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Effects of Clinoptilolite on Growth Performance and Antioxidant Status in Broilers

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Abstract The objective of this study was to compare the effects of natural clinoptilolite and modified clinoptilolite on growth performance and antioxidant capacity in broiler chicks. Two hundred forty 1-day-old commercial Arbor Acres broilers were randomly distributed into three treatments, each of which had eight replicates. Each replicate contains 10 chicks. Control (CON) group fed with the basal diets, natural clinoptilolite (NCLI) group fed basal diets with 2 % natural clinoptilolite, and modified clinoptilolite (MCLI) group fed basal diets with 2 % modified clinoptilolite for 42 days. The results showed that the 2 % supplementation of natural clinoptilolite and modified clinoptilolite had no adverse effect on growth performance of broilers at 42 days of age. Relative weights of organs were not influenced by dietary treatments at 21 and 42 days. The activity of total nitric oxide synthase was significantly ($P<0.05$) decreased in MCLI group than CON group at 21 days of age. At 21 and 42 days, the activities of glutathione peroxidase, catalase, total superoxide dismutase, total antioxidant capacity (T-AOC) were significantly ($P<0.05$) increased in NCLI and MCLI groups than the CON group while there was no difference in T-AOC between CON and NCLI groups. The malondialdehyde content was significantly ($P<0.05$) decreased in NCLI and MCLI groups than the CON group. It was concluded that the addition of 2 % natural clinoptilolite and modified clinoptilolite to diet can improve antioxidant capacity in broilers, although their effects on growth performance was negligible.

Keywords Broilers · Natural clinoptilolite · Modified clinoptilolite · Growth performance · Antioxidant status

Introduction

The current intensive poultry production system has significantly increased the meat quantity, but there are lot of stress factors that impairing both growth performance and health status of broilers. Redox homeostasis is a usual denominator of the responses to these stresses which is maintained by the balance between the production of reactive oxygen species (ROS), reactive nitrogen species, and antioxidant defense system. Oxidative stress results when production of ROS exceeds the capacity of antioxidant defense system to remove these toxic molecules [1]. Broilers are easily to get oxidative stress, and such situation can be increased under certain environmental, physiological, or dietary conditions. Specific feeding strategies have significant influence on oxidative metabolism in broilers. In chickens, supplementation of certain feed additives is significant to keep the balance between antioxidants and oxidative stress. Feed additives have direct influence on the productive and reproductive performances of animals [2].

In the last few decades, natural zeolite, used as a feed additive into the diets of animals, has been applied with enormous success in animal breeding for many purposes. Zeolites are tectosilicates with three dimensional aluminosilicates structure that contain water molecules, alkaline, and alkali earth metals in the structural framework [3]. Currently, there are at least 50 different types of known zeolites. Clinoptilolite is the best type which is suitable for the large numbers of applications. Natural clinoptilolite contains most of the major and trace minerals which are essential for the growth for chickens, livestock, and aquatic animals. These minerals are in an ionic state and can be used in animal diets for improving health conditions for animals [4]. Clinoptilolite appears to be stable in gastrointestinal tract of animals [5], and

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as unique selective adsorbers, they can adsorb heavy metals, toxins, and free radicals in the body and excretes them from animal bodies [6–8]. The absorptive characteristics of clinoptilolites are because of their high cation-exchange capacity, which affects tissue uptake and utilization of NH_4^+, Cu^{2+}, Pb^{2+}, Cd^{2+}, Cs^+, and other cations in animals [4, 6, 9]. Besides these characteristics, clinoptilolites have also shown anti-cancerous and antioxidative effects [10–12]. Natural clinoptilolites have positive influence on the immunity and the inflammatory processes by diminishing the synthesis of nitric oxide and superoxide anions [12]. They enhance the immune activity in animals [13, 14].

Zeolites possess net negative structural charge as a result of isomorphic substitution of cations in crystal lattice; this negative charge leads to the favorable ion exchange selectivity for certain cations of zeolites. However, the negative charge also leads zeolites to have little or no approximation for anions [15]. Recent studies have shown that zeolites modified with some surfactants or other cationic sorbents have an approximation affinity for heavy metal anions. These surfactant-modified zeolites are effective sorbents for many types of contaminants [16, 17].

Few existing studies concerning the effects of natural zeolite on performance of the production, total flora, Salmonella, and blood biochemical parameters of broilers [18–20], but limited data have been published on the supplementation of modified zeolites, especially clinoptilolite modified by formic acid on the oxidant/antioxidant status in healthy broiler chickens. Lots of dietary adjustments and management schemes have been attempted to promote growth performance, antioxidant status, and immunity of broiler. The objectives of this present study were to determine the effects of clinoptilolite and modified clinoptilolite on growth performance, antioxidant status, and immunity in broiler chicks, thus supporting scientific basis for the use of clinoptilolite in the area of animal raising and production.

Materials and Methods

Bird Treatment and Diets

A total of two hundred forty 1-day-old commercial Arbor Acres broilers were obtained from a local commercial hatchery (Hewei, Anhui, China) and randomly allocated into three experimental groups consisting of eight replicates. Each replicate contained ten birds with similar average body weights (BW). Chickens were fed with corn–soybean basal diets. Birds, in control group, were fed with basal diet or the basal diet with 2 % natural clinoptilolite (NCLI) group or the basal diet with 2 % modified clinoptilolite (MCLI) group for 42 days. The diets were formulated based on the NRC (1994) to meet the nutrient requirements of broilers. The birds were fed with a starter diet until 21 days of age followed by a grower diet from 22 to 42 days (Table 1). All birds were placed in wire cages in a three-level battery and housed in an environmentally controlled room maintained at 34 to 36 °C during 1 to 14 days of age and gradually decreased to 26 °C, after which, it was held at room temperature and kept unchanging at the end of the experiment. The light regimen was a 12 h light–dark cycle (0600 to 1800 h light). Birds were allowed to consume feed and water ad libitum. Fresh diets were prepared once a week and were stored in sealed bags at 4 °C.

The clinoptilolite used in the experiment was provided by Nanjing Institute of Geology and Mineral Resources (Nanjing, China). All experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Nanjing Agricultural University (Nanjing, People's Republic of China).

Preparation of Clinoptilolite Modified by Formic Acid

The clinoptilolite sample used in this study was collected from the Center of China Geological Survey (Nanjing, People's

Table 1 Ingredients and nutrient composition of broiler diets, as-fed basis

Item	1 to 21 days	22 to 42 days
Ingredient (g/kg)		
Maize	578	625
Soybean meal	325	265
Corn gluten meal	30	35
Soyabean oil	27	35
Limestone	9.5	105
Dicalcium phosphate	17.5	165
Salt	3	3
Premix[a]	10	10
Total	1,000	1,000
Calculated nutrition levels[b]		
AME (MJ/kg)	12.51	12.93
Crude protein (g/kg)	211.5	192.5
Calcium (g/kg)	9.7	9.0
Available phosphorus (g/kg)	4.2	4.0
L-lysine (g/kg)	10.8	9.5
DL-methionine (g/kg)	4.8	4.3
Met+Cys (g/kg)	8.1	7.1

[a] Premix provided per kilogram of diet: limestone, 3.3 g; L-lysine HCl, 1.5 g; DL-methionine, 1.3 g; VA, 10,000 IU; VD3, 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; pyridoxine HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B12 (cobalamine), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulfate), 8 mg; Mn (from manganese sulfate), 110 mg; Zn (Bacitracin Zn), 65 mg; iodine (from calcium iodate), 1.1 mg; Se, (from sodium selenite) 0.3 mg

[a] The nutrient levels were on an as-fed basis

Republic of China). The NCLI grain size ranged from 0.15 to 0.2 mm. The MCLI was prepared by using the ion exchange method. Clinoptilolite was first calcined at 500 °C for 4 h in a muffle oven. After cooling down, clinoptilolite was added into a formic acid solution of 3 mol/L. The mixture was blended at 80 °C at pH 4.0 and 151 rpm/min within a constant temperature oscillated instrument for 4 h. The lower sediments were repeatedly washed by deionized water until the pH of the washed solution is 7. Finally, the washed materials were collected and dried at around 105 °C for 2 h in an air oven, and then ground through a 100-mesh sieve. The clinoptilolite used in the experiment was provided by Nanjing Institute of Geology and Mineral Resources (Nanjing, P. R. China) and was sieved through a 100-mesh sieve. The content of clinoptilolite used in the experiment including 2 % clinoptilolite, 8 % mordenite, 5 % montmorillonite, and 2 % SiO_2. The textural properties of clinoptilolite are listed in Table 2.

Sample Collection and Procedures

The growth performance of broilers was evaluated by calculation of the average feed intake, average body weight gain, and feed conversion ratio. The individual body weights of broilers were recorded at the beginning of the trial on 21 and 42 days. The feed intake was calculated on a per pen basis on 21 and 42 days of the experiment. The feed conversion ratio (F:G) was calculated by the average of feed consumed per unit of body weight gain on 21 and 42 days.

At 21 and 42 days, one bird per replicate was randomly selected and weighed after feed deprivation for 12 h. All birds were killed by exsanguination and necropsied immediately. After decapitation, liver tissues were excised, frozen in liquid nitrogen at first, and then stored at −20 °C for further analysis. After that, the bursa, thymus, and spleen organs were collected and weighed.

Growth Performance and Relative Weights of Immune Organs

Body weights were recorded for each replicate at 1, 21, and 42 days of age. Feed was withdrawn for 12 h, and water was provided for drinking before weighing at 21 and 42 days. Feed intake was recorded during the 42-day trial. The average daily gain (ADG), average daily feed intake (ADFI), and F/G ratio were calculated in broiler chickens. The relative immune organ weights like spleen, bursa, and thymus were calculated using the following formula: relative immune organ weight= immune organ weight (g)/BW (kg).

Table 2 Textural properties of zeolites

Clinoptilolite	BET surface area (m^2/g)	Pore volume (cm^3/g)
Natural clinoptilolite	19.4852	0.0023
Modified clinoptilolite	24.9931	0.0050

Preparation of Whole Liver Homogenate and Protein Assay

Approximately 0.3 g liver was used to prepare the whole liver homogenate (WLH). The liver pieces were diluted 1:9 (wt/vol) with 60 mM potassium phosphate buffer, pH 7.4, and homogenized using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH). Protein concentrations of the WLH were determined by using a kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China).

Biochemical Assay Determination of Liver

The enzyme activities of glutathione peroxidase (GSH-Px), catalase (CAT), total superoxide dismutase (T-SOD), total antioxidant capacity (T-AOC), inducible nitric oxide synthase (*i*NOS), total nitric oxide synthase (TNOS), and nitric oxide (NO) content of the liver homogenate were determined using corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P. R. China) according to the instructions of the manufacturer. The activities of NOS, T-SOD, GSH-Px, CAT, and T-AOC were expressed as unit per milligram of protein of liver; the content of NO was expressed as micromole per gram of protein of liver.

Malondialdehyde Content of Liver

Malondialdehyde (MDA) content of the liver homogenate was determined by using a kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China) according to the instructions of the manufacturer. The MDA concentrations were expressed as nanomoles per milligram of protein of liver tissue.

Statistical Analysis

All data was analyzed by one-way ANOVA with the post hoc Duncan multiple comparison tests using SPSS statistical software (ver.16.0 for windows, SPSS Inc., Chicago, IL). The means and total standard errors were presented. Significance (*P* value) was evaluated at the 0.05 level.

Results

Growth Performance and Relative Weights of Organs of Broilers

The data of effects of natural clinoptilolite and modified clinoptilolite on growth performance of broilers chickens is

Table 3 Effects of natural clinoptilolite and modified clinoptilolite on the growth performance of broiler chickens

Items	Period (day)	Dietary treatments		
		CON	NCLI	MCLI
ADG g/bird per day	1 to 21	37.47±0.81	36.86±0.86	36.33±0.71
	22 to 42	79.86±1.43	80.62±2.71	81.24±1.95
	1 to 42	58.67±0.69	58.74±0.98	58.79±0.26
ADFI g/bird per day	1 to 21	61.67±2.11	61.37±3.13	61.29±2.10
	22 to 42	163.58±1.12	163.96±1.05	163.95±1.27
	1 to 42	112.63±1.33	112.67±0.91	112.61±0.65
F:G	1 to 21	1.65±0.03	1.665±0.03	1.69±0.04
	22 to 42	2.05±0.069	2.03±0.062	2.02±0.09
	1 to 42	1.92±0.05	1.92±0.06	1.92±0.09

F:G feed/gain ratio; *CON* control with no clinoptilolite added; *NCLI* 2 % natural clinoptilolite; *MCLI* 2 % modified clinoptilolite

presented in Table 3. There was no significant difference ($P > 0.05$) found on ADG, ADFI, and G:F in chickens either supplemented with NCLI or MCLI or without supplementation of natural clinoptilolite during the whole growth period of 42 days of age. It showed that there was no toxic effect of supplementation of NCLI and MCLI in broiler chickens.

The results of effects of clinoptilolite and modified clinoptilolite on the relative weights of immune organs spleen, thymus, and bursa of Fabricius on broilers are given in Table 4. The results of our present study showed that the relative weights of immune organs were not influenced by dietary supplementation of NCLI and MCLI when we compared them with control (CON) group at 21 and 42 days of age in broiler chickens.

The data of liver *i*NOS, TNOS, NO, antioxidant capacity, and lipid peroxidation in broiler chickens are shown in Table 5 and 6. There were no significant difference ($P > 0.05$) found in *i*NOS enzyme activity and NO concentrations of liver supplemented with NCLI and MCLI at 21 and 42 days age of broiler chickens. The liver TNOS enzyme activity was significantly decreased in the MCLI group (23.43 %) than CON group at 21 days ($P < 0.05$). There was no significant difference ($P > 0.05$) found in liver TNOS concentration between CON and NCLI groups at 42 days. It showed that both the NCLI and MCLI supplementation in poultry diets had no injurious effects on chicken health especially the liver and can be used in poultry diets.

The enzyme activity of GSH-Px in liver was significantly increased ($P < 0.05$) in NCLI (3.45 %) and MCLI (4.85 %) groups as compared to CON group in chickens at 21 days of age. The increased in the liver GSH-Px enzyme activity was 3.45 % in NCLI supplemented chickens, while in MCLI fed chickens, it was 4.85 %. Similarly, the liver CAT enzyme activity of broiler chickens was also significantly increased ($P < 0.05$) after supplementation of NCLI and MCLI in poultry diets as compared with chickens in CON group at 21 days of age. The increased in liver CAT enzyme activity was 16.90 % in NCLI supplemented chickens, while it was 33.47 % in MCLI supplemented chickens. However, when we compared NCLI and MCLI treatments, the CAT activity of liver was significantly ($P < 0.05$) increased in MCLI group than the NCLI group in chickens. The increased was 14.2 % in MCLI treatment as compared to NCLI supplemented chickens at 21 days of age. The NCLI and MCLI supplementations significantly increased ($P < 0.05$) the T-SOD enzyme activity of liver in broiler chickens at 21 days of age. The increased was 2.5 % in NCLI supplemented chickens, while it was 3.56 % in MCLI supplemented chickens as compared to the CON group. We also found that the T-AOC of liver was significantly increased ($P < 0.05$) in NCLI and MCLI supplemented

Table 4 Effects of natural clinoptilolite and modified clinoptilolite on the relative weights of immune organs of broiler chickens

Age	Relative organ weight (g/kg)	Dietary treatments		
		CON	NCLI	MCLI
21 days	Spleen	0.708±0.028	0.744±0.030	0.835±0.191
	Thymus	2.159±0.036	2.197±0.033	2.206±0.070
	Bursa of Fabricius	1.888±0.393	1.912±0.047	2.006±0.036
42 days	Spleen	1.189±0.036	1.197±0.004	1.209±0.027
	Thymus	2.447±0.059	2.556±0.110	2.627±0.111
	Bursa of Fabricius	1.705±0.201	1.761±0.331	1.768±0.018

CON control with no clinoptilolite added; *NCLI* 2 % natural clinoptilolite; *MCLI* 2 % modified clinoptilolite

Table 5 Effects of natural clinoptilolite and modified clinoptilolite on liver *i*NOS, TNOS, and NO concentrations in broiler chickens

Age (days)	Items	Dietary treatments		
		CON	NCLI	MCLI
21	*i*NOS (U/mgprot)	0.515±0.048	0.417±0.025	0.416±0.027
	TNOS (U/mgprot)	0.790±0.049[a]	0.697±0.023[ab]	0.640±0.037[b]
	NO (μmol/gprot)	0.579±0.066	0.553±0.121	0.436±0.069
42	*i*NOS (U/mgprot)	0.415±0.017	0.394±0.019	0.364±0.028
	TNOS (U/mgprot)	0.614±0.061	0.581±0.027	0.562±0.034
	NO (μmol/gprot)	0.497±0.019	0.473±0.039	0.4217±0.033

Means with different letters within a row differ significantly ($P<0.05$)

chickens when we compared it with the CON group in broiler chickens. The increased in liver T-AOC was 7.09 % in NCLI and 7.87 % in MCLI supplemented chickens at 21 days of age. The results of our present study showed that the MDA concentration in liver was significantly ($P<0.05$) decreased in NCLI (6.80 %) and MCLI (9.71 %) groups as compared with the CON group in broilers. It showed that MCLI supplementation was more effective to increase the antioxidant capacity and reduced the production of MDA concentration in liver of broiler chickens as compared to NCLI and CON groups at 21 days of age.

At 42 days of age, the liver GSH-Px enzyme activity was significantly increased ($P<0.05$) after supplementation of NCLI and MCLI in broiler chickens as compared to the CON group. The increased in the liver GSH-Px enzyme activity was 5.71 % in NCLI while it was 6.75 % in MCLI supplemented broilers chickens. The CAT enzyme activity of liver was significantly increased ($P<0.05$) in both NCLI and MCLI supplemented broiler chickens as compared to the CON group chickens. The increased in the liver CAT enzyme activity was 4.93 and 9.99 % in NCLI and MCLI supplemented broiler chickens, respectively. However, when we compared the liver CAT enzyme activity in MCLI group with NCLI group, we found that it was significantly increased ($P<0.05$) in MCLI group (4.81 %) than the NCLI group. The T-SOD enzyme activity of liver was significantly increased ($P<0.05$) in NCLI and MCLI supplemented chickens at 42 days of age. The increased in the liver T-SOD enzyme activity was 3.14 % in NCLI and 4.42 % in MCLI supplemented broiler chickens as compared with CON group. The T-AOC of liver was significantly increased ($P<0.05$) in NCLI (6.04 %) and MCLI (7.38 %) groups as compared to the CON group in broilers. The MDA concentration of liver was significantly decreased ($P<0.05$) in both NCLI (11.65 %) and MCLI (14.56 %) groups as compared to the CON group in broilers. The decrease in liver MDA concentration was more in MCLI supplemented chickens as compared to NCLI and CON broiler chickens at 42 days of age. It showed that MCLI supplementation in poultry diets was more beneficial as compared to NCLI supplementation for enhancing antioxidant capacity and reducing lipid peroxidation.

Discussion

Researchers are exploiting chemicals that act as antioxidants that are nontoxic and not detrimental for health. It has been reported that zeolites are promising additives in poultry and other animals. Clinoptilolites are the most abundant forms of

Table 6 Effects of clinoptilolite and modified clinoptilolite on antioxidant capacity and MDA concentrations in broiler chickens

Age (days)	Items	Dietary treatments[1]		
		CON	NCLI	MCLI
21	GSH-Px (U/mgprot)	295.08±0.85[a]	305.26±6.54[b]	309.39±2.51[b]
	CAT (U/mgprot)	7.23±0.16[a]	8.45±0.10[b]	9.65±0.15[c]
	T-SOD (U/mgprot)	246.11±1.86[a]	252.22±1.75[b]	254.88±1.69[b]
	T-AOC (U/mgprot)	1.27±0.03[a]	1.36±0.031[b]	1.37±0.19[b]
	MDA (nmol/mgprot)	1.03±0.01[a]	0.96±0.02[b]	0.93±0.03[b]
42	GSH-Px (U/mgprot)	292.78±4.43[a]	309.51±5.09[b]	312.55±6.42[b]
	CAT (U/mgprot)	9.11±0.15[a]	9.56±0.14[b]	10.02±0.10[c]
	T-SOD (U/mgprot)	248.03±2.69[a]	255.83±1.95[b]	258.99±2.47[b]
	T-AOC (U/mgprot)	1.49±0.02[a]	1.58±0.02[ab]	1.60±0.05[b]
	MDA (nmol/mgprot)	1.03±0.02[a]	0.91±0.03[b]	0.88±0.03[b]

Means with different letters within a row differ significantly ($P<0.05$)

zeolites. It has been reported that there are no toxic effects of clinoptilolite in layers and has influenced on egg production [21]. To our knowledge, this is the first study on the effects of dietary supplementation of the natural clinoptilolite and clinoptilolite modified by formic acid on the growth performance, antioxidant capacity, and lipid peroxidation in the liver of broiler chickens.

In our present study, there was no influence of supplementation of both NCLI and MCLI on growth performance in broiler chickens at 42 days of age. The study of Oliver has shown that adding zeolite into layer diets increase feed intake in laying hens [22], while the study of Roland et al. has concluded that there is no significant effect on feed intake in laying hens fed diets supplemented with zeolites [23]. However, Miles et al. has shown that adding zeolite into diets has adverse effect on feed intake [24]. Evans [25] has concluded that except in few experiments, there is slight improvement in growth performance as a result of zeolite supplementation in the diets. Most of the experiments show that there was no significant improvement in growth performance in different species of animals. There are very few experiments even show that the zeolite has adverse effects in animals. The exact reason is still unknown on how clinoptilolite will affect the palatability in animals. Further molecular studies are required to understand the exact mechanism. On the other hand, clinoptilolite has adverse affects on food intake. It might be due to imbalance of diets. Adding clinoptilolite up to 10 % in the diets leads to changes in dietary composition that eventually imbalance the energy, protein, and amino acid contents of the diets. The addition of zeolites also changes the calcium, aluminum, sodium, and other mineral contents which could affect mineral imbalance.

The bursa of Fabricius and thymus are two primary or central lymphoid organs of the immune system. These organs produce T cells and B cells and play vital role in cellular and humoral immunity [26]. The spleen, another main peripheral lymphoid organ of systemic immunity in birds, plays an important role in disease resistance [27]. The relative weights of these immune organs in broiler chickens were increased in both NCLI and MCLI groups than the CON group at 21 and 42 days, although there were no statistical significant differences between groups. Previous studies reported that the supplemental clays and zeolites did not significantly affect the relative weights of the spleen, liver, and bursa of Fabricius during 21 days of feeding period in broiler chickens [28, 29]. In contrast, Prvulovic suggested that the supplementation with 5 g/kg of hydrated aluminosilicate influenced organ weights. This might be associated with slower passage of ingesta through the digestive tract [30]. The differences in the results might be due to the types, usage, and levels of zeolites in broiler chickens.

Nitric oxide is known as an important radical and cellular signaling molecule that plays an important role in cellular processes and diverse physiological including neurotransmission, platelet inhibition, vasodilation, inflammation, and host defense [31]. During infection, inflammatory cytokines elaborated by the immune system can stimulate a lot of cell types such as primed host macrophages to synthesize huge quantities of NO by an enzyme which is known as *i*NOS [32]. Nitric oxide synthase could catalyze the production of NO. Both *i*NOS and NO concentrations were not influenced by NCLI and MCLI supplementation in broiler chickens, while the TNOS enzyme activity was decreased in the MCLI group than CON group at 21 days in this experiment which indicates that adding clinoptilolite may relieve oxidative status through decreasing the activity of TNOS enzyme. Šverko et al. suggested that the zeolites show the ability to bind NO, 4-hydroxy-noneal, and oxygen which may also have an antioxidative effect [33]. Pavelic et al. reported that adding 3 mg micronized zeolite into the diet of mice would strongly decrease the production of NO by macrophages [13].

Free radicals can generate ROS in cells. It can contribute to cell and tissue damage in living body. The ability of a cell to keep functional homeostasis depends on the fast induction of protective antioxidant enzymes. The antioxidants may prevent these damages induced by oxidation of protein and lipid. The antioxidant enzymes include GSH-Px, SOD, and CAT. These are the three main antioxidant enzymes in the body, which remove unwanted $\bullet O_2^-$, ROOH, hydrogen peroxide (H_2O_2) and produced by free radicals. Superoxide dismutase catalyze superoxide radical dismutation; GSH concentration plays an important role in protecting cells against oxidative stress [34, 35]. Glutathione peroxidase decomposes H_2O_2 [36]. GSH and SOD protect cells against toxic compounds and oxygen radicals [37]. Decreased activity of these enzymes will induce increased free radicals and then lead to damage of the corresponding tissue. Malondialdehyde is an ending product of lipid peroxidation, so the amounts of MDA could be used to assess the extent of lipid peroxidation [38]. The activities of liver GSH-Px, CAT, and T-SOD were increased in the broiler chickens fed with 2 % NCLI and MCLI. The liver CAT activity was higher in MCLI supplemented chickens than NCLI supplemented chickens at 21 days. Malondialdehyde concentration of the liver decreased in broiler chickens when supplemented with 2 % NCLI and MCLI in poultry diets. These findings suggested that the NCLI and MCLI supplementation in broilers is beneficial to protect tissues against lipid peroxidation. A previous study of Saribeyoglu reported that the diets supplemented with 5 mg/kg of clinoptilolite for 10 days lowered the MDA concentrations of plasma and liver tissues of rats. The liver GSH concentration and Cu–Zn SOD enzyme activity of rats were also higher in without supplemented clinoptilolite than clinoptilolite supplemented group. It is suggested that the adding of clinoptilolite may support antioxidant system [39]. Wang et al. found that the supplementation with zinc-bearing clinoptilolite increased SOD enzyme activity in ileal mucosa and decreased intestinal

MDA concentrations, thus reducing the oxidative stress induced by Salmonella infection. These results are in agreement with our results [40]. Yarovan also found that plasma MDA concentration was lowered in dairy cows that have been supplemented with zeolite under oxidative stress induced by diseases [41].

In conclusion, from our animal health point of view, the 2 % inclusion levels of both NCLI and MCLI in poultry diets in this study threatened neither the chicken's growth performance nor the relative weights of immune organs but their utilization could have beneficial impacts on antioxidant capacity which would lead to better health in broiler chickens. So, natural clinoptilolite and its modified product can be used as new antioxidant agents in poultry feed.

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References

1. Celi P (2010) The role of oxidative stress in small ruminants' health and production. Rev Bras Zootec 39:348–363
2. Wang Y, Wang J, Wang C, Chen B, Liu J, Cao H, Guo F, Vázquez-Añón M (2010) Effect of different rumen-inert fatty acids supplemented with a dietary antioxidant on performance and antioxidative status of early lactation cows. J Dairy Sci 93:3738–3745
3. Thiruvenkatachari R, Vigneswaran S, Naidu R (2008) Permeable reactive barrier for groundwater remediation. J Ind Eng Chem 14:145–156
4. Mumpton F, Fishman P (1977) The application of natural zeolites in animal science and aquaculture. J Anim Sci 45:1188–1203
5. Galarnau A, Di Renzo F, Faujula F, Vedrine J (2011) Clinoptilolite-heulandite: applications and basic research.
6. Papaioannou D, Katsoulos P, Panousis N, Karatzias H (2005) The role of natural and synthetic zeolites as feed additives on the prevention and/or the treatment of certain farm animal diseases: a review. Microporous Mesoporous Mater 84:161–170
7. Tiwari J (2007) Zeolite as natural feed additives to reduce environmental impacts of swine manure. McGill University.
8. Tao YF, Qiu Y, Fang SY, Liu ZY, Wang Y, Zhu JH (2010) Trapping the lead ion in multi-components aqueous solution by natural clinoptilolite. J Hazard Mater 180:282–288
9. Oguz H (2011) A review from experimental trials on detoxification of aflatoxin in poultry feed. Eurasian J Vet Sci 27:1–12
10. Pavelić K, Hadžija M, Bedrica L, Pavelić J, Đikić I, Katić M, Kralj M, Bosnar MH, Kapitanović S, Poljak-Blaži M (2001) Natural zeolite clinoptilolite: new adjuvant in anticancer therapy. J Mol Med 78:708–720
11. Zarcovic N, Zarcovic K, Kralj M, Borovic S, Sabolovic S, Blazi MP, Cipak A, Pavelic K (2003) Anticancer and antioxidative effects of micronized zeolite clinoptilolite. Anticancer Res 23:1589–1596
12. Visnja S, Sandra S, Tihomir B, Miroslav C, Tatjana M (2004) Natural micronised clinoptilolite and clinoptilolite mixtures with *Urtica dioica* L. extract as possible antioxidants. Food Technol Biotechnol 42(3):189–192
13. Pavelic K, Katic M, Sverko V, Marotti T, Bosnjak B, Balog T, Stojkovic R, Radacic M, Colic M, Poljak-Blazi M (2002) Immunostimulatory effect of natural clinoptilolite as a possible mechanism of its antimetastatic ability. J Cancer Res Clin 128:37–44
14. Jung BG, Toan NT, Cho SJ, Ko J, Jung YK, Lee BJ (2010) Dietary aluminosilicate supplement enhances immune activity in mice and reinforces clearance of porcine circovirus type 2 in experimentally infected pigs. Vet Microbiol 143:117–125
15. Cruz-Guzman M, Celis R, Hermosin M, Koskinen W, Nater E, Cornejo J (2006) Heavy metal adsorption by montmorillonites modified with natural organic cations. Soil Sci Soc Am J 70:215–221
16. Ghiaci M, Kia R, Abbaspur A, Seyedeyn-Azad F (2004) Adsorption of chromate by surfactant-modified zeolites and MCM-41 molecular sieve. Sep Purif Technol 40:285–295
17. Pérez Cordoves AI, Granda Valdés M, Torres Fernández JC, Pina Luis G, García-Calzón J, Díaz García ME (2008) Characterization of the binding site affinity distribution of a surfactant-modified clinoptilolite. Microporous Mesoporous Mater 109:38–48
18. Mallek Z, Fendri I, Khannous L, Hassena AB, Traore AI, Ayadi M-A, Gdoura R (2012) Effect of zeolite (clinoptilolite) as feed additive in Tunisian broilers on the total flora, meat texture and the production of omega 3 polyunsaturated fatty acid. Lipids Health Dis 11(1):1–7
19. Al-Nasser AY, Al-Zenki SF, Al-Saffar AE, Abdullah FK, Al-Bahouh ME, Mashaly M (2011) Zeolite as a feed additive to reduce Salmonell and improve production performance in broilers. Int J Poult 10:448–454
20. Safaeikatouli M, Jafariahangari Y, Baharlouei A (2011) An evaluation on the effects of dietary kaolin and zeolite on broilers blood parameters, T4, TSH and growth hormones. Pak J Nutr 10:233–237
21. Argun ME (2008) Use of clinoptilolite for the removal of nickel ions from water: kinetics and thermodynamics. J Hazard Mater 150(3):587–595
22. Oliver M (1989) Effect of feeding clinoptilolite (zeolite) to three strains of laying hens. Br Poult Sci 30(1):115–121
23. Roland SD, Rabon H, Frost T, Laurent S, Barnes D (1990) Response of commercial Leghorns to sodium aluminosilicate when fed different levels and sources of available phosphorus. Poult Sci 69:2157
24. Miles R, Harms R, Laurent S (1986) Influence of sodium zeolite A (Ethacal) on laying hen performance. Nutr Rep Int 34:1097–1103
25. Evans M (1989) Zeolites: do they have a role in poultry production
26. Sharma J (1999) Introduction to poultry vaccines and immunity. Adv Vet Med 41:481–494
27. John JL (1994) The avian spleen: a neglected organ. Q Rev Biol 69:327–351
28. Ledoux D, Rottinghaus G, Bermudez A, Alonso-Debolt M (1999) Efficacy of a hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poult Sci 78(2):204–210
29. Kececi T, Oguz H, Kurtoglu V, Demet O (1998) Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br Poult Sci 39(3):452–458
30. Prvulovic D, Kojic D, Grubor-Lajsic G, Kosarcic S (2008) The effects of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. Turk J Vet Anim Sci 32(3):183
31. Bredt D, Snyder S (1994) Nitric oxide: a physiologic messenger molecule. Annu Rev Biochem 63:175–195
32. Liew F, Cox F (1991) Nonspecific defence mechanism: the role of nitric oxide. Immunol Today 12:A17–A21
33. Šverko V, Sobočanec S, Balog T, Čolić M, Marotti T (2004) Natural micronized clinoptilolite and clinoptilolite mixtures with 40% *Urtica*

dionica L. extract as possible antioxidants. Food Technol Biotech 42:189–192
34. Ermak G, Davies KJ (2002) Calcium and oxidative stress: from cell signaling to cell death. Mol Immunol 38:713–721
35. Sies H (1999) Glutathione and its role in cellular functions. Free Radic Biol Med 27:916–921
36. Wills E (1966) Mechanisms of lipid peroxide formation in animal tissues. Biochem J 99:667
37. Nita DA, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L (2001) Oxidative damage following cerebral ischemia depends on reperfusion–a biochemical study in rat. J Cell Mol Med 5:163–170
38. Satoshi S, Kiyoji T, Hiroyo K, Fumio N (1989) Exercise-induced lipid peroxidation and leakage of enzymes before and after vitamin E supplementation. Int J Biochem 21:835–838
39. Wang L, Zhang T, Wen C, Jiang Z, Wang T, Zhou Y (2012) Protective effects of zinc-bearing clinoptilolite on broilers challenged with *Salmonella pullorum*. Poult Sci 91(8):1838–1845
40. Saribeyoglu K, Aytac E, Pekmezci S, Saygili S, Uzun H, Ozbay G, Aydin S, Seymen HO (2011) Effects of clinoptilolite treatment on oxidative stress after partial hepatectomy in rats. Asian J Surg 34(4):153–157
41. Yarovan NI (2008) Effect of zeolites on adaptation processes in cows. Russ Agric Sci 34:120–122