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Dietary Effects of Natural and Modified Clinoptilolite Supplementation on Growth Performance, Fat Deposition and Carcass Characteristics of Broilers

[1, 2]Qiu Jue Wu, [1]Yu Qin Wang, [2]Yan Min Zhou and [2]Tian Wang
[1]College of Animal Science and Technology, Henan University of Science and Technology, No. 70, Tianjin Road, Jianxi District, 471003 Luoyang, Henan, P.R. China
[2]College of Animal Science and Technology, Nanjing Agricultural University, No. 6, Tongwei Road, Xuanwu District, 210095 Nanjing, Jiangsu, P.R. China

Abstract: The objective of this study was to investigate the effects of natural and modified clinoptilolite in broiler chicks' diets on growth performance, fat deposition and carcass characteristics. For the stated purpose, two hundred forty, 1 day old Arbor Acres broiler chicks of mixed sex were randomly divided into four treatment groups and eight replications on the basis of randomized complete block design for a period of 42 days. The broilers were fed control diet and the same diet added with 2% Natural Clinoptilolite (NCLI) and Modified Clinoptilolite (MCLI), respectively. Based on the results obtained, natural and modified clinoptilolite did not affect the growth performance of broiler chickens, the relative weight of breast, thighs and spleen, abdominal fat, subcutaneous fat thickness, intermuscular fat width and the meat quality of breast and thigh muscle. However, heart as haematopoietic organ, the addition of NCLI and MCLI can significantly reduced the relative weight of heart in broiler chicks ($p<0.01$). Furthermore, there is significant difference was observed for gizzard index and pH_{24h} of breast muscle for modified clinoptilolite treatment ($p<0.05$). It seemed that natural and modified clinoptilolite did not have any major impact on the growth performance, fat deposition and carcass characteristics has a certain impact on the relative weight of organs.

Key words: Clinoptilolite, growth performance, fat deposition, carcass characteristics, gizzard index

INTRODUCTION

Clinoptilolite is a member of the naturally occurring zeolite family of minerals is crystalline, hydrated alluminosilicates of alkali and alkaline earth cations having three-dimensional infinite crystalline structures with interconnecting channels and large pores (Mumpton and Fishman, 1977) and therefore, possesses its own set of adsorption properties. Depending on the physical and chemical properties such as Ion Exchange Capacity (IEC), related molecular sieve properties, catalysis and adsorption, clinoptilolite has widespread applications in agriculture and agricultural sectors (Mumpton, 2006).

Clinoptilolite is a most widely used natural zeolite in animal studies due to its structural stability under high temperatures and acidic conditions (Shurson *et al*., 1984). When used as dietary supplements, it improve feed efficiency thus leading to a beneficial growth response in animal and egg productivity (Elliot and Edwards, 1991; Olver, 1997; Yannakopoulos *et al*., 2000; Papaioannou *et al*., 2002). But because these beneficial effects seems to be related to the species and the geographical source of the involved zeolite, its purity and physicochemical properties as well as the dietary, environmental conditions and the supplemental level used in the diets, therefore some researchers have observed no response on growth (Pearson *et al*., 1985) or an adverse effect (Poulsen and Oksberg, 1995). To improve the effect of zeolite applications which require the zeolite selected and processed and was modified by physical or chemical treatment, greatly improve its application effect. In the present, the modified clinoptilolite on animal performance and carcass characteristics of broiler have not been reported. This experiment was carried out to determine possible effects of supplementation at the 2% inclusion levels of natural (Jiangsu) and modified clinoptilolite (Formic Acid modified clinoptilolite) on the performance, fat deposition and carcass characteristics of broilers.

MATERIALS AND METHODS

Animals and management

Chickens, diets and experimental design: In this study, 240 Arbor Acres broiler chicks of 1 day old were allocated

Corresponding Author: Qiu Jue Wu, College of Animal Science and Technology, Henan University of Science and Technology, No. 70, Tianjin Road, Jianxi District, 471003 Luoyang, Henan, P.R. China

to three treatments and eight replications on the basis of randomized complete block design for a period of 42 days. The experiment was conducted in a completely randomized design of three dietary treatments with 8 replicates in each treatment. The dietary treatments were:

- Basal diet
- Basal diet+2% NCLI
- Basal diet+2% MCLI

All birds were housed in wire cages in a 3-level battery. Birds were housed in an environmentally controlled room. The initial temperature of 32°C was gradually reduced according to the age of the birds, reaching 20°C at the end of the experiment. The lighting cycle was 24 h from 1-3 days of age, 18 h from 4-20 days of age, 21 h from 21-35 days of age and 23 h from 35-42 days of age.

The based diets were of the corn-soybean type. Experimental diet in mash form and water were available for *ad libitum* consumption throughout the experimental period. The starter (21.15% CP and 2990 kcal ME kg^{-1}) and grower (19.25% CP and 3090 kcal ME kg^{-1}) basal diets were both supplemented with amino acids, minerals and vitamins and formulated by considering nutrient requirements of broilers for starter (up to 21 days) and grower (up to 42 days) periods (National Research Council, 1994) according to the chinese poultry breeding conditions) (Table 1). The natural clinoptilolite used in this study was provided by the Center of China Geological Survey (Nanjing). Nature clinoptilolite was calcined and stirred, blended, washed, finally a new organic acidifier (Formic acid) modified clinoptilolite was constructed.

Table 1: Compodition and nutritive value of broiler diets on fed basis

Ingredients (g kg^{-1})	1-21 days	22-42 days
Corn	578.00	625.00
Soyabean meal (43%, crude protein)	325.00	265.00
Corn gluten meal	30.00	35.00
Soya-bean oil	27.00	35.00
Limestone	9.50	10.50
Dicalcium phosphate	17.50	16.50
Salt	3.00	3.00
Premix*	10.00	10.00
Total	1,000.00	1,000.00
Calculation of nutrients (g kg^{-1})		
Apparent metabolism energy (MJ kg^{-1})	12.51	12.93
Crude protein	211.50	192.50
Ca	9.70	9.00
Available P	4.20	4.00
Lys	10.80	9.50
Met	4.80	4.30
Met+Cys	8.10	7.10

*Premix provided per kg of diet: limestone, 3.3 g; L-LysineHCl, 1.5 g; Dl-Methionine, 1.3 g; VA 10,000 IU, VD_3 3,000 IU, VE 30 IU, menadione, 1.3 mg, thiamine 2.2 mg, riboflavin, 8 mg, nicotinamide 40 mg, choline chloride 600 mg, calcium pantothenate 10 mg, pyridoxineHCl, 4 mg, biotin 0.04 mg, folic acid 1 mg; vitamin B12 (cobalamine) 0.013 mg, Fe (from Ferrous sulphate) 80 mg, Cu (from Copper sulphate) 8 mg, Mn (from Manganese sulphate) 110 mg, Zn (Bacitracin Zn), 65 mg, iodine (from Calcium iodate) 1.1 mg, Se (from Sodium selenite), 0.3 mg

Sample collection and procedures: At days 21 and 42 of the experimental period, body weight of birds were individually measured in pens each and feed intake was recorded for the feed conversion rate. Eight male broilers per group (one birds per replicate) were selected and individually weighed and slaughtered after 12 h feed withdrawal in a conventional processing plant at 6 weeks of age. The liver, heart, spleen, gizzard and abdominal fat of broiler chicks were also removed and weighed. The relative organ weights (weight of organ/100 g live body weights) were calculated. The subcutaneous fat thickness and intermuscular fat width were measured.

Breast muscles and thigh muscles from the right side of each carcass were trimmed of the adipose tissue and tendon, weighed and then held at 2±2°C for meat quality measurement. The ultimate pH values of both pectoralis muscles were determined 24 h post mortem, using a portable pH meter (IQ150, IQ Scientific Instruments Inc., Carlsbad, CA, USA) equipped with an insertion glass electrode (pH57-SS). Before measurement, the electrode was calibrated using three buffers with pH of 4.01, 7.00 and 9.01. The samples' pH was always measured at the same place of the muscle. For all drip loss determinations sampling was performed 24 h after slaughter. For the bag drip method, the Pectoralis major was excised and cut perpendicular to the fibre direction in the widest part. A slice of 1 cm was taken from the anterior part then weighed (weight approx. 25 g), placed in a non-absorbing net mounted in a plastic bag and allowed to hang for 48 h then wiped of with a paper towel and weighed again. Drip loss is expressed in relative weight loss (Rasmussen and Andersson, 1996). Cooking loss was measured after heat treatment. Breast meat and thigh meat was heat-treated in plastic bags separately in a water bath (82°C), the core temperature of samples was kept at 80°C for 30 min. Samples were cooled at room temperature and stored overnight at 4±2°C (Coro *et al*., 2003). To determine shear force a Texture Analyzer and a Warner-Bratzler device (CLM_2, Northeast Agric Univ., Ltd. China) were used. Muscle samples were stored at 4°C for 24 h and were then individually cooked in a water bath at 80°C in plastic bags to an internal temperature of 70°C. Next, the samples were removed and chilled to room temperature. Strips (1.0×0.5×2.5 cm) parallel to the muscle fibers were prepared from the medial portion of the meat and sheared vertically (Molette *et al*., 2003). Color measurements of the skinless muscle surfaces were determined using a portable

Minolta5 colorimeter and reported according to the CIELAB Color System values of (L*) lightness, (a*) redness and (b*) yellowness.

Statistical analyses: Analyses variance was done using the General Linear Model procedure of Statistical Package for Social Sciences 18.0 (SPSS Inc., Chicago, IL, USA) as a completely randomized design and expressed as mean ±pooled standard errors of means. Differences were considered significant at $p<0.05$ and differences were considered trends when $0.05\leq p\leq 0.10$ and expressed as mean±pooled standard errors of means. The significant differences among different treatment means were separated using the Duncan's new multiple range test.

RESULTS AND DISCUSSION

Effect of natural and modified clinoptilolite on growth performance: The effects of the dietary treatments on broiler chicks feed intake, weekly average Body Weight Gain (BWG) and Feed intake/body weight Gain (F/G) data in the periods of starter, grower and the whole period are presented in Table 2. In the present study, dietary supplementation with NCLI and MCLI had no clear effect on the growth performance or F/G of broiler chicks. There may be explained by the hypothesis that NCLI and MCLI which has different ion exchange capacity may exhibit different effects in digestive system (Elliot and Edwards, 1991). Moreover, it may be attributed to experimental environment. In the present experiment, the experimental subdivisions in the broiler house were separated with 60 cm high barriers which prevented air flow between sections, a factor not controlled in the other two studies. This division of the sections to prevent airflow, so the concentration of odour and ammonia was higher.

Table 2: Effects of NCLI (2%) and MCLI (2%) on the growth performance of broilers

	Diet treatments[1]		
Items	Control[3]	NCLI[3]	MCLI[3]
1-3 weeks			
FI[2] (g/bird/day)	61.67±2.1100	61.37±3.1300	61.29±2.1000
BWG[2] (kg/bird)	0.787±0.017	0.774±0.018	0.763±0.015
F/G[2] (kg kg^{-1})	1.645±0.031	1.665±0.027	1.686±0.037
4-6 weeks			
FI[2] (g/bird/day)	163.58±1.1200	163.96±1.0500	163.95±1.2700
BWG[2] (kg/bird)	1.677±0.030	1.693±0.057	1.706±0.041
F/G[2] (kg kg^{-1})	2.049±0.069	2.034±0.062	2.018±0.091
1-6 weeks			
FI[2] (g/bird/day)	225.25±2.6600	225.33±1.8100	225.22±1.2900
BWG[2] (kg/bird)	2.464±0.029	2.467±0.041	2.469±0.011
F/G[2] (kg kg^{-1})	1.920±0.046	1.918±0.059	1.916±0.088

[1]Data represent means from 6 replicates per treatment and SEM = Standard Error of Mean; [2]BWG = Body Weight Gain; FI = Feed Intake; F/G = Feed Intake/BW gain; [3]Control = Basal diet; NCLI = Basal diet+2% Natural Clinoptilolite; MCLI = Basal diet+2% formic acid Modified Clinoptilolite. [4]Means with different superscript letters in the same line differ significantly; Lowercases represent $p<0.05$; Capital letters represent $p<0.01$

Effect of natural and modified clinoptilolite on carass parameters: Natural and modified clinoptilolite has no effect on muscle percentage. This means that zeolite may have no impact on muscle percentage. Similarly, dietary use of zeolite showed no influence on performance and carcass quality of growing and fattening hogs (Pearson *et al*., 1985). Yalcin *et al*. (1995) also found that adding zeolite to diet did not affect the net carcass weight percentage of broiler chicks. Lotfollahian *et al*. (2004) using two types of aluminosilicates reported that net carcass weight percentage was not influenced by the investigated substances. Furthermore, Tatar (2006) comparing the effect of perlite and zeolite on carcass of broilers showed that adding 2.5 and 5% of these materials to diet did not cause any significant difference on net carcass weight. Azar *et al*. (2011) also comparing the effect of different levels and particle sizes on carcass characteristics of broilers showed that there was no difference among treatments. Tatar (2006) reported that using perlite did not influence the weight of thigh of broiler chicks. Christaki *et al*. (2006) showed that using 2% natural zeolite (60% clinoptilolite) to diet have no any significant differences on the average weights of breast meat, thigh meat. Some other researchers also showed that adding 1.5-5% of aluminosilicates to broilers dietary rations had no major effect on weight percentage of thigh (Mirabdolbagi *et al*., 2007a, b). However, Palic *et al*. (1993) concluded that using dietary zeolite increased the meat production of broilers and consequently yielded higher thigh weight percentage.

As shown in Table 3, diet inclusion of natural and modified clinoptilolite had a lower the relative weight of heart ($p<0.01$) and liver ($p<0.05$) than the control treatment. These findings contracted with the results of

Table 3: Effects of NCLI (2%) and MCLI (2%) on the carass characteristics in broilers at 42 days of age

	Dietary treatments[1]		
Items	Control[2]	NCLI[2]	MCLI[2]
Breast muscle weight (g)	197.57±3.39	197.51±1.08	197.49±0.94
Breast muscle (%)	19.82±0.38	19.92±0.25	19.96±0.07
Thigh muscle weight (g)	160.60±1.68	161.90±1.72	162.44±2.37
Thigh muscle (%)	14.11±0.44	14.33±0.72	14.23±0.45
Heart index (g kg^{-1})	5.58±0.32[A]	3.97±0.27[B]	4.29±0.31[B]
Liver index (g kg^{-1})	18.87±1.25[a]	15.39±0.87[b]	17.45±0.16[ab]
Gizzard index (g kg^{-1})	25.14±0.96[ab]	22.33±1.41[a]	26.46±1.41[b]
Spleen index (g kg^{-1})	1.20±0.12	1.21±0.02	1.25±0.03
Abdominal fat weight (g)	23.59±4.57	23.13±3.58	20.63±1.52
Abdominal fat (%)	0.99±0.16	0.98±0.16	0.98±0.08
Subcutaneous fat thickness (cm)	0.86±0.14	0.75±0.24	0.63±0.08
Intermuscular fat width (cm)	1.40±0.10	1.29±0.10	1.26±0.12

[1]Data represent means from 6 replicates per treatment and SEM = Standard Error of Mean; [2]Control = Basal diet; NCLI = Basal diet+2% Natural Clinoptilolite; MCLI = Basal diet+2% formic acid Modified Clinoptilolite; [3]Means with different superscript letters in the same line differ significantly; Lowercases represent $p<0.05$; capital letters represent $p<0.01$

Santurio *et al.* (1999) and Hamid *et al.* (2011) who concluded that using bentonite in broilers diets did not affect the relative weight of heart and liver. It most likely due to the environment stress experienced by broiler in the control (as discussed in the broiler performance section of the results and discussion) in the present study. Pearson *et al.* (1985) reported that the addition of clinoptilolite to the diet of pigs did not significantly improve their growth performance and carcass quality, citing the fact that disease-control effect is a widely accepted explanation for the growth responses observed in pigs after zeolites. The ameliorative effects of clinoptilolite was also reported by Vrzgula and Bartko (1984) who observed that pigs having diarrhea after having been given a feed mixture supplemented with clinoptilolite, produced compact feces after 48 h and the appetite of the pigs became normal and they began to gain weight normally. In contrast, diarrheic pigs in a control group continued to scour and lose weight. During the entire course of this feed trial, the broiler chickens appeared to be healthy and showed no visible signs of diarrhoea or any other adverse health symptoms. Moreover, based on the findings of this study, natural and modified clinoptilolite did not affect the spleen weight of broilers (Table 3). These findings correspond with the results of Mirabdolbagi *et al.* (2007a, b) and Azar *et al.* (2011). They reported that using aluminosilicates in broilers diets did not have any effect on their spleen weight. This could also explain why less significant differences were observed in broiler performance as compared to other trials. On the other hand, heart as haematopoietic organ, the addition of nature and modified clinoptilolite can significantly reduced the relative weight of heart in broiler chicks, it was further confirmed that the ameliorative efficacy of dietary adsorbent (nature and modified clinoptilolite) on the detrimental effects.

Weight percentage of gizzard was significantly affected by modified clinoptilolite than the natural clinoptilolite (Table 3), it caused the highest gizzard weight. This was possibly due to the effect of modified clinoptilolite remaining and staying longer in gizzard, increasing its wall muscle activity and subsequently making it bulkier (Kilburn and Edwards, 2004). In the present study, modified clinoptilolite has no shown better effect than natural clinoptilolite on the other considered parameters. There was no significant difference among the treatments in terms of abdominal fat weight, subcutaneous fat thickness and intermuscular fat width (Table 3). Tatar (2006) investigating the effect of perlite on weight percentage of abdominal fat also showed that aluminosilicate did not cause any difference between the experimental groups. Other studies reported that adding aluminosilicates to the rations did not have major influence on the abdominal fat of broilers (Yalcin *et al.*, 1995; Mirabdolbagi *et al.*, 2007a, b; Azar *et al.*, 2011). These finding showed that aluminosilicate did not affect energy absorption. But Christaki *et al.* (2006) reported that the reduction on the abdominal fat pad weight in treatments where natural zeolite was added into the diet was 47.5 and 44.7% when compared to the control group, these differences in the abdominal fat pad content can be attributed to the different metabolic use of the dietary fat sources (Sanz *et al.*, 1999).

Effect of natural and modified clinoptilolite on meat parameters: Means and standard errors of meat quality of breast and thigh muscle are reported in Table 4. The data clearly show that natural and modified clinoptilolite has no effect on the meat quality of thigh muscle. In contrast, a significant difference among three treatments were observed for pH_{24} and pressure loss of breast muscle, pH_{24} of control birds ($p<0.01$), natural clinoptilolite birds ($p<0.05$) was lower significantly than modified clinoptilolite birds, respectively. No effect of natural and modified clinoptilolite were found for drip losses, cooking loss, shear force, L*, a* and b*. A significant relationship has earlier been observed between pH and drip loss in turkeys and broiler chickens (Barbut, 1997a, b). The present study only showed that meat with a lower pH had a lower water holding capacity in the breast muscle. So, it is unlikely that a difference in pH entirely explains the difference in drip loss because there was significant relationship of pH and drip loss of thigh muscle was not observed. Further, natural and modified clinoptilolite has

Table 4: Effects of NCLI (2%) and MCLI (2%) on the meat quality of breast and thigh muscle in broilers at 42 days of age

	Dietary treatments[1]		
Parameters	Control	NCLI	MCLI
Breast muscle			
pH_{24h}	5.87±0.05^{A}	5.95±0.06^{ABa}	6.17±0.09^{Bb}
Drip loss of 24 h (%)	1.21±0.14	1.13±0.27	1.14±0.20
Drip loss of 48 h (%)	0.47±0.04	0.57±0.08	0.51±0.07
Cooking loss (%)	12.47±1.45	13.57±1.68	12.03±1.65
Shear force (kg)	26.58±1.95	27.71±4.22	27.07±2.52
Luminance (L*)	47.90±1.72	49.43±0.76	49.06±0.86
a*	3.73±0.71	4.10±0.57	4.05±0.26
b*	16.17±1.81	15.77±1.19	14.72±0.66
Thigh muscle			
pH_{24h}	6.70±0.10	6.68±0.12	6.41±0.08
Drip loss of 24 h (%)	2.64±0.18	2.19±0.53	2.15±0.20
Drip loss of 48 h (%)	1.06±0.26	1.16±0.15	1.13±0.14
Cooking loss (%)	9.24±0.67	11.12±1.46	10.97±1.40
Shear force (kg)	16.53±1.02	19.18±1.07	19.99±1.75
Luminance (L*)	52.45±1.73	49.27±0.72	50.58±1.44
a*	9.23±2.52	7.88±1.92	4.56±0.76
b*	15.93±2.42	14.70±1.74	14.56±1.31

[1]Data represent means from 6 replicates per treatment and SEM = Standard Error of Mean; [2]Control = Basal diet; NCLI = Basal diet+2% Natural Clinoptilolite; MCLI = Basal diet+2% formic acid Modified Clinoptilolite; [3]Means with different superscript letters in the same line differ significantly; Lowercases represent $p<0.05$; Capital letters represent $p<0.01$

no effect on meat quality, it is impossible that natural and modified clinoptilolite had no effect on meat quality due to their absorption capacity on toxic and heavy metal concentrations in kidney, liver and muscle tissues. These were further confirmed that less significant differences were observed in broiler performance and the relative weight of spleen.

The results was corresponds with Nestorov (1984), Fokas *et al*. (2004) and Pond *et al*. (1993). They also think that the toxic cation and lead (Pb), Arsenic (As) and Cadmium (Cd) absorption capacity of clinoptilolite prevented adverse effect on metabolic functions in animal resulting in no effect on meat quality and edible parts of muscles (carcass). On the other hand, natural and modified clinoptilolite seems to increase water holding capacity, cooking loss and shear force of meat. These results were not contracted with Prvulovic *et al.* (2012) but the reasons for these effects are still not clear. Because there is very limited information about meat quality of animal fed aluinosilicates in the present. In addition, natural and modified clinoptilolite have no effect on the L*, a* and b* which may be due to diet inclusion of CLI that has no influence on the total antioxidant capacity of meats. Some studies have demonstrated that the total antioxidant capacity of meats was a strong positively correlated with meat color (L*, a* and b*) (Rowe *et al*., 2004).

CONCLUSION

From these results, it is concluded that although rearing chickens by natural and modified clinoptilolite can caused a raise in gizzard weight, a reduce in the relative weight of heart, they had no major impact on broilers growth performance, carcass improvement, fat deposition and meat quality. Further researches are proposed to clarify the mechanisms likely involved in the use of aluminosilicates on meat quality and carcass characteristics.

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