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Ameliorative effect of bentonite-montmorillonite binder in turkey poult fed diets contaminated with aflatoxin

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Abstract

The effect of different levels of bentonite montmorillonite binder (BB) to reduce aflatoxicosis in turkey was studied. Eighty 21-day old Nicholas turkey poults were randomly assigned to five treatments of four replicates with each replicate having four poults. The experimental diets were: positive control (PC) with no aflatoxin or BB, negative control (NC) with 200µg/kg of total aflatoxin, NC + 2g/kg BB, NC + 4g/kg BB and NC + 6g/kg BB. The experiment was observed for 21 days. Aflatoxin significantly reduced feed intake and body weight gain in poults that were treated with aflatoxin and BB. Mortalities were 0%, 56%, 44%, 56% and 38% for diets 1, 2, 3, 4 and 5 respectively. Serum protein and albumin were also reduced significantly in poults that received aflatoxin alone and with binder. In summary, there was no significant effect of inclusion of bentonite-montmorillonite binder up to 6g/kg on the response criteria measured in this study.

Keywords: Aflatoxicosis; turkey poults; bentonite-montmorillonite.

Introduction

Aflatoxin is a class of mycotoxin produced mainly by two fungal species, *Aspergillus flavus* and *A. parasiticus*. This mycotoxin has been implicated in the aetiology of many disorders in poultry some of which are symptomatic in listlessness, anorexia, low growth rate, poor feed utilization, decreased egg production and increased mortality (Miazzo*et al*., 2000). In addition to these, anaemia (Oguz*et al*., 2000), reduction of immune function (Oguz*et al*., 2003), hepatotoxicosis, haemorrhage (Ortatatli and Oguz, 2001) are associated with aflatoxicosis.

The use of mycotoxin binders to ameliorate the effect of aflatoxicosis in turkey and other poultry species has become very important owing to the fact that it is practically impossible to have diets that is completely free from aflatoxin contamination (Oyegunwa*et al*., (2015). This is because the tropical climate provides a favourable environment for the proliferation of the aflatoxin producing fungi which abound in the tropics and the susceptibility of some feed ingredients (e.g. maize and groundnut cake) to aflatoxin contamination is high. Bentonite is a natural clay that comes from volcanic ash (Walz*et al*., 1998). Because of its adsorptive properties and accessibility, bentonite is widely used as a feed additive to eliminate aflatoxin, cadmium and radiocaesium from the gastro intestinal tract of the animal. It is also used to ameliorate food allergies, mucus colitis, spastic colitis, viral infections, such as stomach flu, and parasites that are unable to reproduce in the presence of the clay (Walz*et al*., 1998; Santurio*et al*., 1999;). The basis for the use of bentonite in this study refers to earlier information that bentonite has strong adsorptive powers and its role in binding aflatoxin in the gastrointestinal tract. Studies have shown that the use of sodium bentonite in broiler chickens diet would improve their weight gain (Tauquir*et al*., 2001; Prvulovic*et al*., 2008). Salari*et al*. (2006) indicated that chickens fed diets containing 10 and 20g/kg sodium bentonite consumed more feed, had more weight gain and less feed conversion ratio. Pasha *et al*. (2008) reported birds fed diets containing sodium bentonite treated with either 5g/kg or 10g/kg acetic acid significantly increased protein efficiency ratio and protein digestibility, as compared to the control.

Bentonite has been used to ameliorate the effect of aflatoxin in broiler chicks and other domestic animals with success, but information on the use of bentonite as aflatoxin binding agent in turkey is scarce considering the fact that turkey is one of the most

susceptible species of poultry to aflatoxin. The main objective of this study is to assess the efficacy of graded levels of bentonite-montmorillonite binder to ameliorate the effect of dietary aflatoxin in turkey poults.

Materials and methods

Ethical consideration

Prior to the commencement of this study, the ethics committee of the department of Animal Science, University of Ibadan approved this study to be conducted after a rigorous seminar where the Ph.D. proposal was presented in August 2011.

Production of aflatoxins on maize grains

Aflatoxin was produced from the pure culture of *A. flavus* N3228, which was obtained from the International Institute of Tropical Agriculture Ibadan. The culture was grown on autoclaved clean yellow maize which was purchased from open market in Bodija, Ibadan by the method of Shotwell *et al.* (1966) with slight modifications as follows:

The maize grains were soaked for six hours to soften and allow for proper autoclaving. After soaking, the grains were autoclaved at 121^0C for 20minutes. The purpose of autoclaving was to ensure that the maize was free from any microbes before being used for the intended purpose. A 1ml aliquot of tween 20 was added to 1,000ml of distilled water for the purpose of sticking the spores of the fungi spores to the maize grains firmly. The tween 20 mixture was then poured gently into each plate containing the fungi while the substrate was stirred gently with a spreader and poured back into the 1,000ml jar and mixed vigorously. Five plates of the fungi were poured into 1,000ml jar while spore count was performed to determine the spore load per ml by multiplying the average count by a factor of 50,000. Aflatoxin in the maize grains was produced by inoculating the autoclaved maize with 100ml of spore solution per 1kg bag of maize. The inoculated maize was shaken vigorously until no visible water was seen in the bag. Samples of maize were then kept for two weeks to allow for high production of aflatoxin in the maize samples. After two weeks, samples of maize showed high contamination characterized by dark greenish to black colouration. The mouldy maize grains were washed with tween 80 prior to drying of the maize grains. The maize grains were sundried on concrete floor in a green house.

Quantification of aflatoxins in maize grains

Portions of the dried grains were collected homogenized and ground into powder with size less than 2mm. A 20g sub-sample from a bulk sample of 200g was ground and extracted with 100ml of 70% methanol using a high-speed blender (Waring Commercial, Springfield, MO) for 3 minutes. The mixture was then passed through Whatman paper No 1, and the extract collected in a 250ml separating funnel and 100ml of distilled water was added to ease separation. The solution was extracted twice with 25ml methylene chloride. Following separation, the methylene chloride layer was filtered through 40g of anhydrous sodium sulphate to remove residual water. The extract was collected in a polypropylene cup and evaporated to dryness in a fume hood. The residue was dissolved in 200ul of methylene chloride and either diluted or concentrated to allow accurate densitometry. Extracts and aflatoxin standards were separated on thin-layer chromatography (TLC) plates (silica gel 60, 250 um) with diethyl ether-methanol-water (96:3:1), visualized under ultra violet light, and scored visually for presence or absence of aflatoxin with a 2mg limit of detection. Aflatoxins were quantified using scanning densitometer, CAMAG TLC Scanner 3 with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland) as described previously (Suhagia*et al*., 2006)

All laboratory procedures culminating in production and quantification of aflatoxin were carried out at the pathology laboratory of International Institute of Tropical Agriculture, Ibadan.

Source of bentonite-montmorillonite binder

The bentonite-montmorillonite binder used in this study is a grey powder obtained from Fusion Biosystems in Germany. Its brand name is Bioad.

Diet preparation

The corn-based diets used in this study was formulated based on the nutritional requirements recommended by the National Research Council (NRC) (1994) with crude protein adjusted to 28.44% and metabolizable energy at 3021.37kcal/kg. The culture material with 1,000µg/kg of total aflatoxin was added to each ration to reach the desired aflatoxin concentration in the diet (Table 1). A simple proportion for calculating the quantity of contaminated grains to be added to the finished feed to give the desired aflatoxin concentration in the diet as developed by Ewuola and Oyegunwa (2015) is as follows:

Required amount of contaminated maize (kg)
= Qty of finished feed (kg) ×conc. required in finished feed (µg/kg)

Conc. of aflatoxin in maize carrier (µg/kg)

Conc. = concentration

Birds and experimental design

Eighty 21-day old turkey poults were weighed and randomly allotted to five dietary treatments in a completely randomized design. Each treatment had 4 replicate and 4 birds per replicate. Treatment 1 was the positive control diet prepared from clean maize that were assumed free of aflatoxin and without inclusion of BB, treatment 2 was the negative control diet with 200µg/kg of total aflatoxin but with no binder, treatments 3, 4 and 5 were the negative control diets with 0.2, 0.4 and 0.6g bentonite montmorillonite binder per kg diet respectively.

Estimation of anti-aflatoxin effects in turkey poults

Feed intake was obtained by subtracting the leftover feed from the quantity served and body weight gain for each replicate group was estimated at day 21 using standard procedure while feed conversion ratio was calculated as the ratio of feed intake to weight gain. On day 21 of the experiment, blood samples were obtained from the jugular vein of two poults per replicate at day 21. Blood samples were collected in bottles and allowed to stand for 30 minutes while serum is separated for biochemical analyses. The serum total protein of the turkey poults was determined following the method described by Kohn and Allen (1995) using Randox^{R} kits. The serum albumin was determined using Bromocresol Green (BCG) method as described by Peters *et al*. (1982). Mortality figures obtained from this study was obtained on day 25 of the experiment when it had risen above 50%, hence the need to terminate the experiment.

Table 1: Composition (g/100g) of turkey poults starter diets

Ingredients	**Diet 1 (PC)**	**Diet 2 (NC)**	**Diet 3 (NC+2g/kgBB)**	**Diet 4 (NC+4g/kgBB)**	**Diet 5 (NC+6g/kgBB)**
Maize	52.40	32.40	32.40	32.40	32.40
Contaminated Maize	-	20.00	20.00	20.00	20.00
Soybean meal	40.00	40.00	40.00	40.00	40.00
Fish Meal (72%)	5.00	5.00	5.00	5.00	5.00
Dicalcuim Phosphate	1.20	1.20	1.20	1.20	1.20
Limestone	1.00	1.00	1.00	1.00	1.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
*Vit/mineral premix	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude Protein (%)	28.44	28.44	28.44	28.44	28.44
*ME (kcal/kg)	3021.37	3021.37	3021.37	3021.37	3021.37
Crude Fibre (%)	3.90	3.90	3.90	3.90	3.90
Calcium (%)	0.92	0.92	0.92	0.92	0.92
Phosphorus	0.55	0.55	0.55	0.55	0.55

PC = positive control without aflatoxin or binder , NC = negative control with 200µg/kg aflatoxin *1kg premix contains: Vitamin A – 13340 I.U; Vitamin D3 – 2680 I.U; Vitamin E – 10 I.U.; Vitamin K – 2.68mg; Calcium pantothenate – 10.68mg; Vitamin B12 – 0.022mg; Folic acid – 0.668mg; Choline chloride – 400mg; Chlorotetracycline – 26.68mg; Manganese – 13mg; Iron – 66.68mg; Zinc – 53.34mg; Copper – 3.2mg; Iodine –
ME = metabolizable energy

Data collection and statistical analysis
Data obtained were analyzed using ANOVA in SAS (SAS, 1999) as completely randomized and means were separated using Duncan multiple range test of the same software

Results and Discussion
In this study, values obtained for feed intake and body weight were statistically similar in birds that were fed with diets 2 to 5 compared with birds on the positive control diet which was higher (Table 2). Mortality was also high in diets 2 to 5 (56, 44, 56 and 38% respectively). Results herein may indicate the failure of bentonite-montmorillonite to ameliorate the toxic effect of 200µg/kg total aflatoxin on the turkeys.

Table 2: Performance of turkey poults fed dietary aflatoxin and bentonite-montmorillonite binder.

Parameters	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**SEM**
	PC	**NC**	**(NC+2g/kgBB)**	**(NC+4g/kgBB)**	**(NC+6g/kgBB)**	
Initial weight (g/poult)	232.25	231.50	206.75	230.75	231.00	0.01
Final weight (g/poult)	699.75^{a}	470.50^{b}	353.25^{b}	441.75^{b}	329.25^{b}	38.78
Weight gain (g/poult)	467.50^{a}	239.00^{b}	146.50^{b}	211.00^{b}	98.25^{b}	39.75
Feed intake (g/poult)	1187.80^{a}	734.50^{bc}	681.50^{bc}	528.30^{c}	769.00^{b}	64.45
Feed conversion ratio	2.58	6.35	5.83	2.58	9.10	1.83
Mortality (%)	0.00	56.00	44.00	56.00	38.00	

^{a,b,c} means on the same row with different superscript are significantly different (P<0.05)
PC = positive control without aflatoxin or binder, NC = negative control with 200µg/kg aflatoxin

Reduced feed intake as seen in turkeys fed with aflatoxin may be associated with injury in the gastrointestinal tract caused by the aflatoxin. Another factor that may have caused reduced feed intake in turkeys fed with aflatoxin-treated diets is low palatability as a result of the presence of the toxin. Feed consumption and body weight gain were significantly reduced in diets 2, 3, 4 and 5. This results contrast to the findings of (Rosa *et al*., 2001; Aravind *et al*., 2003; Miazzo *et al*., 2005; Kermanshahi *et al.,* 2009) which reported that adding Sodium bentonite and yeast glucomannan to the diet is effective in reducing aflatoxin toxicity in terms of growth performance, haematological, serum biochemical and microscopic-histopathological analyses. Similarly, Aidin *et al*. (2011) also found that addition of 1.5g/kg Na-bentonite to diets containing 0.25mg/kg of aflatoxin was effective in improving the feed intake and body weight gain of broiler chicks compared to broilers that were fed with aflatoxin without binders.

The effect of dietary supplementation of aflatoxin B_1 on serum biochemical parameters in this study shows that serum total protein and albumin were significantly reduced in turkey poults fed with diets 2, 3 4 and 5 (Table 3). This again may indicate failure of bentonite-montmorillonite to counteract the effect of aflatoxin. Serum total protein and albumin in positive control diet were significantly higher than other diets that were treated with aflatoxin with or without binder The reduced levels of total protein and albumin in aflatoxin treated birds were indicative of the toxic effect of aflatoxin B_1 on hepatic and renal tissues and are consistent with previous literature reporting aflatoxicosis (Kubena *et al*., 1993; Tejeda-Castaneda *et al*., 2008). The addition of BB was not significantly effective in the protection against aflatoxin B_1 in this study as reflected in the decreased serum protein and albumin observed in diets supplemented with bentonite-montmorillonite. The result obtained in this study contrasts with previous reports by Kubena *et al*. (1990), Ledoux*et al*. (1999) and Gowda *et al*. (2008) who recorded improved serum parameters in broilers fed with Aflatoxin diets supplemented with binders. Wafaa *et al*. (2013) also reported that the addition of hydrated sodium calcium aluminosilicate and tumeric powder as supplement in turkey diet improved the serum protein and general performance of turkeys that were fed with 2.5ppm of aflatoxin.

Table 3: Serum parameters of turkey poults fed dietary aflatoxin and bentonite-montmorillonite binder.

Parameters	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**	**SEM**
	PC	**NC**	**(NC+2g/kgBB)**	**(NC+4g/kgBB)**	**(NC+6g/kgBB)**	
Total protein (g/dl)	5.34[a]	3.75[bc]	4.10[b]	3.10[c]	3.50b[c]	0.17
Albumin (g/dl)	3.42[a]	1.95[b]	2.67[ab]	1.40[b]	1.86[b]	0.24
Globulin (mg/dl)	1.92	1.80	1.43	1.70	1.64	0.14
AST (IU/L)	178.40	191.50	189.33	185.00	194.60	15.34
ALT (IU/L)	42.80[a]	40.00[ab]	31.67[bc]	43.00[a]	25.80[c]	1.95

PC = positive control without aflatoxin or binder, NC = negative control with 200µg/kg aflatoxin
[a,b,c] means on the same row with different superscript are significantly different.
AST = aspartate aminotransferase, ALT = alanine aminotransferase

The values of alanine aminotransferase in diets 1, 2, 3 and 4 were similar (42.80, 40.00, 31.67 & 43.00) but significantly higher than the value in diet 5 (25.80). Elevated serum ALT in birds fed with diets 1, 2, 3 and 4 may not suggestive of liver damage since diet 1 which is the control diet was free of aflatoxin.Although the ameliorative effect of bentonite has been studied in broilers (Bailey e*t al*., 2006) and rats (Abdel-Wahhab*et al*., 1998) with remarkable improvement in performance, information on the use of bentonite montmorillonite in turkey is still scanty (Oyegunwa*et al*., 2015). It has been reported that not all binders will bind aflatoxin because it may take multiple electrical sites to hold the aflatoxin molecule or because the dosage necessary for the BB to work is very high. It could also be that the level of inclusion of the aflatoxin is too high to be sequestered by the binder. This may be the reason for the failure of the BB to mitigate the effect of aflatoxin.

Conclusion

It can be concluded from this study based on our findings that bentonite-montmorillonite binder at levels of 2, 4 and 6g/kg could not ameliorate the negative effect of aflatoxin 200µg/kg of total aflatoxin on turkey poults. However our next study would be geared towards reducing the concentration of the total aflatoxin in the diets from 200µg/kg to 150µg/kg.

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