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The Use Clinoptilolite in Broiler Diet to Decrease of Aflatoxin Effects

Majid goodarzi[1], Davood modiri[2]

[1]Broujerd Branch, Islamic Azad University, Iran

[2]Broujerd Branch, Islamic Azad University, Iran

Abstract. This A study was conducted to evaluate the efficacy of clinoptilolite (most abundant natural zeolite) to reduce the effect of aflatoxin in diets of broiler chicks. In this study 900 one day-old male chicks were used in experimental as factorial (completely randomized design) with three levels of clinoptilolite (0, 3 and 5%) and three levels of aflatoxin (0,1 and 2ppm) from 1 day to 7 weeks of age . The result showed that the live weight , carcass weight and weekly weight gain were the highest (2215,1583,and53.8 respectively) for broilers fed ration 7(contain 5 percent clinoptilolite and 0 ppm aflatoxin) and were the lowest for ration 3 contain 0% clinoptilolite and 2 ppm aflatoxin (1571, 1043 and 37.25 respectively).The liver weight , feed conversion ratio and the percentage of mortality were markedly higher for ration 3 (45.8gr, 2.23 and 15.9% respectively) and were lower for ration 7 (40.08gr, 1.78 and 6.98% respectively) than the other ration . The highest feed intake was related to diet 4 containing three percent clinoptilolite and 0 ppm aflatoxin (4060gr) and the lowest feed intake was related to diet 3 (3633gr). In general the results showed that aflatoxin in level of more than 1ppm resulted in reducing of broiler performance and increasing feed conversion ratio. The usage of clinoptilolite in diet can reduce the effects of aflatoxin and in conditions of this experiment the level of 5% clinoptilolite was better.

Keywords: broiler; clinoptilolite; aflatoxin

1. Introduction

Aflatoxins (AF), a class of mycotoxins produced by fungal species of the genus Aspergillus (A.flavus and A.parasiticus), are contaminants in feed ingredients routinely used for poultry rations. Major forms of AF include B1, B2, G1, and G2, with AFB1 being the most common and biologically active component (27). Aflatoxins damage the liver, kidney, and thymus resulting in a variety of effects including decreased growth rates, poor productivity, immunosuppression, and disruption of carbohydrate, protein, and lipid metabolism (23).

Additionally, AF have been shown to be potent carcinogens, strong mutagens, and potential teratogens(3).Measures used by the livestock industry to protect animals from the toxic effects of AF include grain testing, use of mold inhibitors, fermentation, microbial inactivation, physical separation, thermal inactivation ,irradiation, ammoniation(2),ozone degradation(16),and the use of adsorbents(14) Unfortunately ,most of these measures are costly, time consuming, and only partially effective. At the present time, one of the more promising and practical approaches is the use of adsorbents. Selected adsorbents added to AF-contaminated feeds can sequester AF during the digestive process, allowing the mycotoxin to pass harmlessly thr ough the animal (14). The major advantages of these adsorbents include expense, safety, and easy administration through addition to animal feeds.

However, not all adsorbents are equally effective in protecting poultry against the toxic effects of AF and several adsorbents have been shown to impair nutrient utilization (25). The objectives of this research were to determine the efficacy of clinoptilolite (most abundant natural zeolite) to ameliorate the toxic effects of

+ Corresponding author. Tel.: + 0098-6623514272; fax: +0098-662 4453013.
E-mail address: goodarzi_ms@yahoo.com.

AFB1 present in poultry rations and to demonstrate that adding of clinoptilolite to poultry diets would not negatively affect poultry performance.

2. MATERIAL AND METHOD

2.1. Preparation of Aflatoxin

To aflatoxin produce, we used a standard strain Aspergillus parasiticus (NRRL-2999). For primary culture of the fungus was used on Sabouraud dextrose agar medium (22). In order to the mass production of fungi and increase amount of toxin was used from the one-liter flask, thus was poured per flask the amount of 150 grams of rice with 150 ml of water. The mixture was mixed for 2 hours by a mixer. The Flasks were autoclaved for 15 minutes at pressure of 15 pounds per square inch and then cooled. Then under a hood system in completely sterile conditions, was inoculated the amount of 6.5*106 to 7*106 spores per ml (2 ml) of fungal suspension into the flasks .The Flasks were placed 5 days at 28 º C in a incubator. Contaminated rice containing fungal spores (the contents of two flasks) was mixed with 40 kg of wet corn and was maintained for 7 days at normal temperature. After this period the infected corns were dried and was conducted the next stage of extraction and measurement (22).To measurement of aflatoxin in the samples, from thin-layer chromatography and high-performance liquid chromatography was used (20).

2.2. Diets and Expermental Design

Experimental diets were formulated based on nutrient requirements of broilers (NRC1994) and nutrients found in foods (Table 1). In this experiment, nine diets were used that included three levels of the clinoptilolite (0, 3 and 5 percent of the diet) and three levels of the aflatoxin (0, 1 and 2 ppm). Diets were similar in terms of energy and protein and other nutrients and the only different was related to the presence of aflatoxin and clinoptilolite in the diet. In this experiment four replications were considered for each diet, and each replicate were contained 20 pieces of broilers.

Clinoptilolie physicochemical properties and elements of constituting are given in tables 2 and 3 respectively. Its purity was 85 percent. Weight of chicks and feed intake were measured weekly. At the end of the experiment were slaughtered chickens and were measured the whole carcass, thigh, chest, abdominal fat, liver and heart.

2.3. Statistical Analysis

To analysis of experimental data SAS and MINITAB software was used. Data on mortality initially using the formula ArcSin√Y were converted and then were analyzed (1).

TABLE 1. DIET FORMULATION

Ingredient	Starter*(0- 14))*	Grower*(14 – 28)*	Finisher*(28-42)*
fish meal	49.50	57.50	65.70
corn grain	1.55	00.00	00.00
soy bean meal	35.52	30.00	24.33
oil	5.00	3.8 0	1.95
oyster shell	1.25	1.86	1.48
DCP	1.12	0. 95	0 .73
salt	0.39	0.34	0.29
mineral premix	0.25	0.25	0.25
vitamin premix	0.25	0.25	0.25
DL Methionine	0.12	0.05	0.02
Zeolite or sand	5.00	5.00	5.00
Calculated composition			
Metabolic Energy(Kcal/Kg)	2900.00	2900.00	2900.00
Crud protein (%)	20.84	18.12	16.30
Calcium (%)	0. 90	1.00	0.80
Available phosphorus (%)	0.41	0.32	0.27
Methionine (%)	0.45	0.40	0.38
Lysine (%)	1.31	1.09	0.93
Argenine (%)	1.51	1.39	1.22

TAB LE2. CLINOPTILOLIE PHYSICOCHEMICAL PROPERTIES

Physical shape	granule
Color	Light green
Density	$1.4 gr/cm^3$
Thermal stability	650 – 700 °c
Water absorption	30% of weight
Ion exchange capacity	170-190 meq/100gr
pH in water	7-8
acidic stability	stable in pH 2-8
pores size	3.5- 4 Angstrom

TABLE 3. CHEMICAL COMPOSITION OF CLINOPTILOLITE

Item	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	TiO_2	P_2O_5	MnO	SO_3	Na_2O	K_2O
(%)	66.10	11.50	1.30	3.10	0.80	0.30	0.10	0.40	0.10	2.10	2.20

3. Results and Discussion

Clinoptilolite main effects and aflatoxin were significant on all traits measured. About all traits level of 5 percent of clinoptilolit was the best and level of 2ppm of aflatoxin was the worst. Because the purpose of this experiment was mainly clinoptilolite and aflatoxin interaction so here was investigated clinoptilolite and aflatoxin interaction.

3.1.The Average Feed Intake

Mean data of clinoptilolite and aflatoxin interaction were significant on feed intake (Table 4) in the periods of starter, grower, and finisher, and the whole period ($P<0.05$). Clinoptilolite in the diet increased,and aflatoxin reduced feed intake. The Chickens which were fed with diets containing the highest clinoptilolite and the lowest aflatoxin (diet 7) had the highest feed intake.

The effects of clinoptilolite and aflatoxin were more prominent in starter and grower periods than the finisher. Clinoptilolite perhaps with increasing of the digestibility of feeds increased passage rate of material from the gastrointestinal tract and this in turn of increased feed consumption. Moreover clinoptilolite can irritate the digestive tract and increase different parts of its muscle layers (17). The clinoptilolite can reduced intestinal enzyme motility and thereby increased their stability and activity and help to better digest of feed (8). Also clinoptilolite due to increasing of intestinal pH provide a more suitable environment for the digestion of starch by pancreatic alpha-amylase enzymes (6) Recent trial results are consistent with results of other researchers (4,12,26). Aflatoxin also decreased growth and feed intake by disrupting of the immune system (10). Hydrated Sodium Calcium Aluminosilicate combined with aflatoxin and create stability complex and thereby the availability of aflatoxin decrease for absorption from gastrointestinal tract. HSCA also with absorbing of some elements in the building of aflatoxin caused deformation and inactivation of aflatoxin (7). Lon et al (5) observed in their studies that 5 percent clinoptilolite in diets contain mycotoxins has been increased broiler performance. These results are consistent with results of other researchers (12, 11).

TABLE4. AVERAGE VALUES OF FEED INTAKE(GR)

Cp	**Af**	**period**			
(%)	*(ppm)*	*starter*	*grower*	*finisher*	*total period*
0	0	619.6^{a}	2199^{a}	1155^{ab}	3973^{ab}
0	1	560.3^{b}	1981^{b}	1060^{cd}	3602^{c}
0	2	553.7^{b}	1956^{b}	1123^{b}	3633^{c}
3	0	628.9^{a}	2233^{a}	1198^{a}	4060^{ab}
3	1	574.7^{b}	2034^{b}	1089^{c}	3698^{ab}
3	2	559.7^{b}	1978^{b}	1011^{a}	3549^{c}
5	0	635.2^{a}	2218^{a}	1174^{ab}	4021^{a}
5	1	627.7^{a}	2228^{a}	1165^{ab}	4020^{a}
5	2	581.1^{b}	2057^{b}	1079^{c}	3718^{b}

Values in the same column with different superscripts are significantly different. ($p<0.05$)
Cp= clinoptilolite (see table 2) Af = Aflatoxin

3.2.Average Daily gain

Mean data clinoptilolite and aflatoxin interactions about daily gain during the starter, grower, finisher and total period (Table 5) showed that differences of this trait in chickens which received various diets had significant difference with each other ($P<0.05$).Also the effects of different levels of aflatoxin and clinoptilolite were significant(P<0.05). In all periods, maximum body weight was related to diet containing the highest clinoptilolite and the lowest aflatoxin(diet 7) and minimum body weight related to diet containing lowest clinoptilolite and highest aflatoxin(diet3).The clinoptilolite effects on body weight gain is probably due to the effects of this substance on the feed intake, digestibility and body cation - anion difference(15). Increasing of daily growth and final body weight in chickens fed with natural zeolite could be due to increasing of digestibility of organic matter, fat and carbohydrate (17). Elliot et al (19) increasing of body weight gain in chicks fed with zeolite contributed to increasing of gastrointestinal pH that can help to the absorption of some nutrients.The adding of natural zeolite to the diet of broilers will increase 2-2.5 hours transmission time of gastrointestinal contents and ultimately will be lead to increase of nutrient uptake(24).

3.3.Feed Conversion Ratio

Mean data clinoptilolite and aflatoxin interactions about FCR during the starter, grower, finisher and total period were not significantly different from each other. But in numerical aspect in all periods maximum FCR was related to diet 7 and minimum FCR was related to diet 3 (Table 6). Reasons for

TABLE5.AVERAGE VALUES OF WEIGHT GAIN (GR)

Cp (%)	Af (ppm)	period starter	grower	finisher	total period
0	0	449.7[d]	1199[d]	350[d]	1999[d]
0	1	368.4[g]	988[g]	317[h]	1674[g]
0	2	341.1[b]	895[i]	329[g]	1565[i]
3	0	488.0[b]	1301[b]	366[b]	2155[b]
3	1	405.0[e]	1083[e]	332[e]	1820[e]
3	2	343.0[h]	922[h]	307[i]	1573[h]
5	0	515.0[a]	1363[a]	380[a]	2259[a]
5	1	457.0[c]	1217[c]	353[c]	2027[c]
5	2	403.0[f]	1077[f]	331[f]	1810[f]

Values in the same column with different superscripts are significantly different. (p<0.05)
Cp= clinoptilolite (see table 2) Af = Aflatoxin

TABLE6. AVERAGE VALUES OF FEED CONVERSION RATIO

Cp (%)	Af (ppm)	period starter	grower	finisher	total period
0	0	1.38[f]	1.84[bc]	3.30[a]	1.99[f]
0	1	1.52[c]	2.00[ab]	3.34[a]	2.15[c]
0	2	1.62[a]	2.19[a]	3.45[a]	2.32[a]
3	0	1.29[h]	1.72[c]	3.28[a]	1.89[h]
3	1	1.42[e]	1.88[b]	3.28[a]	2.03[e]
3	2	1.63[b]	2.14[a]	3.29[a]	2.26[b]
5	0	1.23[i]	1.62[d]	3.12[a]	1.78[i]
5	1	1.37[g]	1.83[bc]	3.30[a]	1.98[g]
5	2	1.44[d]	1.90[ab]	3.26[a]	2.06[d]

Values in the same column with different superscripts are significantly different. (p<0.05)
Cp= clinoptilolite (see table 2) Af = Aflatoxin

improve feed conversion can be same reasons for other characters. These results are consistent with results of other researchers.(11, 18).

3.4.Traits related to liver

Trial data showed that effects of different levels aflatoxin and clinoptilolite on these traits are significant (Table 7) and the maximum liver weight was related to diet contain the highest aflatoxin level. The usage of the clinoptilolite reduced the liver weight significantly so that chicks received diet 7(the highest level of the Clinoptilolite and the lowest level of the aflatoxin) had the lowest liver weights. One of the effects of toxic agents on the body is increasing of liver weight and enlargement of hepatic and renal cells and necrosis renal tubular cells (13). Zeolite added to AF-contaminated feeds can sequester AF during the digestive process, allowing the mycotoxin to pass harmlessly through the animal (14).

3.5.Abdominal fat

Clinoptilolite and aflatoxin interaction (Table 7) on abdominal fat weight and percentage of its weight for different treatments were significant (P<0.05). Clinoptilolite and aflatoxin in the diet reduced abdominal fat. Aflatoxin reduce enzymes and bile acids are needed to digestion of fat, and so inhibit fat digestion, therefore diet with high fat and protein reduces the effect of aflatoxin strongly (4). Hepatic toxicity resulting from aflatoxin raised fat in the liver (10). Clinoptilolite probably decreased body and abdominal fat with pushing more nutrients for the production of the meat.

3.6.Mean Percentage of Mortality

Clinoptilolite reduced mortality and aflatoxin increased it. Aflatoxin as a poisoning factor resulted in weakness in the immune system and increased mortality of chickens (10). Oguze and. Kurtoglu (11) observed that aflatoxin decreased serum total protein, inorganic phosphorus, uric acid, total cholesterol, hematocrit, red blood cells, hemoglobin, thrombocyte, the percentage of monocytes and increased the numbers of white blood cells and heterophile. When the aflatoxin was added to broilers diet, it reduced the number of leukocytes (26). Zeolite prevented from aflatoxin absorption in the gastrointestinal tract, and also by absorbing nest carbon dioxide and ammonia improved the ventilation conditions for broiler chickens (9).

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TABLE7.AVERAGE VALUES FOR WEIGHT OF LIVER AND ABDOMINAL FAT AND MORTALITY PERCENTAGE

Cp*(%)*	Af*(ppm)*	liver wt.*(gr)*	liver wt.*(% of BW)*	Ab. Fat*(gr)*	ab. Fat*(% of BW)*	Mor(*(%)*
0	0	41.40^{bc}	2.96^{e}	32.02^{a}	2.36^{a}	9.20^{de}
0	1	45.75^{a}	4.06^{a}	23.90^{g}	2.12^{bc}	12.95^{b}
0	2	45.80^{a}	3.87^{b}	21.70^{h}	2.08^{c}	15.85^{a}
3	0	41.50^{bc}	2.74^{e}	33.03^{a}	2.18^{b}	7.03^{e}
3	1	43.13^{ab}	3.42^{cd}	29.30^{d}	2.32^{a}	11.60^{c}
3	2	45.83^{a}	3.97^{ab}	27.30^{f}	2.36^{a}	15.08^{a}
5	0	40.08^{c}	2.60^{f}	32.60^{b}	2.06^{c}	6.98^{e}
5	1	42.40^{b}	3.01^{d}	32.30^{c}	2.29^{ab}	9.90^{d}
5	2	43.70^{ab}	3.50^{c}	28.50^{e}	2.29^{ab}	13.00^{b}

Values in the same column with different superscripts are significantly different. ($p<0.05$)
Cp= clinoptilolite (see table 2), Af = Aflatoxin, ab= abdominal, mor = mortality

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