^{d}	2275.00^{d}	968.15	3784.53^{a}	4752.68^{a}	1.68	2.22^{a}	2.08^{a}
A	564.85	1752.08^{dc}	2316.94^{dc}	921.71	3534.53^{b}	4456.25^{b}	1.62	2.01^{bcd}	1.92^{bc}
B	616.50	1953.94^{a}	2570.45^{a}	952.11	3606.69^{ab}	4558.80^{ab}	1.54	1.84^{e}	1.77^{d}
C	598.00	1815.56^{bc}	2413.56^{bc}	983.59	3632.60^{ab}	4616.19^{ab}	1.64	1.99^{dc}	1.90^{bc}
D	592.50	1785.25^{dc}	2377.76^{dc}	1005.61	3789.14^{a}	4794.75^{a}	1.69	2.11^{b}	2.01^{ab}
E	594.55	1890.07^{ab}	2484.63^{ab}	970.44	3644.73^{ab}	4615.17^{ab}	1.62	1.92^{de}	1.85^{dc}
F	573.52	1747.72^{dc}	2321.25^{dc}	913.22	3642.70^{ab}	4555.92^{ab}	1.58	2.08^{bc}	1.96^{bc}
SEM	16.46	24.49	36.21	53.03	76.88	70.27	0.04	0.03	0.04
P-value	NS	P < 0.01	P < 0.01	NS	P < 0.05	P < 0.05	NS	P < 0.01	P < 0.01

The means within the same column with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
NS: non significant and SEM: standard error of the means.
BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.
The treatments included various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F= 3% clinoptilolite with particle size of 1-2 mm.

Morphological measurements of the small intestine

The length and weight of small intestine in different segments are presented in Table 6.

Table 4 Effect of clinoptilolite on protein efficiency ratio (PER) of broilers

Treatments	PER (7-21 d)	PER (22-42 d)	PER (7-42 d)
Control	2.81	2.33^{e}	2.43^{d}
A	2.87	2.57^{bcd}	2.64^{bc}
B	3.05	2.84^{a}	2.88^{a}
C	2.86	2.61^{bc}	2.67^{bc}
D	2.78	2.46^{de}	2.52^{cd}
E	2.89	2.70^{ab}	2.75^{ab}
F	2.98	2.51^{cd}	2.61^{bc}
SEM	0.10	0.04	0.05
P-value	NS	P<0.01	P<0.01

The means within the same column with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
NS: non significant and SEM: standard error of the means.
BWG: body weight gain; FI: feed intake and FCR: feed conversion ratio.
The treatments included various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F= 3% clinoptilolite with particle size of 1-2 mm.

The weight and length of the duodenum, jejunum and ileum were not affected by treatments (P>0.05). The effect of clinoptilolite on villus height was considerable in the duodenum and jejunum (Table 7). Treatments including clinoptilolite had significantly longer villus height in the duodenum (3% with particle size of 0.4-0.8 mm) and jejunum (1.5% with particle size of 0.4-0.8 mm) than the control group (P<0.05). However, villus width in different segments of the small intestine was not affected by treatments (P>0.05). Using clinoptilolite significantly decreased crypt depth of the small intestine of broilers (P<0.05). Effects of clinoptilolite on ratios of villus height to villus width and crypt depth of the small intestine of broilers are presented in Table 8. The ratios of villus height: villus width in the duodenum and jejunum was significantly lower in the control group than in other treatments (P<0.05), but the ratio of villus height: villus width in the ileum of the small intestine was not significantly influenced by treatments (P>0.05). Villus height to crypt depth at the small intestinal mucosa was significantly higher with clinoptilolite supplementation than in the control (P<0.05). Among different dietary treatment groups, the group receiving 1.5% clinoptilolite with particle size of 0.4-0.8 mm had the greatest ratio of villus height to crypt depth.

Zeolites are used in animal nutrition for a number of beneficial effects they providing better utilization of feed nutrients, positive effects on intestinal microflora and the mechanism of digestion (Miroslav *et al.* 2010). The present study found that supplementation with clinoptilolite did not impact feed intake, but significantly improved body weight gain and decreased feed conversion ratio. This is consistent with other reports that have shown that zeolite improves the production characteristics of layers and broilers (Evans *et al.* 2005; Herzig *et al.* 2008).

The observed effects of zeolites may be due to such factors as nature, concentration, the aluminum content of the zeolite and the level of calcium and phosphorus in the diet (Ozturk *et al.* 1998). The effect of dietary zeolites on feed intake varies, according to researchers, with an increase in feed intake reported by Olver (1989), no effect (Roland *et al.* 1990) and reduced feed intake (Miles *et al.* 1986). The best attribute of zeolite is its beneficial effect on feed efficiency in both layers 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 Koelliker, 1981). Different mechanisms have been proposed to explain the effect of zeolite on the digestive and productive results of the animals. 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).

Table 5 Effect of clinoptilolite on breast meat quality indexes

Treatments	WHC (%)	pH	Moisture (%)	TBARS (mg of malonaldehyde/kg)
Control	54.54	6.26	76.15	0.68
A	57.55	5.99	72.38	0.53
B	57.26	6.24	72.03	0.64
C	55.83	6.07	74.01	0.62
D	55.91	6.17	79.80	0.51
E	58.59	6.09	73.88	0.68
F	54.24	5.96	75.15	0.60
SEM	1.4	0.14	1.01	0.06
P-value	NS	NS	NS	NS

WHC: water holding capacity and TBARS: 2-thiobarbituric acid reactive substances.
NS: non significant and SEM: standard error of the means.
The treatments included various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F= 3% clinoptilolite with particle size of 1-2 mm.

Table 6 Effect of clinoptilolite on length and weight of small intestine of broilers

Treatments	Duodenum		Jejunum		Ileum	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)
Control	11.17	5.84	26.69	11.50	29.04	11.31
A	11.33	5.79	27.66	11.27	28.91	10.60
B	11.50	5.61	27.64	11.37	31.33	11.60
C	11.74	5.52	28.42	11.24	29.64	11.17
D	11.42	5.52	27.27	11.18	29.45	11.41
E	11.82	5.77	28.70	10.90	30.25	10.90
F	12.32	5.77	27.32	11.37	29.07	11.37
SEM	0.43	0.17	0.76	0.24	0.75	0.29
P-value	NS	NS	NS	NS	NS	NS

NS: non significant and SEM: standard error of the means.
The treatments included various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F= 3% clinoptilolite with particle size of 1-2 mm.

Table 7 Effect of clinoptilolite on villus height (VH), villus width (VW) and crypt depth (CD) of small intestine of broilers

Treatments	Duodenum			Jejunum			Ileum		
	VH (µm)	VW (µm)	CD (µm)	VH (µm)	VW (µm)	CD (µm)	VH (µm)	VW (µm)	CD (µm)
Control	1265.88^{d}	277.00	188.04^{a}	963.85^{c}	210.07	152.29^{a}	620.62	171.82	114.25^{a}
A	1316.44^{bcd}	256.60	155.77^{b}	1012.29^{bc}	195.60	133.44^{b}	632.30	156.10	108.44^{ab}
B	1387.73^{ab}	268.52	156.11^{b}	1157.40^{a}	202.39	130.36^{b}	674.83	172.64	101.86^{b}
C	1348.58^{bc}	277.07	161.57^{b}	1123.58^{a}	203.07	129.75^{b}	665.09	162.82	107.25^{ab}
D	1290.25^{cd}	252.87	165.36^{b}	1075.25^{ab}	208.62	138.36^{ab}	647.87	184.37	108.36^{ab}
E	1429.48^{a}	270.57	163.79^{b}	1118.90^{a}	195.57	131.29^{b}	655.86	158.07	108.79^{ab}
F	1311.85^{bcd}	260.01	170.05^{ab}	1078.58^{ab}	200.01	136.52^{ab}	620.00	160.01	107.61^{ab}
SEM	21.28	8.17	6.89	28.07	6.79	5.63	17.68	10.21	2.86
P-value	P < 0.01	NS	P < 0.05	P < 0.01	NS	P < 0.05	NS	NS	P < 0.05

The means within the same column with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
NS: non significant and SEM: standard error of the means.
VH: villus height; VW: villus width and CD: crypt depth.
The treatments included various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F= 3% clinoptilolite with particle size of 1-2 mm.

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 increased 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 suggest that using clinoptilolite with a physical size of 0.4-0.8 mm produces beneficial performance effects compared to other particle sizes.

There are many approaches to improving feed efficiency. It is known that details of production, handling and processing of a feed ingredient can affect its feeding value. One of these methods is using different feed particle sizes in animal feeding (Parson *et al.* 2006). There is evidence that coarser grinding improves the performance of birds maintained on mash diets. 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).

Table 8 Effect of clinoptilolite on ratios of villus height to villus width (VH:VW) and crypt depth (VH:CD) of small intestine of broilers

Treatments	Duodenum		Jejunum		Ileum	
	VH:VW	VH:CD	VH:VW	VH:CD	VH:VW	VH:CD
Control	4.56[b]	6.74[b]	4.58[b]	6.35[d]	3.66	5.44[c]
A	5.13[a]	8.48[a]	5.19[ab]	7.60[c]	4.08	5.82[bc]
B	5.17[a]	8.96[a]	5.72[a]	8.90[a]	3.89	6.62[a]
C	4.88[ab]	8.40[a]	5.55[a]	8.67[ab]	4.10	6.21[ab]
D	5.12[a]	7.87[ab]	5.15[ab]	7.78[bc]	3.55	5.99[abc]
E	5.29[a]	8.78[a]	5.75[a]	8.52[abc]	4.17	6.02[abc]
F	5.04[ab]	7.72[ab]	5.40[a]	7.96[abc]	3.92	5.75[bc]
SEM	0.16	0.38	0.19	0.22	0.20	0.21
P-value	P < 0.05	P < 0.05	P < 0.05	P < 0.01	NS	P < 0.05

The means within the same column with at least one common letter, do not have significant difference (P>0.01 and P>0.05).
NS: non significant and SEM: standard error of the means.
VH:VW: villus height to villus width and VH:CD: villus height to crypt depth.
The treatments included various levels (1.5 and 3%) and sizes (<0.25 mm, 0.4-0.8 mm and 1-2 mm) of 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 and F= 3% clinoptilolite with particle size of 1-2 mm.

Feed particle size has been shown to influence the development of other segments of the digestive tract in birds fed mash diets. Nir *et al.* (1994a) reported hypertrophy of the small intestine and lowering of intestinal pH when fine mash diets were fed. 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 an increased time which in turn may improve energy and protein utilization and nutrient digestibility (Nir *et al.* 1994b).

Intestinal morphology can reveal some information on gut health. A shortening of the villus reflects villus atrophy and a decrease in surface area for nutrient absorption. The crypt can be regarded as the villus factory and a large crypt indicates fast tissue turnover and a high demand for new tissue (Ma and Guo, 2008). The findings of Maneewan and Yamauchi (2005) showed that there is a positive relation between body weight and villus height in broilers. The condition of intestinal villi is a reliable indicator of nutrient absorption from feed ingredients in chickens (Maneewan and Yamauchi, 2005) and in pigs (Mekbungwan and Yamauchi, 2004).

The short villi were accompanied by reductions in the villi surface area (Park *et al.* 1998), resulting in reduced absorptive functions. These short villi corresponded to reductions in the activities of enzymes, such as lactase and alkaline phosphatase (Zijlstra *et al.* 1997), mucosal lactase and sucrase (Park *et al.* 1998), alkaline phosphatase and disaccharidase (Lopez Pedrosa *et al.* 1998), and total lactase phlorizin hydrolase and mucosal protein concentration (Dudley *et al.* 1998). In the present study, an increase in villus height and a decrease in crypt depth in the small intestinal mucosa of broilers fed on the diets including clinoptilolite were observed. The beneficial effect of clinoptilolite on the duodenum was relatively greater, followed by jejunum and ileum.

The reason for this is that clinoptilolite possibly reduces the numbers of *Clostridium perfringens* and *Escherichia coli* and depresses the activity of bacterial enzymes in the small intestinal digesta of broilers (Xu *et al.* 2003). Olver (1983) reported that natural zeolites reduced colony counts in the gut microflora of the proximal and distal gut and described reduced mortality in broilers and layers. Moreover, it is reported that clinoptilolite, a mucus stabiliser, effectively acts by attaching to the mucus to preserve the mucosa from the toxic effects of drugs and toxins (Albengres *et al.* 1985). This result suggests that the intestinal villi might be activated by feeding clinoptilolite. The villus height to crypt depth ratio is considered to be an important criterion for estimating the likely digestive capacity of the small intestine. A decrease in this ratio is considered deleterious for digestion and absorption, and vice versa (Montagne *et al.* 2003). Therefore, the absorptive function might be reflected by the villus height to crypt depth ratio in the small intestine (Chiou *et al.* 1996).

CONCLUSION

This study demonstrated that dietary clinoptilolite supplementation improves growth indices and intestinal morphology of broilers. Furthermore, using clinoptilolite with a size of 0.4-0.8 mm had nutritional benefits compared to other sizes. But, further work is essential to confirm and extend these findings.

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