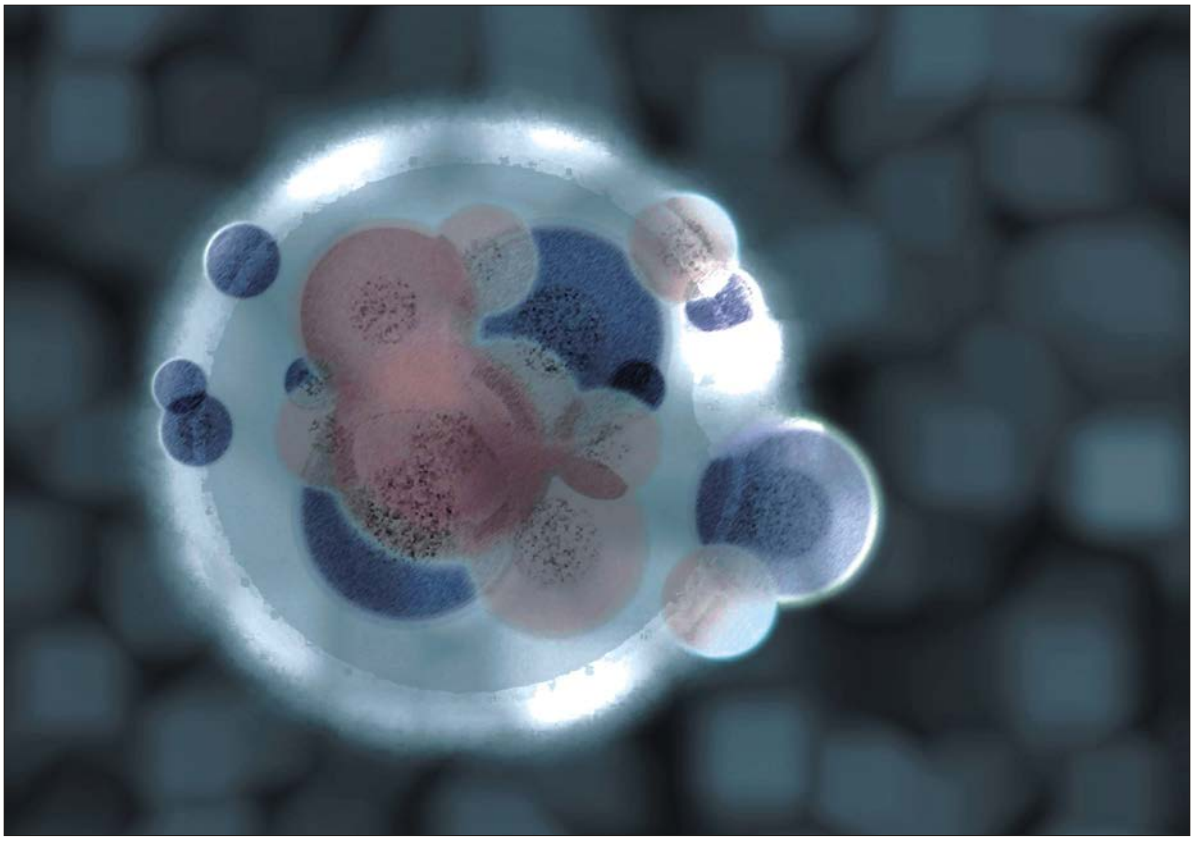

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Response of Broiler Chickens to Different Levels of Nanozeolite During Experimental Aflatoxicosis

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Abstract: This experiment was conducted to evaluate the efficiency of nanozeolite against aflatoxicosis in broiler chickens. For this purpose, 336 male Ross 308 day old broiler chickens were allocated to 6 dietary treatments, including a control corn-soybean meal diet without Aflatoxin (AF) and Nanozeolite (NZ) and five diets contaminated with 500 ppb AF and containing 1 of 5 levels of zero, 0.25, 0.5, 0.75 and 1% Nanozeolite (AFNZ0, AFNZ0.25, AFNZ0.5, AFNZ0.75 and AFNZ1, respectively). Data were analyzed as a completely randomized design with 4 replications and 14 birds per repetition. The results showed that the lowest weight gain and feed intake and the highest feed conversion ratio were related to diet containing 500 ppb AF without NZ (AFNZ0 diet). The birds were fed AFNZ0 showed lower weight gain in 2nd, 3rd and 4th weeks in comparison with control diet ($p<0.05$). AF led to reduce feed intake and increase feed conversion ratio in 3rd week ($p<0.05$). No significant differences observed between control diet and contaminated diets containing different levels of NZ for body weight gain, feed intake and feed conversion ratio at all weeks. Serum total protein, cholesterol and triglyceride concentrations were significantly lower in AFNZ0 compared to control diet ($p<0.05$). There was no significant difference between dietary treatments for serum albumin. These findings confirm that at least 0.25% NZ is sufficient to reduce the toxicity of AF in broiler chickens.

Key words: Nutrition, performance, toxicity, Aflatoxins, nanozeolite

INTRODUCTION

Aflatoxins (AFs), are hepatotoxic metabolites produced by fungal species of genus *Aspergillus* (Shotwell *et al*., 1966). AFs have been detected as contaminants of feed ingredients routinely used for poultry diets before and during harvesting and drying, in storage and after processing and manufacturing (Arab-Abousadi *et al*., 2007). The manifestation of chronic or acute toxicosis as well as carcinogenicity depends on the dose, duration of exposure and rate of metabolism to less toxic metabolites (Zaghini *et al*., 2005). AFs in chickens caused to impair feed conversion, reduce growth rates (Kiran *et al*., 1998), poor food utilization (Celik *et al*., 2000), increase susceptibility to environmental and microbial stresses, increase mortality (Parlat *et al*., 1999), change in relative organ weights (Arab-Abousadi *et al*., 2007), atrophy of the thymus and bursa of fabricius and lymphocytopaenia (Kiran *et al*., 1998). AF is associated with liver damage, nephrotoxicity (Kiran *et al*., 1998), mutagenicity, carcinogenicity and haemorrhage (Parlat *et al*., 1999). In the liver, AF molecules are exposed complex metabolic processes occurring via diverse cytochrome P450-dependent pathways (CYP) (detoxification or bioactivation processes) (Zaghini *et al*., 2005) and lead to form the reactive AFB_1-8,9-epoxide (AFBO) that binds to DNA and other vital macromolecules, causing toxicity, mutation and cancer (Coulombe *et al*., 2005). AFB_1 is exclusively likely to cause dose-dependent induction or inhibition of liver mixed-function-oxygenize activities, which may affect the liver's metabolism of endogenous and exogenous substrates (Nebbia, 2001). Different methods and strategies have been examined to reduce AF toxicity (Kiran *et al*., 1998). A variety of physical, chemical and biological processes used to detoxify but they followed limited success (Kiran *et al*., 1998). The stability of AF to heat and other physical conditions causes to fail these inactivation processes (Celik *et al*., 2000). Another method to the problem has been the use of nutritionally

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absorptive materials in the diet to sequester mycotoxins and reduce the absorption of AF from the gastrointestinal tract (Miazzo *et al*., 2000). These absorptive materials must not be absorbed from the gastrointestinal tract and must have the capability to bind physically with chemical substances, interrupting their absorption (Oguz, 1997), avoiding the toxin effects in livestock and the carry-over of these fungal metabolites into animal products (Miazzo *et al*., 2000). Recent studies have confirmed that sodium aluminosilicate and sodium calcium aluminosilicate can adsorb aflatoxins (Parlat *et al*., 1999; Miazzo *et al*., 2000; Oguz and Kurtoglu, 2000). *In vitro* studies also detected this adsorptive ability (Miazzo *et al*., 2000; Tomsevic-Canovic *et al*., 2001). Depending on the chemical structure of each adsorbent, there are variable degrees of adsorption (Santurio, 1999). Zeolites are crystalline, volcanic, hydrated aluminosilicates (Elliott *et al*., 1991; Parlat *et al*., 1999). The zeolite structure known as a type is a specific arrangement in which the unit cell contains 24 tetrahedra, 12 AlO_4 and 12 SiO4. When fully hydrated, there are 27 water molecules and there is also one monovalent cation for each present of AL ione (Miazzo *et al*., 2000). The zeolites, with a structure more rigid than other nutritionally absorptive materials could be much more selective with regard to adsorbates (Miazzo *et al*., 2000). Silver nano-particles have antimicrobial properties against bacteria and viruses that depended to size of particles (Sawosz *et al*., 2007). The objective of the present study was to evaluate the nanozeolite effectiveness in reducing the aflatoxin toxicity in broiler chickens.

MATERIALS AND METHODS

Birds, housing and feeding: This experiment was carried out from June 01, 2008 to October 01, 2009 at Poultry Research Center, University of Agricultural Sciences and Natural Resources, Gorgan, Iran. A total of 336 male Ross 308 day old broiler chickens were used in this study. Birds were weighed and randomly distributed into 24 experimental units. They reared on floor 1.5×1 m pens and fed a control corn-soy bean meal diet for the first week of age. Birds had free access to feed and water during and after this adaptation week. They also subjected to a continuous lighting schedule during all rearing period. At 7 days of age, all birds fasted for 4 h and pen body weight measured. The experimental units allotted to 1 of 6 experimental diets from 7 to 42 day. AF and NZ were used as a contamination agent and AF binder in experimental diets, respectively. Treatments consisted of a control corn-soybean meal diet without AF and NZ (AFNZ0) and five diets contaminated with 500 ppb AF which containing zero, 0.25, 0.5, 0.75 and 1% NZ (AFNZ0, AFNZ0.25, AFNZ0.5, AFNZ0.75 and AFNZ1, respectively). The experimental diets were formulated based on NRC (1994) recommendations to be isocaloric (3000 and 3100 Kcal ME/Kg diet) and isonitrogenus (21.5 and 19.3 % CP) for starter (7-21 day) and grower periods (22-42), respectively. Four replicate groups of 14 birds were allocated to each experimental diet.

Aflatoxin and Nanozeolite production: AF was produced by growing Aspergillus Parasiticus, IRCD50, as described by Shotwell *et al.* (1966). The toxin was removed with chloroform and the AF concentration determined by HPLC methods (AOAC, 2005). The chloroform solutions were placed in flasks, kept in a water bath at 60°C until complete evaporation of the diluents and subsequently transferred in 1 L of sterile maize oil (Del Bianchi *et al*., 2005). The maize oil and AF mixture were vortexed for better homogenization of solution and appropriate amount of AF-oil mixtures were substituted for maize oil in feed. The concentration of AF in final mixtures was confirmed by analyzing 100 g samples of feed of each treatment by HPLC methods. NZ was contained 1.5% silver and 98.5% natural zeolite.

Data collection: Body weight of birds was measured weekly after 4 h fasting and feed intake recorded during this period and then feed conversion ratio calculated. On day 18, 4 birds from each treatment groups bled by wing vein. Five milliliter of blood samples were transferred to tubes without anticoagulant. Serum was separated by centrifugation at 1000×g for 15 min and stored at -20°C until used (Santin *et al*., 2003). Samples were analyzed for total protein, albumin, triglycerides and cholesterol using commercial kits according to the manufacturer's recommended procedures.

Statistical analysis: Data were analyzed as a completely randomized design by SAS software (SAS Institute, 1999) software package. Treatment means were compared by Duncan's multiple range test (Duncan, 1955) on the probability level of $p<0.05$.

RESULTS

Growth responses of broiler chickens to experimental diets are shown in Table 1. Broilers fed diet contaminated with 500 ppb AF without NZ (AFNZ0) had significantly lower body weight gain in 2nd, 3rd and 4th weeks of experiment compared to the control diet ($p<0.05$). Also this treatment had significantly lower body weight gain in

Table 1: Effect of experimental diets on body weigh gain during different weeks of experiment*

	Week				
Experimental diet	1	2	3	4	5
Control	220.9±7.74	366.6±4.36^{a}	590.1±9.68^{a}	647.6±11.14	491.6±41.05^{a}
AFNZ0	219.6±10.62	333.0±6.89^{b}	469.6±21.27^{b}	593.8±47.48	329.3±37.23^{b}
AFNZ0.25	207.9±6.40	383.5±8.00^{a}	575.8±9.42^{a}	676.9±18.48	443.9±55.78^{ab}
AFNZ0.5	225.3±8.55	353.1±10.01^{ab}	583.8±3.13^{a}	633.8±31.66	475.0±79.47^{ab}
AFNZ0.75	203.8±9.66	360.2±11.84^{ab}	566.4±13.79^{a}	659.6±6.92	447.9±21.90^{ab}
AFNZ1	227.4±7.13	354.2±12.32^{ab}	551.8±16.28^{a}	647.5±21.78	436.0±32.81^{ab}

*a-b Means±SEM within column with no common superscript differ significantly ($p<0.05$)

Table 2: Effect of experimental diets on feed intake during different weeks of experiment*

	Week				
Experimental diet	1	2	3	4	5
Control	332.1±9.29	590.7±7.40	956.4±14.37^{a}	1251.8±31.44	1143.2±58.23
AFNZ0	343.0±14.55	557.7±14.01	874.0±20.28^{b}	1163.7±30.20	1047.4±16.70
AFNZ0.25	340.9±20.50	576.8±24.19	937.7±11.52^{a}	1238.3±41.97	1206.8±87.68
AFNZ0.5	343.1±12.09	580.7±10.13	961.1±7.52^{a}	1205.9±20.76	1198.8±70.87
AFNZ0.75	323.6±11.00	571.4±23.02	932.9±33.19^{a}	1165.3±20.18	1177.3±13.02
AFNZ1	341.9±11.39	590.7±3.56	951.3±3.14^{a}	1178.0±13.41	1100.0±45.58

*a-bMeans±SEM within column with no common superscript differ significantly ($p<0.05$)

Table 3: Effect of experimental diets on feed conversion ratio during different weeks of experiment*

	Week				
Experimental diet	1	2	3	4	5
Control	1.50±0.019	1.61±0.037	1.62±0.031^{a}	1.93±0.039	2.39±0.295
AFNZ0	1.58±0.141	1.68±0.077	1.87±0.107^{b}	1.98±0.110	3.28±0.285
AFNZ0.25	1.64±0.073	1.51±0.087	1.63±0.021^{a}	1.83±0.089	2.82±0.342
AFNZ0.5	1.52±0.009	1.65±0.068	1.65±0.018^{a}	1.91±0.104	2.68±0.310
AFNZ0.75	1.59±0.035	1.60±0.108	1.65±0.031^{a}	1.77±0.0437	2.65±0.132
AFNZ1	1.50±0.043	1.67±0.059	1.73±0.048^{ab}	1.83±0.076	2.57±0.225

*a-bMeans±SEM within column with no common superscript differ significantly ($p<0.05$)

Table 4: Effect of experimental diets on blood parameter of experimental broilers*

Experimental diet	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Cholesterol (mg dL^{-1})	Triglyceride (mg dL^{-1})
Control	3.37±0.076^{a}	1.50±0.103	132.89±4.704^{a}	105.94±3.894^{a}
AFNZ0	3.04±0.070^{b}	1.41±0.084	115.32±2.322^{b}	91.13±3.505^{b}
AFNZ0.25	3.30±0.102^{a}	1.55±0.111	127.94±3.511^{ab}	115.99±5.718^{a}
AFNZ0.5	3.50±0.082^{a}	1.83±0.087	123.46±4.072^{ab}	112.78±4.223^{a}
AFNZ0.75	3.40±0.092^{a}	1.52±0.123	124.23±4.238^{ab}	111.06±4.925^{a}
AFNZ1	3.38±0.070^{a}	1.46±0.123	119.54±5.554^{ab}	111.51±5.957^{a}

*a-b Means±SEM within column with no common superscript differ significantly ($p<0.05$)

3rd week than the other treatment. The addition of 0.25% NZ to contaminated diets improved body weight gain in 2nd week of experiment ($p<0.05$). No significant difference was found between birds fed AF diets containing different levels of NZ and those fed control diet. As shown in Table 2 there was no significant differences between dietary treatments for feed intake except for 3rd week. Birds were fed AF diet without NZ (AFNZ0) had lower feed intake (469.6 g) and higher feed conversion ratio (1.87) than other dietary treatments during 3rd week of experiment. The use of NZ on AF contaminated diets significantly ameliorated feed conversion ratio in 3rd weeks of experiment (Table 3). Serum blood parameters including total protein, cholesterol, albumin and triglyceride concentrations are shown in Table 4. Birds were fed diet containing AF without NZ (AFNZ0 diet) had lower protein (3.04 g dL^{-1}), cholesterol (115.32 mg dL^{-1}) and triglyceride (91.13 mg dL^{-1}) in serum which they are indicators of aflatoxicosis. No significant differences were observed between treatments in serum ALB. The addition of NZ to contaminated diets resulted to increase serum total protein, cholesterol, albumin and triglyceride concentrations.

DISCUSSION

This study showed that AF had adverse effect on broilers' performance. Our results are agreement with those reported by Verma *et al.* (2004), Miazzo *et al.* (2005), Pandey and Chauhan (2007). Also, Rosa *et al.* (2001) evaluated sodium bentonite for its ability to reduce the effects of AF (5 mg AFB 1 kg^{-1}) in the diet of growing broiler chickens from 30 to 52 day of age. They showed that body weight gains were significantly ($p<0.05$) lower

for broilers fed diets containing AF alone compared with the controls (1,865 vs. 1,552 g) and the AF significantly ($p<0.05$) decreased feed efficiency. Hesham *et al.* (2004) incorporated 0.5% of Kaolin and activated charcoal in diet to evaluate their ability to reduce the deleterious effects of 30 ppb AF in broiler chicken. They reported a significant ($p<0.05$) reduction in body weight gain (7%) in chicken fed diet contaminated with 30 ppb AF compared to the control group. Denli *et al.* (2009) evaluated the ability of AflaDetox in counteracting the deleterious effects of 1 mg kg^{-1} AFB_1 in broiler chicks. They reported that AFB_1 significantly decreased the body weight gain, feed intake and impaired feed conversion rate ($p<0.05$). The utilization of NZ in AF contaminated diets had favorable effects on the performance of broilers which these results were consistent with similar studies carried out with natural and synthetic zeolite (Parlat *et al.*, 1999; Miazzo *et al.*, 2000; Oguz *et al.*, 2000; Oguz and Kurtoglu, 2000). Parlat *et al.* (1999) studied effect of 50 g kg^{-1} Clinoptilolite (CLI, a natural zeolite) to reduce the deleterious effects of 2 mg AF/kg diet on Japanese quail chicks. They showed that food consumption was reduced by 14% in quail chicks consuming the AF diet without CLI, but by only 6% for quail chicks consuming the AF plus CLI diet and body weight gain was reduced by 27% in birds consuming the AF diet without CLI, but by only 8% for birds consuming the AF plus CLI diet. Miazzo *et al.* (2000) related that broilers exposed to the AFB_1 treatment (2.5 mg AFB_1 /kg) had a lower body weight gain (1,432 g) than the control (1,594 g). They reported that the addition of 1% synthetic zeolite into diets containing AF increased the body weight gain in chicks (1,576 g). Oguz *et al.* (2000) reported 100 ppb AF treatment significantly decreased body weight gains of chicks compared to controls. The addition of 15 g kg^{-1} CLI to the 100 ppb AF containing diet reduced the adverse effects of AF on performances of chicks. *In vitro* studies regard to adsorbant ability of AF by clinopetliolite zeolitic, certify our results too. Miazzo *et al.* (2000) showed that under the experimental conditions, sorbent capacity for AFB_1 was 100% for Zeolite NaA. They reported that this complex was stable in water at pH ranging from 2 to 10 and temperatures of 25 and 39°C. Tomsevic-Canovic *et al.* (2001) investigated the adsorption of AFB_1 by cation-exchanged clinoptilolite zeolitic tuff at 37°C and pH 3.8 from an aqueous electrolyte having a composition similar to that of gastric juices of animals. They showed high AFB_1 chemisorption indexes (c_) for different exchanged forms of clinoptilolite (from 0.90 to 0.95). The harmful effects of AF can be related to impairing of metabolizing and protein and nucleic acid synthesis (Tung *et al.*, 1972). On the other hand, AF-contaminated feeds reduce the activities of specific enzymes enrolling in digestion of carbohydrates, proteins, lipids and nucleic acids in broilers and also reduce the absorption of essential nutrients (Arab Abousadi *et al.*, 2007). Kubena *et al.* (1990) expressed that AF induces nutritional deficiency, led to decrease of body weight gain. Dersjantli *et al.* (2003) expressed that reduce of body weight gain in pigs and broilers depend on the levels of AF in the feed. Aflatoxins can be obsorbed from the gastrointestinal tract, so we use Nanozeolite in broilers' diets to preventing its absorption. NZ is a kind of mineral absorptive material which can bind to AFs and form tremendously durable complexes. Hydration of the exchangeable cations generates a hydrophilic environment on the surface of zeolites and this environment affects the adsorption of various organic molecules, such as mycotoxins, on zeolite particles and on the solidity of the adsorbed complexes (Tomsevic-Canovic *et al.*, 2001). *In vitro* results have declared that the ability of zeolite to absorption of toxins depends on the type and composition of zeolite (Miazzo *et al.*, 2000; Tomsevic-Canovic *et al.*, 2001). Addition of AF in absence of NZ resulted in a significant decrease in serum total protein, cholesterol, albumin and triglyceride concentrations. Santurio (1999) related that total protein and cholesterol concentrations decreased significantly (by 15 and 24%, respectively) and triglyceride concentrations increased (by 96%) with diets contaminated by 3 mg AF/kg. Allameh *et al.* (2005) reported significant reduction at levels of cholesterol (95 vs. 151 g L^{-1}), total protein (3.8 vs. 2.5 g dL^{-1}) and albumin (0.86 vs. 1.45 g dL^{-1}) in chicks fed diets containing 1 mg AF/kg compared to control diet. They no observed significant change in serum triglyceride (TG) levels. Also, our results agree with those reported by Batina *et al.* (2005), Bailey *et al.* (2006) and Franciscato *et al.* (2006). Decrease of plasma protein concentration is related to disruption of protein and nucleic acid metabolism (Hesham *et al.*, 2004). Reductions of plasma cholesterol and triglyceride concentrations are consistent with the general decrease of lipogenesis and damaged lipid transport in broilers and prevention of hepatic cholesterol biosynthesis.

CONCLUSION

The obtained results suggest that AF, at level used (500 ppb), decrease performance of broiler chickens as well as total protein, cholesterol and triglyceride concentrations. NZ at all levels can reduce detrimental effects of AF on performance and blood parameters.

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