Zeolite and corn with different compositions in broiler chickens feeding

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ABSTRACT The objective of this work was to evaluate the behavior of zeolite against different types of corn in broiler chickens' diets. 1,200 male broiler chickens were assigned to 6 diets in a completely randomized design and a 2 × 3 factorial arrangement, consisting of 2 types of corn (higher or lower mycotoxin contamination) and the inclusion of zeolite (0; 5,000 and 10,000 g ton^{-1}). In the period from d 1 to 21, there was an interaction ($P = 0.0040$) between types of corn and the inclusion of zeolite for feed conversion ratio (**FCR**). In the phase from 1 to 42 d, there was an interaction ($P = 0.0322$) on the serum levels of creatinine (**CREA**) and digestible gross energy (**dGE**); corn with lower mycotoxin level contamination (**LMLC**), caused a reduction in body weight gain (**BWG**) ($P = 0.0046$) and increase in the relative weight of abdominal fat ($P = 0.0256$). Inclusion rates of zeolite promoted an increase in the digestible CP ($P = 0.0477$) and digestible ash ($P < 0.0001$), as well as an increase ($P < 0.0001$) in hot carcass yield ($P = 0.0433$). The results indicate that the inclusion of zeolite, in the amounts used, did not alter the performance, serum levels, intestinal development and litter quality of the birds at 42 d of age. However, it was responsible for the improvement in the percentage of digestible nutrients (CP, GE, and MM).

Key words: histopathology, metabolism, mycotoxin, poultry farming

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INTRODUCTION

Corn (*Zea mays L.*) is the most produced cereal in the world, being widely used in human and animal nutrition. Corn quality can be verified by analysis such as the search for impurities, mycotoxins, insects, excess of moisture, and specific density. All those parameters may be influenced by crop genetics and processing, therefore both planting, harvesting, transportation, drying, and storage are mandatory to the final product quality (Gehring et al., 2013; Yin et al., 2017; Adeyeye, 2019).

Fungi contamination of corn used in animal feed causes a decrease in productivity and can endanger animal and human health (Adeyeye, 2019). Its presence impairs the visual appearance of the grains and is an indicator of higher risk of increased risk of mycotoxins contamination (Alshannaq and Yu, 2017).

Mycotoxins are harmful to animals mostly because their metabolization generates toxic compounds to the cells, specially to the hepatocytes, as most of this process occurs in the liver. In an attempt to reduce this negative effect, animal nutritionists have incorporated adsorbents in the feed that chelate mycotoxins and prevent its absorption in the gastrointestinal tract. Among those adsorbents, zeolites, a type of clay, are receiving attention.

Zeolites are highly reactive molecules with three essential properties: water absorption, ion adsorption and ion exchange. Considering adsorption as the adhesion of a solute to the surface of a solid material, zeolites can be compared to "molecular sieves" that show a microporous structure and their selective capacity to attach to molecules and ions (Schneider et al., 2017). Positive effects of zeolites have been demonstrated in the growth performance and intestinal morphology of broiler chickens (Wawrzyniak et al., 2017), in the enzymatic activity on the digestive system (Wu et al., 2013), and in the quality of poultry litter (Nikolakakis et al., 2013).

However, results may vary according to factors such as feed concentration of zeolites, type of zeolite being tested and other particular experimental conditions

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(Schneider et al., 2017). Therefore, this study aimed to evaluate the effects of the inclusion of clinoptilolite, a type of zeolite, into broiler chickens' diets formulated with corn from different bromatological profiles.

MATERIAL AND METHODS

This study was carried out based on the rules issued by the National Council for Animal Control and Experimentation (**CONCEA**) and approval by the local Ethics Committee on the Use of Animals (**CEUA**), under the protocol # 43-18.

Two different batches of corn were select according to their visual aspect: batch 1——with no perceptive alterations; batch 2——with significant color and grain integrity issues. Corn batches were analyzed for chemical composition using NIRS (Tango FT-Nir, Bruker Optics, Ettlingen, BW, GER) and mycotoxins using a commercial ELISA kit (Table 1).

Then, a metabolic assay was conducted, aiming to determine the metabolizable energy content of corn batches according to the total excreta collection technique (Sibbald and Slinger, 1963), to calculate the energy content of the rations used in the growth performance assay. For that, 150 male broiler chickens (Cobb-Vantress Ltd., Cascavel, PR, BR) from 14 to 24 d old were weighed individually and distributed according to weight in a completely randomized design in 3 treatments, 10 repetitions and 5 birds per experimental unit (**EU**). The diets were: a reference ration based on corn and soybean meal following the recommendations of Rostagno et al. (2017) and two different types of corn (Higher mycotoxin level corn (**HMLC**) and Lower mycotoxin level corn (**LMLC**)) replaced in 40% of the total corn, receiving water and feed ad libitum. The experimental period consisted in 5 d for adaptation and 5 d for sampling (Sibbald and Slinger, 1963).

In the growth performance assay, a total of 1,200 one-day-old male broiler chickens (Cobb 500) (43.48 ± 0.63 g) were raised on floor pens and assigned to a completely randomized design, in a 2 × 3 factorial arrangement, totalizing 6 treatments and 8 replications of 25 birds each. The factors under study were corn batch 1 (HMLC) and 2 (LMLC) and dietary inclusion of clinoptilolite zeolite (0; 5,000 or 10,000 g ton^{-1}).

Feed was supplied in mash form and formulated to reach or exceed broiler chickens' nutritional requirements according to Rostagno et al. (2017) for the phases: 1 to 7 (preinitial), 8 to 21 (initial), 22 to 28 (growth 1), 29 to 35 (growth 2) and 36 to 42 d of age (finisher). Clinoptilolite was included replacing the inert filler (sand). Finisher diets were supplemented with 1 kg ton^{-1} of celite to determine the indigestibility coefficient (Table 2).

Table 1. Bromatological composition and amount of mycotoxins from two different corns.

Corn	DM %	CP	MM	GE kcal kg^{-1}	FUM μg kg^{-1}	DON	ZEA	AFLA
HMLC	86,94	7,60	1,19	3,899	>6,000	0	0	0
LMLC	87,31	7,09	1,25	3,906	4,200	160	0	0

AFLA: aflatoxins; CP: crude protein; DM: dry matter; DON: deoxynivalenol; FUM: fumonisin; GE: gross energy; HMLC: higher mycotoxin level corn; LMLC: Lower mycotoxins level corn; MM: mineral matter; ZEA: zearalenone.

Mean feed intake (**FI**), body weight gain (**BWG**), and feed conversion ratio (**FCR**) were evaluated at 7, 21, and 42 d by weighing birds and feed leftovers in a pen basis. Mortality was checked daily for corrections in feed conversion and feed consumption (Sakomura and Rostagno, 2016).

At 20 and 41 d, 2 broiler chickens per pen were randomly selected for blood collection and analysis of serum aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), gamma glutamyl transferase (**Gamma GT**), creatinine (**CREA**), total proteins (**TP**), and albumin (**ALB**) (Nunes et al., 2018), using an automatic biochemical analyzer by spectrophotometry (Flexor EL200, Elitech Latin America, BR), reagents, calibrators (Elical II) and measurement standards (Elitrol I) from Elitech.

At 21 and 42 d, two broiler chickens per pen were euthanized. After the removal of the digestive system, samples of approximately 1 cm^2 of the larger lobe of livers were taken, fixed in 10% buffered formalin and destined for histopathological evaluation. After histological processing, livers were visualized and ranked by a pathologist in a scale ranging from 0 (normal condition) to 3 (marked alterations). Also, at 42 d, 3 birds per pen were randomly selected and fasted for 6 h to assess carcass yield, cuts, and percentage of abdominal fat.

Carcass yield was obtained according to the weight of the eviscerated carcass (without the feet, head, and neck), in relation to the live weight before slaughter. For the yield of commercial cuts, weights of boneless and skinless breast (*Pectoralis major, Pectoralis minor*, legs (thigh + drumstick) and wings were considered. For abdominal fat, the fat deposited around the cloacal bursa, gizzard, proventricle, and adjacent abdominal muscles was considered. Cut yields were calculated in relation to the weight of eviscerated carcass, and the percentage of abdominal fat in relation to the bird live weight.

At 28 d, one broiler chicken per pen was selected and euthanized, Samples of jejunum and ileum were taken, fixed in 10% buffered formalin and destined for histopathological evaluation. After histological processing, intestinal segments were transversely cut and digitalized images of their mucosa were generated to determine mean values (n = 30) of villus height and crypt depth, following the methodology of Luna (1968).

Litter quality was assessed at 24 and 40 d, collecting samples of each pen to determine dry matter and ammonia content. The determination of ammoniacal nitrogen was performed according to the methodology described by Hernandez and Gazetta (2001) and, for dry matter, the AOAC technique 930.15 (1990) was used.

Finisher diet and the ileal content of three birds per pen at 42 d were sampled, predried and analyzed for dry matter (**DM**), crude protein (**CP**) and ash, following the methodologies 930.15, 954.01, and 942.05 described

Table 2. Percentage and calculated composition of the basal diet containing two types of corn for broiler chickens.

	1 to 7 d		8 to 21 d		22 to 28 d		29 to 35 d		36 to 42 d	
Ingredients (kg t^{-1})	HMLC	LMLC	HMLC	LMLC	HMLC	LMLC	HMLC	LMLC	HMLC	LMLC
Corn (HMLC)[1] (80 g/Kg CP)	507.3	0.0	511.9	0.0	574.1	0.0	600.8	0.0	639.1	0.0
Corn (LMLC)[2] (75 g/Kg CP)	0.0	493.3	0.0	498.9	0.0	557.8	0.0	583.3	0.0	620.4
Soybean meal (480 g/Kg CP)	361	372	331	341	271	283	250	263	223	237
Soybean oil	50.68	54.32	76.67	80.34	74.80	78.90	71.90	76.20	67.00	71.60
Meat and bone meal (450 g/Kg CP)	47.0	47.0	52.00	52.00	52.00	53.00	49.00	50.00	42.00	43.00
Limestone (385 g/Kg Ca)	4.40	4.10	4.27	3.96	4.56	4.22	4.61	4.25	4.50	4.12
Dicalcium phosphate	2.50	2.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
DL−Methionine (990 g/Kg)	3.94	4.01	3.31	3.38	2.89	2.97	2.81	2.90	2.64	2.73
L-Lysine (517 g/Kg)	3.83	3.45	2.69	2.31	2.85	2.42	3.41	2.96	3.67	3.19
L-Threonine (980 g/Kg)	1.05	1.01	0.77	0.72	0.73	0.68	0.81	0.76	0.83	0.77
NaCl	3.90	3.89	3.11	3.10	3.13	3.11	2.66	2.65	2.35	2.34
Choline Chloride (600 g/Kg)	0.88	0.84	0.74	0.69	0.70	0.65	0.79	0.73	0.81	0.76
Enzymatic Blend[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin[4] and mineral[5] premix	1.80	1.80	1.80	1.80	1.50	1.50	1.50	1.50	1.50	1.50
ADISODIUM[6]	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	1.35	1.35
Coccidiostatic[7]	0.65	0.65	0.65	0.65	0.56	0.56	0.56	0.56	0.00	0.00
Inert filler[8]	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Celite™[9]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
Calculated composition (Kg t^{-1})										
Metabolizable energy (MJ kg^{-1})	127.7	127.7	135.2	135.3	137.3	137.3	137.8	137.8	138.2	138.1
Crude protein	237	237	224	224	201	201	192	193	180	180
Dig. Methionine	7.07	7.13	6.31	6.37	5.66	5.73	5.49	5.57	5.20	5.28
Dig. Lysine	13.30	13.30	12.02	11.98	10.71	10.71	10.49	10.49	9.90	9.91
Dig. Threonine	8.61	8.60	7.93	7.91	7.11	7.11	6.90	6.90	6.52	6.52
Dig. Tryptophan	2.56	2.51	2.40	2.35	2.12	2.06	2.02	1.97	1.89	1.83
Calcium	9.80	9.71	9.65	9.56	9.61	9.61	9.24	9.25	8.42	8.42
Dig. Phosphorus	5.03	4.98	4.83	4.78	4.80	4.81	4.62	4.63	4.21	4.21

[1]HMLC: Higher mycotoxin level corn.
[2]LMLC: lower mycotoxin level corn.
[3]Amilase 400000 U; Fitase 2000000 U; Protease 8000000 U; Xilanase 4976000 U; Glucanase 1216000 U. [2] Supplied per kilogram of product: Vit. A (min) 2.7 g, Vit. D3 (min) 0.75 g, Vit. E (min) 0.06 g, Vit. K3 (min) 2.5 g, Vit. B1 (min) 1.5 mg, Vit. B2 (min) 6 g, Vit. B6 (min) 3 g, Vit. B12 (min) 0.0012 μg, Pantothenic acid (min) 12 g, Niacin (min) 25 g, Folic acid (min) 800 mg, Biotin (min) 60 mg, Selenium (min) 0.25 g.
[4]Supplied per kilogram of product: Copper (min) 20 g, Iron (min) 100 g, Manganese (min) 160 g, Cobalt (min) 2 g, Iodine (min) 2 g, Zinc (min) 100 g.
[5]Sodium sulfate, 32% Sodium and 22% sulfur.
[6]Enradin 8%.
[7]Coxistac 12%.
[8]Sand.
[9]Insoluble acid ash.Inclusion of zeolite was performed by replacing the inert with 5,000 or 10,000 g ton^{-1} of zeolite.

by AOAC (1990). To determine the gross energy (**GE**), samples were subjected to combustion in a calorimetric pump (IKA C200 IKA Werke GmbH & Co.Kg, Staufen, BW, DE). To determine the indigestibility factor, an analysis of acid insoluble ash (**AIA**) was performed, according to the methodology described by Van Keulen and Young (1977) and adapted by Carvalho et al. (2013). Using the results obtained from diets and digesta, total digestible nutrients were determined: acid insoluble ash (**digAIA**), digestible crude protein (**digCP**), digestible crude energy (**digCE**), digestible ash (**digAsh**), and digestible dry matter (**digDM**).

Statistical Analysis

All the experimental results were analyzed considering each pen as an independent observation (experimental unit). Data were first tested for the homogeneity of variances (Levene test) and normality of residuals (Shapiro-Wilk test). After that, data were submitted to a two-way ANOVA using the PROC GLM of SAS (SAS Institute, version 9.0, 2009), according to the model $Yijk = \mu + C + Z_j + C \times Z_j + e_{ijk}$, where: Y_{ijk} = dependent variable; μ = general mean; C = corn effect; Zj = effect of the inclusion of zeolite; $C \times Zj$ = effect of the interaction between corn and zeolite; e_{ijk} = residual error. In case of significance, means were compared by the SNK test.

For the results of histopathological analysis of liver, non-parametric Wilcoxon's test was adopted. When there was interaction, the degrees of freedom were deployed through the Kruskal-Wallis test within each factor. Results were expressed as mean and standard error of the mean (**SEM**). A 5% significance ($P < 0.05$) was adopted at all steps.

RESULTS

In the growth performance, an interaction ($P = 0.0040$) was observed between the different types of corn and the inclusion of zeolite for feed conversion ratio (**FCR**) at 21 d (Table 3). The other growth performance variables (BWG and FI) did not show significant interaction. However, there was a main effect of types of corn on weight gain (BWG) ($P = 0.0046$) and on FCR

Table 3. Growth performance of broiler chickens fed diets containing two types of corn and supplemented with clinoptilolite zeolite.

Types of corn	21 d of age			42 d of age		
	FI (g)[1]	BWG (g)[2]	FCR ($g\ g^{-1}$)[3]	FI (g)	BWG (g)	FCR ($g\ g^{-1}$)
HMLC[4]	1,326	1,154	1.152	5,082	3,228[a]	1.575[b]
LMLC[5]	1,346	1,156	1.155	4,976	3,123[b]	1.600[a]
Zeolite (g ton^{-1})						
0	1,336	1,151	1.145	5,036	3,153	1.598
5000	1,331	1,155	1.158	5,007	3,198	1.574
10000	1,341	1,160	1.156	5,045	3,177	1.589
P Corn	0.1452	0.8581	0.7743	0.0593	0.0046	0.0244
P Zeolite	0.8422	0.7101	0.3531	0.8408	0.5869	0.1761
P Interaction	0.0811	0.8672	0.0040	0.4066	0.9331	0.1158
SEM	47.20	30.68	0.02	189.82	121.51	0.04
CV (%)	2.66	3.53	2.13	3.77	3.83	2.23

[1]Feed intake.
[2]Body weight gain.
[3]Feed conversion ratio; P: probability; SEM: standard error of the mean; CV (%): coefficient of variation.
[4]HMLC: Higher mycotoxin level corn.
[5]LMLC: lower mycotoxin level corn.Means with different letters in the same column, differ by F test.

(P = 0.0244) at 42 d, where birds fed HMLC showed higher BWG and lower FCR compared to birds receiving LMLC.

In the interaction deployment for FCR at d 21 (Table 4), when receiving LMLC (lower mycotoxin level corn) at an inclusion rate of 5,000 g ton^{-1} of zeolite, birds showed better FCR (P = 0.0459) compared to those receiving inclusion of 10,000 g ton^{-1} of zeolite (1,142 vs. 1,170). Birds receiving diets with HMLC, and inclusion of 10,000 g ton^{-1} of zeolite showed better FCR than those receiving LMCL (P = 0.0238) (1,150 vs. 1,170). Birds receiving LMCL and inclusion of 5,000 g ton^{-1} of zeolite showed better FCR (P = 0.0483) than birds receiving corn with higher levels of mycotoxin (1.112 vs. 1.176).

There was no interaction ($P > 0.05$) between types of corn and zeolite concentrations, but there was an isolated effect of corn for percentage of litter DM at 24 d (P = 0.0437), where birds receiving diets with LMLC obtained a higher percentage of DM in the litter when compared to birds that received HMCL. At 40 d, there was an isolated effect of corn on the content of ammonia in the litter (P = 0.0146). Birds that received HMLC had a lower content of NH_3 in the litter than those that received LMLC (Table 5).

At 42 d of age, there was an interaction (P = 0.0322) between types of corn and the concentrations of clinoptilolite zeolite on the serum creatinine concentrations (Table 6). The other blood variables showed no difference ($P > 0.05$) between treatments.

In the interaction deployment for creatinine, it was observed that the birds consuming LMLC supplemented

Table 4. Interaction deployment between types of corn (HMLC and LMLC) and the inclusion of clinoptilolite zeolite on feed conversion ratio ($g\ g^{-1}$) at 21 d of age.

Corn	Inclusion of Clinoptilolite Zeolite g ton^{-1}			P value
	0	5,000	10,000	
HMLC	1.140[B]	1.176[Aa]	1.150[ABb]	0.0401
LMLC	1.152[AB]	1.142[Bb]	1.170[Aa]	0.0459
P value	0.3771	0.0483	0.0238	

[ABab]Means with different lowercase letters in the same column and capital letters in the same line, differ by SNK test at 5% probability.

Table 5. Dry matter and ammonia released from the litter of broilers fed diets containing two types of corn and clinoptilolite zeolite at 24 and 40 d of age.

Types of corn	24 d		40 d	
	NH_3 (mg $100g^{-1}$)	DM (%)	NH_3 (mg $100g^{-1}$)	DM (%)
HMLC	1.35	67.24[b]	1.56[b]	67.59
LMLC	1.24	70.49[a]	1.91[a]	68.80
Zeolite (g ton^{-1})				
0	1.33	68.12	1.66	67.78
5000	1.29	68.89	1.67	68.60
10000	1.26	69.50	1.85	68.24
P Corn	0.1923	0.0437	0.0146	0.4133
P Zeolite	0.8012	0.7980	0.5117	0.8911
P Interaction	0.3031	0.1433	0.1130	0.7997
SEM	0.29	5.41	0.47	4.70
CV (%)	22.36	7.86	27.15	6.89

CV, coefficient of variation; DM (%), dry matter; HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; NH_3, ammonia concentration; P, probability; SEM, standard error of the mean.
[ab]Means with different letters in the same column differ by the F test.

Table 6. Serum concentration of blood metabolites in broilers fed diets containing two types of corn and clinoptilolite zeolite.

	21 d of age						42 d of age					
Types of corn	ALB ($g L^{-1}$)	TP ($g L^{-1}$)	CREA ($mg dL^{-1}$)	ALT ($UI L^{-1}$)	AST ($UI L^{-1}$)	GGT ($UI L^{-1}$)	ALB ($g L^{-1}$)	TP ($g L^{-1}$)	CREA ($mg dL^{-1}$)	ALT ($UI L^{-1}$)	AST ($UI L^{-1}$)	GGT ($UI L^{-1}$)
HMLC	15.37	25.85	0.22	11.24	186	20.01	16.91	28.16	0.20	14.41	441	31.64
LMLC	15.09	25.47	0.21	10.36	184	18.88	16.39	27.53	0.20	13.37	404	33.63
Zeolite ($g ton^{-1}$)												
0	15.11	25.31	0.21	11.74	175	19.35	16.51	27.50	0.20	13.63	424	33.95
5000	15.06	25.11	0.21	10.63	191	18.80	16.90	28.26	0.20	14.25	423	29.43
10000	15.50	26.57	0.23	10.03	188	20.26	16.53	27.75	0.20	13.80	420	34.88
P Corn	0.4952	0.5969	0.1291	0.4442	0.6667	0.4929	0.1241	0.2797	0.4021	0.2622	0.1450	0.3612
P Zeolite	0.6170	0.2112	0.0946	0.4705	0.4380	0.7980	0.5455	0.5293	0.3798	0.8505	0.9928	0.0632
P Interaction	0.9573	0.5894	0.7286	0.6491	0.3016	0.6368	0.2550	0.5200	0.0322	0.7928	0.1818	0.8771
SEM	1.35	2.50	0.03	3.97	40.11	5.45	1.14	2.01	0.02	3.15	88.11	6.64
CV (%)	8.86	9.73	12.54	36.75	21.67	27.98	6.82	7.22	10.52	22.68	20.86	20.33

ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CREA, creatinine; CV, coefficient of variation; GGT, gamma glutamyltransferase; HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; P, probability; SEM, standard error of the mean; TP, total proteins.

with 0 and 10,000 g ton^{-1} of zeolite had higher concentrations of creatinine as compared to birds receiving diets with 5,000 g ton^{-1} of zeolite (Table 7). Within the levels, birds that received a diet with HMLC without inclusion of zeolite had lower serum creatinine concentrations when compared to birds fed with LMLC.

There was an interaction ($P = 0.0340$) for digestible crude energy (CEdig) (Table 8), where different levels of zeolite in the diets improved the digestibility of CP ($P = 0.0477$) and ash ($P < 0.0001$).

The results for interaction deployment for digestible gross energy (Table 9) showed that within the zeolite levels, diets formulated with HMLC without inclusion of zeolite provided a lower digCE when compared to diets formulated with LMLC. A similar result occurred with diets formulated with 10,000 g ton^{-1}.

There were no differences ($P > 0.05$) with the different inclusions of zeolites and types of corn on villus height, crypt depth and villus:crypt ratio (Table 10).

There was an interaction between types of corn (HMLC and LMLC) and zeolite concentrations (0; 5,000 and 10,000 g ton^{-1}) ($P = 0.0354$) for percentage of abdominal fat (**AF**) at 42 d of age (Table 11). However, there was no interaction ($P > 0.05$) on carcass and cut yield. An isolated effect of zeolite was observed for hot carcass yield (**HCY**) ($P = 0.0433$), where the treatment with the highest inclusion (10,000 g ton^{-1}) provided the highest values.

In the interaction deployment for AF (Table 12), it is observed that within the zeolite levels, birds receiving corn with higher mycotoxin level (HMLC) and inclusion of 10,000 g ton^{-1} of zeolite showed a lower amount of AF, when compared to birds that received diets formulated with corn with lower mycotoxin level (LMLC). Birds that received LMLC and 10,000 g ton^{-1} of zeolite in the diet had a higher amount of AF when compared to birds that did not receive zeolite in the diet.

There was no effect of the types of corn and zeolite levels on the histopathological characteristics of the liver at 21 and 42 d (Table 13).

Some visual characteristics were observed in the different stages of liver lesions, as showed on Figure 1.

DISCUSSION

The rations supplied in the present study were formulated with corn containing fumonisin and the results found for BWG and FCR were opposite to those of Rauber et al. (2013), who using contamination levels of 0, 100 and 200 mg kg^{-1} of fumonisin for broiler chickens from 1 to 28 d of age, and observed a difference for BWG and FCR, where birds that did not receive fumonisin showed better results of BWG and FI at 14 and 28 d of age and better FCR at 28 d of age. However, Fernandes et al. (2017), when comparing broiler chickens fed different types of corn (classified by densimetric table and not classified), did not observe significant differences in BWG, FCR, and FI in the phase from 1 to 42 d of age, diverging from the results found in this study,

Table 7. Interaction deployment for corn type and inclusion rates of clinoptilolite zeolite on serum creatinine concentration (mg dL^{-1}) at 42 d of age.

Corn	Inclusion of Clinoptilolite Zeolite g ton^{-1} 0	5,000	10,000	P value
HMLC	0.19^{b}	0.20	0.20	0.6734
LMLC	0.22^{Aa}	0.19^{B}	0.21^{A}	0.0051
P value	0.0402	0.1500	0.2992	

HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; [ABab]Means followed by different letters, lowercase in the column and upper case in the row, differ by the SNK test at 5%.

This divergence of results may be linked to the amount of crude protein (**CP**) present in the different corn supplied to the birds, since HMLC, which contained a greater amount of fumonisin, also had a greater amount of CP in its composition, which may have the biggest BWG and the best FCR.

The challenge status of the farm can enhance the action of mycotoxins, that is, the higher the stress level of animals, the lower the amount of mycotoxin necessary to modify their performance (Filazi et al., 2017). Housing conditions (density), air quality (ammonia), litter quality, temperature and air humidity were controlled to provide the best possible welfare for birds, and the amounts of mycotoxins found in corn were probably not sufficient to negatively affect the birds' performance.

Broiler chickens' growth performance is directly linked to the quality of the offered feed and diets with high levels of protein and energy, with excess of nitrogen, are generally provided to ensure that the nutritional requirements of animals are met (Schneider et al., 2017). This may have been the reason for the increase in nitrogen excretion by birds fed with LMLC, since in the formulation of this ration, it was necessary to add more soybean meal to balance the dietary protein.

Table 8. Dry matter, crude protein, crude energy and digestible ash in broiler chickens fed different types of corn and clinoptilolite zeolite.

Types of corn	digDM (%)	digCP (%)	digCE (kcal kg^{-1})	digAsh (%)
HMLC	17.69	12.97	3465	2.19
LMLC	17.89	13.20	3565	2.27
Zeolite (g ton^{-1})				
0	17.80	12.70^{b}	3510	1.95^{c}
5000	17.60	13.03^{ab}	3537	2.18^{b}
10000	17.97	13.52^{a}	3501	2.56^{a}
P Corn	0.6143	0.4032	0.0510	0.3003
P Zeolite	0.7125	0.0477	0.8079	<0.0001
P Interaction	0.2451	0.0742	0.0340	0.2818
SEM	1.25	0.91	175.27	0.29
CV(%)	7.03	6.97	4.98	12.85

CV, coefficient of variation; digAsh, digestible mineral matter; digCP, digestible crude protein; digCE, digestible gross energy; digDM, digestible dry matter; HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; P, probability; SEM, standard error of the mean.
[ab]Means followed by different lowercase letters in the same column differ by the F test.

Table 9. Interaction deployment for corn type and the inclusion rates of clinoptilolite zeolite on digCE (kcal kg^{-1}) at 42 d of age.

Corn	Inclusion of Clinoptilolite Zeolite g ton^{-1} 0	5000	10000	*P* value
HMLC	$3{,}385^{b}$	3,581	$3{,}419^{b}$	0.1337
LMLC	$3{,}620^{a}$	3,493	$3{,}582^{a}$	0.2595
P value	0.0171	0.4342	0.0287	

HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; [ab]Means with different lowercase letters in the same column differ by the F test.

in which body weight gain and feed intake in this phase differed between birds fed HMLC when compared to birds fed LMLC.

Table 10. Morphometric parameters of jejunum and ileum of 28 d old broilers fed diets containing two types of corn and clinoptilolite zeolite.

Types of corn	Jejunum VH (μm)	Jejunum CD (μm)	Jejunum V:C	Ileum VH (μm)	Ileum CD (μm)	Ileum V:C
HMLC	731.77	34.07	20.90	656.83	29.47	22.12
LMLC	699.17	32.02	22.34	642.74	29.42	22.07
Zeolite (g ton^{-1})						
0	712.66	32.68	22.01	636.65	28.46	22.35
5000	717.57	33.01	21.92	624.41	27.95	22.64
10000	719.48	33.62	20.74	690.44	32.17	21.30
P Corn	0.2594	0.2155	0.2246	0.7457	0.9382	0.9139
P Zeolite	0.9900	0.8712	0.5587	0.2611	0.1598	0.5883
P Interaction	0.2934	0.5633	0.0735	0.8868	0.4592	0.3745
SEM	97.00	5.65	3.99	120.42	6.75	3.76
CV (%)	13.54	17.06	18.50	18.52	22.93	17.03

CD, Crypt depth; CV, coefficient of variation; HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; SEM, standard error of the mean; VH, Villus height; V:C, villus:crypt ratio.

Table 11. Carcass and cuts yield (%) of broiler chickens fed diets containing two types of corn and inclusion rates of clinoptilolite zeolite at 42 d of age.

Corn	HCY	CCY	PMJ	LQ	Wing	PM	AF
HMLC	70.38	71.21	27.31	31.05	9.73	5.43	1.72
LMLC	70.03	70.04	27.23	30.76	9.45	5.50	1.85
Zeolite (g ton^{-1})							
0	70.04^{b}	71.24	27.28	31.10	9.62	5.52	1.67
5,000	69.77^{b}	70.88	26.86	31.16	9.67	5.43	1.81
10,000	70.79^{a}	71.25	27.64	30.45	9.48	5.44	1.85
P Corn	0.2653	0.5664	0.7101	0.4682	0.0753	0.4662	0.0256
P Zeolite	0.0433	0.5778	0.0938	0.2637	0.5734	0.6452	0.0187
P Interaction	0.5148	0.7107	0.5178	0.7760	0.6507	0.4395	0.0354
SEM	1.16	1.17	0.92	1.33	0.53	0.32	0.17
CV (%)	1.65	1.65	3.36	4.32	5.58	5.90	9.56

AF, Abdominal Fat; CV, coefficient of variation; CCY, Cold carcass yield; HCY, Hot carcass yield; HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; P, probability; PMJ, *Pectoralis major*; PM, *Pectoralis minor*; SEM, standard error of the mean.

Means followed by different lowercase letters in the same column differ by F test.

Table 12. Interaction deployment between corn type and the inclusion rates of clinoptilolite zeolite on abdominal fat (%) at 42 d of age.

	Inclusion of Clinoptilolite Zeolite g ton^{-1}			
Corn	0	5,000	10,000	*P* value
HMLC	1.638	1.812	1.699^{B}	0.1222
LMLC	1.697^{b}	1.805^{ab}	2.003^{aA}	0.0162
P value	0.5734	0.9109	0.0081	

HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; ^{ABab}Means with different lowercase letters in the same row and capital letter in the same column differ by SNK test at 5% probability.

Table 13. Histopathological analysis of liver lesions in broiler chickens fed diets containing different types of corn and inclusion rates of clinoptilolite zeolite at 21 and 42 d of age.

	21 d of age	42 d of age
Corn	Histology	Histology
HMLC	1.00	0.50
LMLC	0.92	0.50
Zeolite (g ton^{-1})		
0	1.00	0.56
5,000	0.94	0.38
10,000	0.94	0.56
	Chi-square	
P Corn	0.7561	0.7838
P Zeolite	0.9519	0.7357
P Interaction	0.7200	0.8043
SEM	0.12	0.10

HMLC, Higher mycotoxin level corn; LMLC, Lower mycotoxin level corn; P, probability; SEM, standard error of the mean. Livers classified as 0 presented: absence of diffuse and discreet alterations or hydropic and/or fatty degeneration. Those classified as 1 (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 2 (moderate) displayed: 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 inflammatory infiltration, and mild diffuse congestion. Livers classified as 3 (severe) showed: marked diffuse congestion and marked bile duct hyperplasia.

A decrease in ammonia emission from the litter can be attributed to the positive effect that zeolite has on the use of nutrients, in terms of the adsorption of higher amounts of protein nitrogen. Natural zeolites have been used with different results to control ammonia production, depending on the physical properties of the materials used (Karamanlis et al., 2008); however, a reduction in ammonia emission by litter was not observed in the present study.

One of the ways to assess the health condition of birds is the serological analysis, because the results may indicate possible physiological changes which may have been influenced by nutrition, climatic conditions and animal management (Wein et al., 2017).

Total protein and ALB concentrations are within the normal range according to Nunes et al. (2018), who reported values between 25.63 and 47.92 g L^{-1} for TP and 11.26 to 21.40 g L^{-1} for ALB, as well as GGT concentrations between 11.45 and 97.51 UI L^{-1}.

According to Tayo et al. (2017), hepatotoxic mycotoxins cause an increase in hepatobiliary enzymes GGT, ALT, and AST in the blood of birds. The fact that these enzymes are within the normal range in the evaluated birds may indicate that the amount of mycotoxin present in corn was not sufficient to cause severe liver damage.

Concentrations of TP, ALB, ALT, AST, and GGT were not altered due to the low amounts of fumonisin present in the different types of corn. In contrast, Rauber et al. (2013), when adding fumonisin in the diet at levels of 0, 100, and 200 mg kg^{-1}, found differences in the serum concentration of blood metabolites in broiler chickens at 14 and 28 d of age, with an increase in the concentrations of TP, ALB, ALT, AST, and GGT in birds that received fumonisin. Similar results to the obtained in this study were presented by Maciel et al. (2007), who evaluated 0.25 and 0.50% of clinoptilolite zeolite associated with 5 ppm of aflatoxin in broiler chickens diets, and promoted a 30% decrease in serum creatinine levels at 42 d of age in relation to the control group.

Creatinine comes from the breakdown of creatine in muscle tissue, so its blood concentrations are related to the amount of creatine present in the muscle. Elevated levels may be related to disorders that elevate muscle catabolism, such as kidney disease, hepato-renal syndrome, urinary obstruction, reduced renal flow, dehydration, hypotension, intense exercise and muscle damage. On the other hand, the decrease in serum creatinine concentration may indicate diseases such as excessive hydration, liver failure and muscle diseases (Córdova-Noboa et al., 2018).

According to Nunes et al. (2018), creatinine is present in small concentrations in avian serum, as creatine is excreted by the kidneys before being converted into creatinine, with normal values of this metabolite in birds

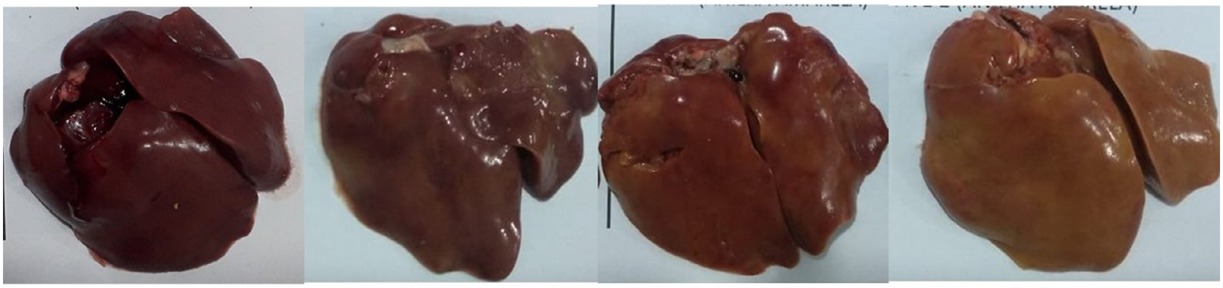

Figure 1. Visual characteristics of broiler chickens' livers classified according to the histopathology analysis.

being constant, between 0.05 and 0.35 mg dL^{-1}. Thus, creatinine levels detected in the birds in this study are within the normal range, which suggests that there was no liver damage.

Aluminosilicates included in the feed can act in the digestion processes and nutrients absorption, being responsible for prolonging the retention time of the feed, improving its digestion and absorption (Schneider et al., 2017).

Intestinal pH, transit time, and enzyme secretion can also be influenced by aluminosilicates and thus improve the diet digestibility. Yalçin et al. (2017) found an increase in ileal digestibility of protein and dry matter in broiler chickens. Such improvement in digestibility was also observed by Pasha et al. (2008), using bentonite and including 3% of zeolite in the diet. Ouhida et al. (2000) obtained better nutrients use as a result of lower intestinal flow rate and longer retention time with an increase in the action of digestive enzymes.

Even though there were no significant differences between treatments, there was a desirable relationship between villi and intestinal crypts. When the villi are high and the crypts are shallow, the absorption of nutrients will be better and the energy costs for cell renewal will be lower (Singh and Kim, 2021).

Wu et al. (2013), using 2% natural clinoptilolite zeolite, 2% modified clinoptilolite zeolite and a control diet on intestinal parameters of broiler chickens fed for 42 d, observed greater jejunum villus height for birds fed the diet containing modified zeolite. For the height of the ileum villus, the two diets containing zeolites were higher than the control group, and there were no differences between the two groups. However, supplementation with natural and modified zeolite did not show significant differences in the crypt depth of the jejunum and ileum compared to the control diet.

There was no effect of corn type on carcass and cut yields, with the exception of percentage of abdominal fat. Results similar to those found in this work for carcass and cuts yield were described by Rossi et al. (2010), who supplied intoxicated corn with different levels of aflatoxins, with or without inclusion of adsorbent, and did not obtain difference ($P > 0.05$) in the yields of carcass and cuts (breast, thigh, and drumstick). However, there was no effect of treatments on abdominal fat percentage.

The different levels of zeolite inclusion did not improve the cold carcass yield and cuts but improved hot carcass yield and increased the amount of abdominal fat. These results are similar to those described by Tatar et al. (2012), who did not observe significant differences in the carcass, breast, legs, back and abdominal fat yield of broilers supplemented with levels of 2 and 4% of zeolites in the diet. However, Christaki et al. (2006) by including 2% of zeolite in the diet, obtained positive results for leg yield and decrease in the percentage of abdominal fat.

The results found in this study can be explained by the reduction in intestinal transit caused by zeolite. According to Safaeikatouli, et al. (2012), this occur when the silicate mineral included on the diet makes temporary connection with the nutrients, which provides the body with more time to absorb the nutrients present in feed, causing greater protein deposition and consequently higher carcass yield. These nutrients, when in excess, are stored as fat in the body which explains the greater amount of abdominal fat present in birds that ingested zeolite with the feed.

These results reinforce the fact that the performance of broiler chickens fed with zeolite depends on some factors such as the type of zeolite, its physical and chemical properties and percentage rate of addition of this ingredient in the diet.

Liver is the target organ of mycotoxins in all animal species (Rauber et al., 2013), so it is the organ chosen for histopathological analysis. The different types of corn and the different zeolite inclusions did not cause histopathological damage in birds at 21 and 42 d. However, besides normal-looking livers, changes such as fat accumulation, diffuse atrophy, bile duct hyperplasia, inflammation around the bile ducts and/or deteriorated bile ducts have also been observed.

The occurrence of the same or similar histopathological lesions in the livers of birds in the control group and birds that received zeolite in the diet may indicate that the mycotoxin contamination of corn was not enough to cause severe histological changes in this organ.

Similar results were found by Rauber et al. (2013) who included 0, 100, and 200 mg kg^{-1} of fumonisin in the diet of broiler chickens and observed histopathological lesions in the liver at 14 and 28 d of age, with the main changes being bile duct hyperplasia, hepatocellular degeneration, lymphoid hyperplasia and proliferation of bile ducts. These lesions were the same regardless of the dose of fumonisin used.

Inclusion of zeolite at the level of 10,000 g ton^{-1} in the diet improves the digestibility of dietary protein, which reflects in a higher hot carcass yield at 42 d. However, the LMLC and the inclusion of zeolite interacts for the abdominal fat deposition with greater fat deposition observed in birds supplemented with higher inclusion rates of zeolite.

The results indicate that the inclusion of zeolite, in the amounts used, did not alter the performance, serum levels, intestinal development, and litter quality of the birds at 42 d of age. However, it was responsible for the improvement in the percentage of digestible nutrients (CP, GE, and MM).

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DISCLOSURES

None.

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