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# Impact of various dietary levels of zeolite on broiler performance, digestibility, and carcass traits

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## Abstract

The objective of this study was to evaluate the use of clinoptilolite zeolite to enhance performance in broiler chickens. Nine hundred male broiler chickens were distributed in a completely randomized design to four treatments with nine repetitions. Treatments included clinoptilolite zeolite in the diet at 0, 2500, 5000 and 10000 grams/ton. Inclusion of zeolite caused a linear increase in ammonia released by the litter after 21 days, with greater effects at 42 days. The digestibility coefficient for dry matter increased, whereas the digestibility coefficients for crude protein, gross energy, and ash were not affected. Zeolite affected the digestibility of dry matter, gross energy and ash, with digestible gross energy and digestible ash showing a quadratic effect. The same effect was shown in the percentage of abdominal fat and the relative weight of the pancreas. Zeolite did not change the performance, serum levels and quality of the litter at 42 days old. It improved the percentage of digestible nutrients and increased pancreas weight.

**Keywords:** histopathology, litter quality, nutrition, serum levels
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## Introduction

Zeolites are minerals whose structure is composed of channels, which largely determine their important properties, including capacity for cation exchange, adsorption, hydration and dehydration (Alvarez *et al.*, 2019). The use of zeolites can influence feed efficiency as a result of a reduced rate of passage in the gastrointestinal tract, immobilization of enzymes, and alteration of the intestinal microflora (Wu *et al.*, 2013). In addition, zeolites may affect apparent and true metabolizable energy, digestible protein, and the number, size, and shape of intestinal villi (Wawrzyniak *et al.*, 2017).

The ability of zeolite to retain water could improve litter conditions. Schneider *et al.* (2017) reported that consumption of zeolite resulted in a lower moisture content in the excreta and therefore in the litter. Zeolite has been included in diets for poultry at various rates. The recommended value for synthetic zeolite is approximately 1% (Papaioannou *et al.*, 2005). For natural zeolite, rates of inclusion as high as 10% have been reported (García, 2010). These differences might reflect the physical and chemical characteristics of natural and synthetic zeolites and impurities in natural zeolites.

Thus, the aim of this study was to evaluate the inclusion of various rates of natural clinoptilolite zeolite in rations for broiler chickens. Effects on growth performance, relative organ weight, liver damage, blood parameters, and carcass and cut yield were reported in addition to effects on the characteristics of the litter on which the birds were kept.

## Material and Methods

This research was based on the rules of the National Council for Animal Control and Experimentation and approved by the Ethics Committee on the Use of Animals (number 43-18).

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The experimental aviary was 20 m long and 8 m wide. Its interior was divided into 36 pens (experimental units) of 1.76 $m^2$. Each pen had a tubular feeder, nipple drinker, concrete floor with a layer of about 10 cm of pinewood shavings prior to being used, and heating source (250 watts resistance).

A total of 900 one-day-old male Cobb 500 broiler chickens with initial mean weight of 43.56 ± 0.73 g were assigned to a completely randomized design with nine replications of four treatments. The diets were formulated with clinoptilolite zeolite at rates of 0, 2500, 5000 and 10000 grams/ton. The experiment was conducted in November and December 2019. The environmental conditions, temperature, humidity, and light schedule were in accordance with the supplier's guidelines (Cobb-Vantress, 2018).

The rations were based on corn and soybean meal and were supplied in mash form. Nutritional requirements were based on the recommendations used by the Brazilian industry for phases 1 to 7 (pre-initial), 8 to 21 (initial), 22 to 28 (growth 1), 29 to 35 (growth 2) and 36 to 42 days old (finisher). Clinoptilolite zeolite of Brazilian origin replaced sand in the ration on a weight basis. Finisher rations were supplemented with 1 kg $ton^{-1}$ of celite, to determine the indigestibility coefficient.

Feed intake (FI) and bodyweight gain (BWG) were evaluated for the periods from 1 to 7 days, 1 to 21 days and 1 to 42 days old by weighing birds and leftover feed. Feed conversion ratio (FCR) was calculated. Mortality was recorded daily to enable corrections in feed conversion and feed consumption (Sakomura & Rostagno, 2016).

At 21 and 41 days old, two birds were randomly selected from each pen. A blood sample was obtained and serum was harvested according to Nunes *et al.* (2018). The readings of the serum levels of AST (aspartate aminotransferase), ALT (alanine aminotransferase), gamma GT (gamma glutamyl transferase), creatinine, total protein, and albumin were performed with an Elitech Flexor EL 200 automatic biochemical analyser with spectrophotometry (Diamond Diagnostics Inc., Holliston, Massachusetts, USA), using reagents from the same supplier.

To determine the relative organ weight of the gastrointestinal tract at 21 and 42 days old, two birds per pen were euthanized and necropsied. The liver was weighed and a 1 $cm^2$ larger lobe sample was fixed in 10% buffered formalin for histopathological evaluation. The liver samples were scored according to severity, with numbers ranging from 0 (normal condition) to 3 (marked changes). Livers classified as 0 had no alterations, discreet diffuse hydropic degeneration, and discreet diffuse fatty degeneration. Those classified as 'mild' presented mild diffuse fatty degeneration, mild focal cholestasis, mild lymphohistiocytic and multifocal granulocytic pericolangitis, mild bile duct hyperplasia and mild lymphohistiocytic perivasculitis. Those classified as 'moderate' showed moderate diffuse atrophy, pericolangitis and moderate lymphohistioplasmocytic cholangitis, mild focal cholangionecrosis, moderate bile duct hyperplasia, moderate diffuse granulocytic perivasculitis, mild necrotic hepatitis with mild lymphohistiocytic infiltrate and mild haemorrhagic inflammation and congestion. Livers classified as 'severe' presented marked diffuse congestion and bile duct hyperplasia.

Samples of the litter were collected to determine its dry matter and ammonia contents after 24 and 40 days. Ammoniacal nitrogen was determined according to Hernandes & Gazetta (2001). Dry matter was measured as prescribed in AOAC technique 930.15 (AOAC, 1990).

Intestinal morphometry was evaluated at 28 days old using three birds per pen. After being euthanized, two centimetre samples were removed from the jejunum and ileum to measure villus and crypt height. Crypts were measured from their base to the region of transition to the villus. Villus height was measured from its basal region to its peak, following the methodology of Luna (1968).

At 42 days old, three birds per pen were randomly selected to assess carcass yield, weight of commercial cuts, and percentage of abdominal fat (AF). Broiler chickens were fasted for six hours with free access to water, weighed individually, stunned by electronarcosis, and exsanguinated. The carcasses were then scalded, plucked, and gutted. Carcass yield consisted of the eviscerated carcass weight (without the feet, head, and neck) in relation to the live weight of the bird. The weights were recorded of boneless and skinless breast (*Pectoralis major*, *Pectoralis minor)*, legs (thigh and drumstick) and wings. The fat deposited around the cloacal bursa, gizzard, proventricle, and adjacent abdominal muscles was weighed as a measure AF. Cut yields were calculated in relation to the weight of eviscerated carcass, and the percentage of abdominal fat was relative to live weight of the bird.

The ileal content was collected and pre-dried, and excreta and feed samples were ground and analysed for dry matter (DM), crude protein (CP) and ash (methodologies 930.15, 954.01 and 942.05 of AOAC, 1990). To determine the gross energy (GE), samples were combusted in a IKA C 200 calorimeter (IKA Works, Inc., Wilmington, North Carolina, USA). To determine the indigestibility factor, an analysis of acid insoluble ash (AIA) was performed according to the methodology described by Van Keulen & Young (1977), adapted by Carvalho *et al.* (2013). Total digestible nutrients were determined from the digestible insoluble acid ash (dAIA), digestible crude protein (dCP), digestible gross energy (dGE), digestible ash (dAsh), and digestible dry matter (dDM) and later from the digestibility coefficients of dry matter (DCDM), crude protein (DCCP), gross energy (DCGE) and ash (DCAsh).

The data were analysed using analysis of variance. The mathematical model was:

$$y_{ij} = \mu + t_i + e_{ij}$$

where: $y_{ij}$ = an observation, $\mu$ = general mean, $t_i$ = diet effect, and $e_{ij}$ = random residual error. For significant effects, the means were compared with the Student–Newman–Keuls procedure at the 5% level of probability. Means were also compared using polynomial contrasts ($P$ =0.05). For histopathological analyses, Wilcoxon nonparametric test was used at 5% of probability.

## Results and Discussion

The inclusion of zeolite in the diet for broiler chickens did not affect ($P$ >0.05) FI, BWG and FCR from 1 to 21 days and from 1 to 42 days old (Table 1). Similarly, Ferreira *et al.* (2005) did not observe significant differences in these variables when feeding 1% aluminosilicate to broiler chickens. However, the use of aluminosilicate showed positive effects in productive performance when birds were given feed contaminated with aflatoxins (Attia *et al.*, 2013; 2016). This was attributed to its binding capacity. Karamanlis *et al.* (2008) added 2% clinoptilolite to the ration for broiler chickens and observed that birds that received the augmented diet had greater BWG during the entire rearing period compared with the basal diet. Increase in BWG of broiler chickens that consume zeolite could depend on the type of zeolite, its purity and physicochemical properties, and the rate of supplementation (Papaioannou *et al.*, 2005). The lack of response in the present study might be attributable to the low level of zeolite in the feed.

**Table 1** Growth performance of broiler chickens fed diets containing clinoptilolite zeolite

| Inclusion level, g/ton | 21 days | | | 42 days | | |
|---|---|---|---|---|---|---|
| | FI, g | BWG, g | FCR | FI, g | BWG, g | FCR |
| 0 | 1321 | 1164 | 1.143 | 5046 | 3193 | 1.581 |
| 2500 | 1353 | 1157 | 1.169 | 4983 | 3148 | 1.584 |
| 5000 | 1340 | 1153 | 1.162 | 5089 | 3209 | 1.574 |
| 10000 | 1321 | 1154 | 1.144 | 5162 | 3222 | 1.584 |
| *P*-value treatment effect | 0.3659 | 0.9045 | 0.2116 | 0.1549 | 0.6355 | 0.9578 |
| *P*-value linear effect | 0.5503 | 0.7773 | 0.6726 | 0.0945 | 0.2646 | 0.6171 |
| *P*-value quadratic effect | 0.4364 | 0.8209 | 0.1624 | 0.1824 | 0.5251 | 0.7351 |
| SE | 44.61 | 32.04 | 0.04 | 160.13 | 126.82 | 0.04 |
| CV, % | 3.34 | 2.77 | 2.68 | 3.16 | 3.97 | 2.67 |

FI: feed intake, BWG: bodyweight gain, FCR: feed conversion ratio, CV: coefficient of variation

Natural zeolites have been used to control ammonia production with variable success, depending on the physical properties of the materials (Karamanlis *et al.*, 2008). The level of zeolite in the birds' diet influenced ($P$ =0.0355) the amount of ammonia released from the litter after 24 days. A linear increase ($P$ =0.0279) was also observed in the amount of ammonia released from the litter with zeolite inclusion after the birds had been housed for 40 days (Table 2). These results are contrary to those of Karamanlis *et al.* (2008), who evaluated the inclusion of 2% of natural zeolites in the diet of broiler chickens at 14 days old, and showed a decrease in ammonia emissions from the litter in the pens of birds supplemented with zeolite. However, as in the present study, the DM content of the litter did not differ between treatments. Decreased ammonia emission could be attributed to the positive effect of zeolite on the use of nutrients in increased adsorption of protein nitrogen.

**Table 2** Dry matter content and ammonia released from the litter housing broiler chickens fed diets containing clinoptilolite zeolite for 24 and 40 days

| Inclusion level, g/ton | 24 days | | 40 days | |
|---|---|---|---|---|
| | $NH_3$, mg/100 g | Dry matter, % | $NH_3$, mg/100 | Dry matter, % |
| 0 | 1.26[ab] | 67.65 | 1.37 | 67.95 |
| 2500 | 1.05[b] | 65.71 | 1.75 | 69.04 |
| 5000 | 1.42[a] | 69.02 | 1.57 | 65.44 |
| 10000 | 1.33[ab] | 67.22 | 2.01 | 70.39 |
| *P*-value treatment effect | 0.0355 | 0.6202 | 0.1119 | 0.3026 |
| *P*-value linear effect | 0.2373 | 0.8885 | 0.0279[1] | 0.4195 |
| *P*-value quadratic effect | 0.4925 | 0.9711 | 0.0904 | 0.3687 |
| SE | 0.26 | 5.13 | 0.55 | 5.59 |
| CV, % | 20.75 | 7.62 | 33.12 | 8.20 |

$NH_3$: ammonia concentration
[a,b] Within a column, means with a common superscript did not differ with probability *P* =0.05
[1]$NH_3$ =1.44513 + 0.00005664CZ ($R^2$ =0.1421), where CZ = clinoptilolite zeolite inclusion level

The amount of zeolite in the diet did not change DM content in the litter (Table 2). Similarly, Basha *et al.* (2016), found that various forms of zeolite did not produce a significant change in the moisture content of the litter, except when aluminosilicate was included in the diet. However, Schneider *et al.* (2017) observed that the amount of DM in the excreta of broiler chickens fed diets augmented with 0.5% zeolite was higher than that of birds that did not receive zeolite. Nikolakakis *et al.* (2013) included doses of 1%, 2% and 3% clinoptilolite zeolite in broiler chicken diets and found a significant decrease in the moisture content of the litter at 30 and 40 days old with 2% and 3% zeolite. Zeolite retains water by osmotic balance or by hydrating cations that are compensating for surface loads. Thus, it is a recommended feed additive for reducing moisture in excreta and thus in the litter (Schneider *et al.*, 2017). However, several other factors alter moisture content of the litter, including temperature, type of drinker, water consumption, feed, and ventilation (Oliveira *et al.*, 2015).

There was an effect (*P* =0.0394) of including clinoptilolite zeolite in the diet on DCDM (Table 3), in that birds that consumed diets without zeolite had lower DCDM compared with the ration that included 2500 g/ton of zeolite. Inclusion of zeolite did not influence the DCGE and DCCP of the diet. Zeolite has been said to increase feed digestibility and the performance of broiler chickens (Mumpton & Fishman, 1977), which reinforces the results of this research. However, when evaluating the effects of including 0%, 1.5% and 3% zeolite in the diet for broiler chickens, Safaeikatouli *et al.* (2012) found that 3% zeolite increased DCCP without altering DCGE. The reason for improved digestibility is the mode of action of mineral silicates, as they bond temporarily with the nutrients and reduce the gastrointestinal passage rate, thus allowing more time for digestion (Safaeikatouli *et al.*, 2012).

**Table 3** Digestibility of dry matter, crude protein, gross energy, and ash of broiler chickens fed with various rates of inclusion of clinoptilolite zeolite

| Inclusion level, g/ton | Dry matter, % | Crude protein, % | Gross energy, % | Ash, % |
|---|---|---|---|---|
| 0 | 66.93[b] | 65.23 | 70.27 | 36.03 |
| 2500 | 72.99[a] | 71.52 | 75.08 | 39.08 |
| 5000 | 69.06[ab] | 66.30 | 72.44 | 34.60 |
| 10000 | 69.20[ab] | 66.44 | 71.43 | 35.41 |
| *P*-value treatment effect | 0.0394 | 0.0837 | 0.0785 | 0.3198 |
| *P*-value linear effect | 0.6227 | 0.4807 | 0.9521 | 0.0999 |
| *P*-value quadratic effect | 0.6733 | 0.6143 | 0.2731 | 0.2563 |
| SE | 4.28 | 5.13 | 3.74 | 5.32 |
| CV, % | 6.16 | 7.72 | 5.17 | 14.67 |

[a,b] Within a column, means with a common superscript did not differ with probability *P* =0.05

The inclusion of various rates of zeolite was reflected in a difference in the variables dCP (*P* =0.0190), dGE (P =0.0198) and dAsh (P =0.0047). There were linear (*P* =0.0019) and a quadratic (*P* =0.0066) effects of zeolite on dAsh and a quadratic effect (P =0.0211) on dGE (Table 4). The results for indigestible CP, GE and ash differed from Macháček *et al.* (2010), who included 0%, 2% and 4% zeolite in the diet of laying hens, and did not observe differences in the amounts of CP, GE and ash in the excreta. However, Ferreira *et al.* (2005), included 1% alumino-silicate in the diet and obtained higher DM in the excreta, compared with the control group. Increased DM values and unchanged CP values in excreta were found by Schneider *et al.* (2017), who used 0.5% natural zeolites in broiler chicken diets.

**Table 4** Indigestible dry matter, crude protein, gross energy, and ash of diets with various rates of inclusion of clinoptilolite zeolite when fed to broiler chickens

| Inclusion level, g/ton | dDM (%) | dCP (%) | dGE, kcal/kg | dAsh (%) |
|---|---|---|---|---|
| 0 | 17.84 | 12.79$^{b}$ | 3,385$^{b}$ | 1.95$^{b}$ |
| 2500 | 17.54 | 14.29$^{a}$ | 3,595$^{ab}$ | 2.18$^{b}$ |
| 5000 | 17.49 | 13.13$^{b}$ | 3,619$^{a}$ | 2.09$^{b}$ |
| 10000 | 17.40 | 12.88$^{b}$ | 3,403$^{ab}$ | 2.53$^{a}$ |
| *P*-value treatment effect | 0.6575 | 0.0190 | 0.0198 | 0.0047 |
| *P*-value linear effect | 0.4237 | 0.2505 | 0.8009 | 0.0019[1] |
| *P*-value quadratic effect | 0.6223 | 0.2278 | 0.0211[3] | 0.0066[2] |
| SE | 0.73 | 1.02 | 183.05 | 0.31 |
| CV, % | 4.17 | 7.72 | 5.22 | 14.16 |

dDM: digestible dry matter; dCP: digestible crude protein; dGE: digestible gross energy; dAsh: digestible ash
$^{a,b}$ Within a column, means with a common superscript did not differ with probability *P* =0.05
[1]dAsh: 2.00221+0.00004811CZ; $R^2$ =0.2945
[2]dAsh: 2.05266 + 0.00000771CZ + 3.843671CZ$^2$; $R^2$ =0.3106
[3]dGE: 3395.10238 + 0.09721CZ – 0.00000951CZ$^2$; $R^2$ =0.2486

The rate at which zeolite was fed did not affect (*P* >0.05) intestinal histology at 28 days old (Table 5). However, Wu *et al.* (2013) found a difference in villus height and villus:crypt ratio of the jejunum and ileum at 21 days old, when they supplied 2% natural or modified zeolite to broiler chickens. Otherwise, Qu *et al.* (2019) evaluated inclusion of 10 g/kg of zeolite in the diet of broiler chickens and observed stimulation of growth of the villi in the small intestine. However, neither of these authors found any effect on crypt depth.

**Table 5** Villus height, crypt depth and their ratio for samples of the jejunum and ileum from 28-day old broiler chickens fed rations that included various amounts of clinoptilolite zeolite

| Inclusion level, g/ton | Jejunum | | | Íleum | | |
|---|---|---|---|---|---|---|
| | VH, µm | CD, µm | V:C | VH, µm | CD, µm | V:C |
| 0 | 726.91 | 36.24 | 19.9 | 641.98 | 29.8 | 22.95 |
| 2500 | 758.79 | 33.64 | 22.84 | 640.53 | 27.89 | 23.44 |
| 5000 | 732.25 | 33.8 | 20.18 | 686.18 | 30.68 | 21.63 |
| 10000 | 688.87 | 30.23 | 24.8 | 630.65 | 26.96 | 23.35 |
| *P*-value treatment effect | 0.6993 | 0.3577 | 0.0985 | 0.8122 | 0.7254 | 0.8492 |
| SE | 114.76 | 6.51 | 4.31 | 129.72 | 7.32 | 4.77 |
| CV, % | 15.78 | 19.34 | 19.83 | 19.94 | 25.36 | 20.89 |

VH: Villus height; CD: Crypt depth; V:C ratio of villus height to crypt depth

The use of zeolite did not influence (P >0.05) carcass and cut yield at 42 days old (Table 6). However, it influenced the percentage of AF (P =0.0359), generating a quadratic effect (P =0.0123). Similar results were obtained by Loft Elahian *et al.* (2004), who reported that the use of 2% zeolite decreased abdominal fat, but 4% and 6% zeolite increased the amount of abdominal fat in broiler chickens at 42 days old. Possibly zeolite increased the digestibility of some nutrients. Basha *et al.* (2016) provided zeolite in various quantities and ways (100 $g/m^2$ suspended, 15 g $kg^{-1}$ in the diet, and 100 g $kg^{-1}$ in the litter), and did not observe significant changes (P >0.05) in carcass yield. Similarly, Safaeikatouli *et al.* (2012) did not observe differences (P >0.05) in the yields of carcass, breast, and legs of birds receiving 0%, 1.5% and 3% zeolite in the diet.

**Table 6** Carcass and cuts yield (%) of broiler chickens fed diets containing various rates of inclusion of clinoptilolite zeolite at 42 days old

| Inclusion level, g/ton | HCY | CCY | PMJ | LQ | Wing | PM | AF |
|---|---|---|---|---|---|---|---|
| 0 | 70.25 | 71.56 | 27.20 | 30.81 | 9.65 | 5.44 | $1.62^{b}$ |
| 2500 | 70.04 | 71.07 | 27.47 | 30.82 | 9.52 | 5.40 | $1.81^{a}$ |
| 5000 | 70.18 | 71.09 | 26.95 | 31.21 | 9.85 | 5.49 | $1.84^{a}$ |
| 10000 | 70.62 | 71.01 | 27.76 | 30.57 | 9.57 | 5.42 | $1.71^{ab}$ |
| *P*-value treatment effect | 0.7156 | 0.7870 | 0.4789 | 0.4635 | 0.5910 | 0.9143 | 0.0359 |
| *P*-value linear effect | 0.5660 | 0.7889 | 0.4896 | 0.9881 | 0.6740 | 0.8046 | 0.5832 |
| *P*-value quadratic effect | 0.7207 | 0.6469 | 0.6450 | 0.2906 | 0.3131 | 0.9593 | 0.0123[1] |
| SE | 1.12 | 1.28 | 1.04 | 0.83 | 0.54 | 0.27 | 0.16 |
| CV, % | 1.59 | 1.80 | 3.82 | 2.69 | 5.56 | 4.90 | 9.14 |

HCY: hot carcass yield; CCY: cold carcass yield; PMJ: *Pectoralis major*; PM: *Pectoralis minor*; AF: abdominal fat

[a,b] Within a column, means with a common superscript did not differ with probability *P* =0.05

[1] $AF = 1.61478 + 0.00009148CZ - 8.2437CZ^2$; $R^2 = 0.2694$.

The relative weight of the pancreas showed a difference (P =0.0327) in birds at 42 days old (Table 7), presenting a quadratic effect (P =0.0117) with the inclusion of clinoptilolite zeolite. Zeolite inclusion did not influence organ weight at 21 days. However, the weight of the pancreas increased at 42 days old. Safameher (2008) supplied 2% zeolite to broiler chickens, and did not obtain significant results for organ weight. However, Ortatatli & Oguz (2001) included 0%, 1.5% and 2.5% of clinoptilolite in a broiler chicken diet, and reported that the inclusion of 2.5% zeolite caused a significant decrease in liver weight at 21 days old compared with the inclusion of 1.5%. Rosa *et al.* (2001) incorporated 0.3% sodium bentonite in the birds' diet and also observed a reduction in liver weight at 21 days old. Oliveira *et al.* (2015) found no difference in the relative weights of pancreas, liver, and intestine at 21 and 35 days old when testing various doses of glucomannan in broiler chickens. These results are similar to those in this study at 21 days old, but differed for relative weight of pancreas at 42 days. The ]pancreas is one of the main engines of the digestive system, where enzymatic production occurs for the digestion of carbohydrates, fats, and proteins. The supply of zeolite in the feed may have influenced the production of enzymes, thus increasing the weight of the pancreas.

There were no differences in histological lesions of the liver for the zeolite doses. However, Safameher (2008) observed a reduction in liver damage with 2% clinoptilolite zeolite. Ortatatli % Oguz (2001) added 1.5% to 2.5% clinoptilolite in diets containing 2.5 mg $kg^{-1}$ of aflatoxin, and observed a reduction in the incidence of severe damage to the liver in broiler chickens.

**Table 7** Relative organ weight (%) of broiler chickens at 21 and 42 days old, fed with diets containing clinoptilolite zeolite

| Inclusion level, g/ton | 21 days | | 42 days | |
|---|---|---|---|---|
| | Liver | Pancreas | Liver | Pancreas |
| 0 | 2.270 | 0.264 | 1.629 | 0.140[b] |
| 2500 | 2.346 | 0.305 | 1.673 | 0.151[ab] |
| 5000 | 2.256 | 0.284 | 1.657 | 0.157[a] |
| 10000 | 2.357 | 0.278 | 1.629 | 0.144[ab] |
| *P*-value treatment effect | 0.5454 | 0.3363 | 0.8601 | 0.0327 |
| *P*-value linear effect | 0.4581 | 0.9237 | 0.8300 | 0.6398 |
| *P*-value quadratic effect | 0.7238 | 0.4382 | 0.7419 | 0.0117[1] |
| SE | 0.18 | 0.05 | 0.13 | 0.01 |
| CV, % | 7.88 | 16.56 | 7.95 | 8.31 |

[a,b] Within a column, means with a common superscript did not differ with probability $P = 0.05$
[1]Pancreas: $0.13951 + 0.00000623CZ - 5.7382CZ^2$; $R^2 = 0.2361$.

The rate of inclusion of zeolite in the diets did not alter the metabolites in the serum of broiler chickens (Table 8). Thus, this study corroborates the results of Watts *et al.* (2003), who supplied hydrated calcium and phosphorus alumino-silicate to birds. Rocha *et al.* (2012) supplemented diets for pigs with seven rates of clinoptilolite zeolite and did not observe any influence on serum constituents. In addition, Maciel *et al.* (2007) found that 0%, 0.25% and 0.5% clinoptilolite in diets for broiler chickens did not alter the protein contents of serum.

**Table 8** Serum concentration of blood metabolites in 21- and 42-day-old broiler chickens fed diets containing clinoptilolite zeolite

| Inclusion level, g/ton | ALB ($g\ L^{-1}$) | TP ($g\ L^{-1}$) | CREA ($mg\ dL^{-1}$) | ALT ($UI\ L^{-1}$) | AST ($UI\ L^{-1}$) | GGT ($UI\ L^{-1}$) |
|---|---|---|---|---|---|---|
| | 21 days old | | | | | |
| 0 | 15.20 | 25.54 | 0.21 | 10.74 | 202 | 19.35 |
| 2500 | 14.86 | 25.32 | 0.21 | 11.38 | 167 | 18.64 |
| 5000 | 14.63 | 25.00 | 0.21 | 11.60 | 200 | 17.34 |
| 10000 | 15.33 | 26.53 | 0.23 | 11.68 | 191 | 20.90 |
| *P*-value treatment effect | 0.7772 | 0.5932 | 0.3007 | 0.9723 | 0.3571 | 0.7329 |
| *P*-value linear effect | 0.9565 | 0.3702 | 0.1711 | 0.8834 | 0.3453 | 0.3377 |
| *P*-value quadratic effect | 0.5623 | 0.2881 | 0.2923 | 0.9895 | 0.6175 | 0.2408 |
| SE | 1.58 | 2.47 | 0.03 | 4.62 | 43.16 | 6.12 |
| CV, % | 10.50 | 9.64 | 13.29 | 40.71 | 22.68 | 31.93 |
| | 42 days old | | | | | |
| Inclusion level, g/ton | | | | | | |
| 0 | 16.60 | 27.60 | 0.19 | 13.24 | 410 | 30.98 |
| 2500 | 16.76 | 27.06 | 0.19 | 11.53 | 386 | 36.94 |
| 5000 | 17.34 | 27.06 | 0.20 | 14.83 | 477 | 29.34 |
| 10000 | 16.87 | 26.21 | 0.20 | 14.37 | 427 | 31.63 |
| *P*-value treatment effect | 0.5775 | 0.9079 | 0.7030 | 0.0562 | 0.1456 | 0.3895 |
| *P*-value linear effect | 0.7656 | 0.6001 | 0.2203 | 0.3624 | 0.2857 | 0.7876 |
| *P*-value quadratic effect | 0.5801 | 0.8717 | 0.4538 | 0.6534 | 0.2508 | 0.9201 |
| SE | 1.15 | 4.04 | 0.02 | 2.53 | 83.54 | 9.67 |
| CV, % | 6.81 | 14.97 | 10.55 | 18.69 | 19.66 | 29.97 |

ALB: albumin, TP: total proteins, CREA: creatinine, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyltransferase

## Conclusion

Feeding zeolite to broiler chickens at up to 10,000 g/ton of feed did not change intestinal morphometry at 28 days old, or growth performance, carcass and cuts yield, serum constituent levels, and litter quality of birds at 42 days old. However, it improved the digestibility of DM and increased the weight of the pancreas. Thus, clinoptilolite zeolite proved an inert and nontoxic material that could be fed to broiler chickens because when added at up to 10,000 g/ton it did not cause deleterious effects on growth performance, relative organ weight, liver attributes, blood parameters, and carcass and cut yield.

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**Authors' Contributions**

MSDP worked on the methodology, investigation and the original draft. RVN and CE designed the study and were the supervisors. AMV and BSV participated in management and discussion of the writing of results and corrected the manuscript. CK, NCCS, FPC and EHC worked on investigation, resources and methodology. NRJ worked on statistical analysis.

**Conflict of Interest Declaration**

The authors declare that there are no conflicts of interest in the content of this paper.

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