# EVALUATION OF THE IMMUNOMODULATORY AND AMELIORATIVE EFFECT OF NEVATOX CLAY BINDER ON LIVER, KIDNEY, AND BURSA OF FABRICIUS OF TURKEY POULTS DURING AFLATOXICOSIS

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**Abstract**: Aflatoxin has become a nightmare in poultry industry. Clay minerals have shown some potency in neutralizing the effect of the toxin on broilers and other class of poultry. However, such information on turkey management is scanty. This study was therefore conducted to evaluate the immunomodulatory and ameliorative effect of Nevatox, a clay mineral on the performance of turkey poults. A total of eighty, 21-day-old turkey poults were randomly allotted to five dietary treatments with four replicates of four poults per replicate in a completely randomized design. Data collected were analysed using SAS while means were separated using Duncan Multiple Range Test. Treatment 1 is positive control diet with no aflatoxin and no nevatox, treatment 2 is the negative control with 0.2mg/kg of aflatoxin and no nevatox, treatment 3 is the negative control containing 0.2mg/kg aflatoxin and 2.0g/kg Nevatox, treatment 4 is the negative control with 0.2mg/kg aflatoxin and 0.4g/kg Nevatox, treatment 5 is the negative control with 0.2mg/kg aflatoxin and 6.0g/kg Nevatox. The feeding trial lasted for 21 days while performance parameters were measured during this period. At the end of the feeding trial, 2 birds per replicate were randomly selected, slaughtered by severing the jugular vein and their liver, kidney and bursa of fabricius harvested, for histopathology examination. Significant reduction ($p<0.05$) in body weight and feed intake were observed in treatments 2 to 5. Varying degree of induced pathological lesions ranging from mild to severe were also observed in the liver, kidney and bursa of the turkey poults while mortalities in treatments 2, 3, 4 and 5 (26%, 14%, 24% and 8%) were also high in all aflatoxin-treated birds except in the control. Supplementation of the diet with Nevatox at 2, 4 and 6g/kg did not ameliorate the effect of 0.2mg/kg aflatoxin on the performance and organs toxicity.

**Keywords:** Aflatoxin B1, nevatox, turkey poults, immunomodulatory, ameliorative

## Introduction

Aflatoxins are secondary toxic metabolites of two fungal species namely *Aspergillus flavus* and *Asperillus parasiticus* (Wilson and Payne, 1994). Aflatoxins are of different types and forms but among the different types of aflatoxins, aflatoxin B1 is the most prevalent and the most carcinogenic and is often found in cereal grains and peanut meals (Gowda *et al*., 2008). Aflatoxicosis in poultry causes, listlessness, anorexia, decrease growth rates, negative feed conversion, fatty liver, decreased egg production mortality among other problems (Leeson *et al*., 1995).

The pathological lesion induced after the ingestion of mycotoxins depends on the dose of the toxin and the resultant effects range from biochemical disorders to loss of cellular functions or even toxic injury of the cells (Bryden, 2012). Digestive, urinary, nervous, reproductive and immune systems of the body are affected even by low doses of mycotoxin but generalized cytotoxicity can be caused by a high dose of mycotoxins (Maresca and Fantini, 2010). Mycotoxins are considered as one of the most important immunosuppressive toxin that increase the rate of susceptibility to diseases and reduce the productivity of animals (Corrier, 1991; Surai and Mezes, 2005). This immune-suppresive effect is due to depression of the activity of T- and B-lymphocyte and inhibition in the activity of natural killer cells (Berek *et al*., 2001)

One important way to reduce the effect of aflatoxin in poultry is the use of adsorbing agents such as bentonite or hydrated sodium and calcium aluminosilicates (HSCAS) (Oguz and Kurtoglu, 2000). While there are several studies in the past to determine the potencies of these adsorbing agents in broilers and other livestock species, information on effects on performance, histopathology of liver, kidney and bursa of fabricius in turkey is scanty. Therefore this study was conducted to evaluate the immunomodulatory and ameliorative effect of Nevatox clay binder on performance of turkey poults and liver, kidney and bursa of fabricius histopathology during aflatoxicosis.

## Material and methods

### *Experimental Site*

The experiment was carried out at the poultry unit of the Tai Solarin University of Education, Ijagun.. Histopathology was carried out at the department of Veterinary Pathology, University of Ibadan, Ibadan Nigeria.

### *Experimental Animals and Design*

A total of eighty 21-day-old turkey poults were used for this study. The turkey poults were brooded for three weeks. The poults were weighed at the third week and randomly allotted to five dietary treatments with four replicates and four poults per replicate in a completely randomized design as follows: Diet 1 (Positive control with no aflatoxin or Nevatox); Diet 2 (negative control with 0.2mg/kg aflatoxin); Diet 3 (negative control + 2.0g/kg Nevatox ); Diet 4 (negative control + 4.0g/kg Nevatox) and Diet 5 (negative control + 6.0g/kg Nevatox).

### *Inoculation of maize with fungi for aflatoxin production*

A pure culture of *Aspergillus flavus* (N3228 strain) was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. Maize inoculum was prepared by a method described by Shotwell *et al*. (1966).

The maize grains were soaked for six hours to soften and allow for proper autoclaving. After soaking, the grains were autoclaved at $121^0$C for 20 minutes. 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 1000ml of distilled water for the purpose of sticking the pores of the fungi 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 1000ml jar and mixed

vigorously. Five plates of the fungi were poured into 1000ml jar while spore count was performed to determine the spores load per ml by multiplying the average count by a factor of 50000. Aflatoxin in the maize grains was produced by inoculating the previously autoclaved maize with 100ml of spore's solution per 1kg 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, the 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 on concrete floor in a green house.

***Quantification of aflatoxin in maize grains***

Portions of the dried grains were collected, homogenized and ground into powder of 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 No1, 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 dentiometry. Extracts and aflatoxin standards were separated in a thin layer chromatography (TLC) plates (silica gel 60, 250um) 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 densitiometer, CAMAG TLC scanner 3 with win-CATS 1.4.2 software as described previously.

***Source of Nevatox***

The clay binder (Nevatox) used in this study was obtained from Fusion Biosystems in Germany.

***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 1,000mg/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 finished feed to give the desired aflatoxin concentration in the diet as developed by Oyegunwa and Ewuola (2015) was used as follows:

Required amount of contaminated maize in the diet (kg) = Qty of finished feed (kg) X conc. Required in finished feed (ppb)/ conc. of aflatoxin in maize carrier (ppb)

**Table 1: Gross composition (g/100g) of turkey poults starter diets**

| Ingredients | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 |
|---|---|---|---|---|---|
| **Pure maize** | 52.40 | 32.40 | 32.40 | 32.40 | 32.40 |
| **Aflatoxinmaize** | 0.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| **Soybean meal** | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 |
| **Fish meal** | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| **Dicalcium 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 |
| **Premix** | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| **Total** | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| **Calculated nutrients** | | | | | |
| **Crude protein (%)** | 28.44 | 28.44 | 28.44 | 28.44 | 28.44 |
| **M. Energy (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 |

Diet 1 = positive control diet with no Aflatoxin or Mevatox, Diet 2 = negative control diet with 200mg/kg Aflatoxin, Diet 3 = negative control + 0.2g/100g Nevatox, Diet 4 = negative control + 0.4g/100g Nevatox, Diet 5 = negative control + 0.6g/100g Nevatox. *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 – 1.86mg; Cobalt – 0.268mg; Selenium – 0.108mg.*M - Metabolizable

***Performance parameters***

Feed intake was determined by the difference between the feed given and the leftover for the period of feeding trial.

Body weight gain is the difference between the weight at the end of the experiment and weight at the start of the experiment.

Feed conversion ratio is the ratio of the feed intake to body weight gain

Mortality is the number of dead birds

***Histopathology and Immunohistochemistry***

At the end of the 21 days feeding trial, 2 poults per replicate were euthanized and the liver, kidney and bursa of fabricius harvested for histopathology. Sections of the liver, kidney and bursa of fabricius were collected and fixed in neutral 10% formalin, embedded in paraffin, and cut into 4 -µm thick sections. The sections were stained with hematoxylin-eosin (H&E) method for microscopic examination (Bancroft and Stevens, 1996). Sections of organs with no, mild, moderate and severe lesions were given scores of 0, 1, 2 and 3, respectively (Gowda *et al*., 2008).

***Statistical Analyses***

Statistical analyses were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple comparison tests (SAS 1999)

***Results and discussion***

**Table 2: Performance of turkey poults fed with aflatoxins and nevatox binder at 21 days**

| Parameters | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | SEM |
|---|---|---|---|---|---|---|
| **Initial weight (g/bird)** | **192.25** | **171.50** | **166.75** | **160.75** | **191.00** | **0.01** |
| **Final weight (g/bird)** | **659.75** | **430.50** | **313.25** | **401.75** | **289.25** | **30.78** |
| **Feed intake (g/bird)** | **1117.80** | **664.50** | **611.50** | **458.30** | **699.00** | **40.45** |
| **Weight gain (g/bird)** | **467.50** | **259.00** | **146.50** | **241.00** | **98.25** | **28.75** |
| **Feed conversion ratio** | **2.39** | **2.57** | **4.17** | **1.90** | **7.11** | **1.83** |
| **Mortality (%)** | **0.00** | **26.00** | **14.00** | **24.00** | **8.00** | |

Diet 1 = positive control diet with no Aflatoxin or Nevatox, Diet 2 = negative control diet with 0.2mg/kg Aflatoxin, Diet 3 = negative control + 2g/kg Nevatox, diet 4 = negative control + 4g/kg Nevatox, Diet 5 = negative control + 6g/kg Nevatox
a, b, c: means on the same row with different superscripts are significantly different ($P<0.05$)

In this study, the lower feed intake, body weight gain, higher feed conversion ratio and mortality in group fed with aflatoxin alone (diet 2) and groups with aflatoxin and Nevatox (diets 3, 4 and 5) showed the depressing effects of aflatoxin compared with positive control group (diet 1). However, the fact that there were no significant differences in the values of performance parameters (feed intake, body weight gain, feed conversion ratio and mortality) measured in diets 2 to 5, clearly indicate the failure of the different levels of the Nevatox (2.0, 4.0 and 6.0g/kg) to ameliorate the effect of 0.2mg/kg of aflatoxin in turkeys.

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 is similar to the findings of Rauber_*et al*. (2007) who reported that aflatoxin concentration of 0.05, 0.10 and 0.20mg/kg significantly reduced the feed intake and body weight gain in turkey poults. The fact that the values of feed intake and body weight gain in diets 2, 3, 4 and 5 were similar but significantly lower that positive control may probably suggest the failure of Nevatox to bind aflatoxin.

Mortality Figures of 26%, 14%, 24% and 8% recorded in this study for diets 2, 3, 4 and 5 respectively again clearly indicates failure of the Nevatox to ameliorate the effect of aflatoxin at that concentration. Mortality is a common phenomenon during aflatoxicosis (Giambrone_*et al*., 1985). In the current study, mortality may have occurred in diets 2, 3, 4 and 5 because of the liver damage associated with necrosis and the interference of the toxin with the immune system of the turkeys as evident in the result of histopathology.

***Histopathology***

Turkeys that were fed with aflatoxin diets showed mild and moderate degeneration of hepatocytes with loss of hepatic cord arrangement in some cases despite the addition of Nevatox binder. Similar lesions have been reported during aflatoxicosis in chickens (Endrington_*et al*., 1997). Significantly higher scores of liver lesion occurred due to aflatoxicosis from $AFB_1$ administration and these were not present in the control diet.

The aflatoxin $B_1$ is the most potent hepatocacinogen known. It is capable of inducing liver cancer in many animal species. The Aflatoxin $B_1$ can cause malignant hepatocellular carcinomas at carcinogenicity amounts as low as 0.01mg/kg in the diet of trout. This makes it one of the most abundant, most toxic and the most potent naturally occurring carcinogenic substance known (Jones *et al*., 1994).

The liver is considered the target organ for aflatoxin $B_1$ because it is the organ where most aflatoxins are bioactivated to reactive 8, 9 epoxide form which is known to bind DNA and proteins, damaging the liver structures and increasing liver weight (Miazzo_*et al*., 2005) Aflatoxin has been known to cause liver congestion during aflatoxicosis due to increased lipid deposits (Hsieh, 1988).

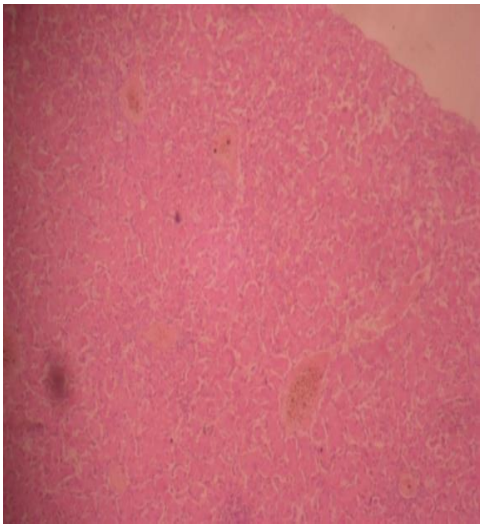

Plate 1: The photomicrograph of liver of turkey poult fed with control diet without aflatoxin showing no significant lesion

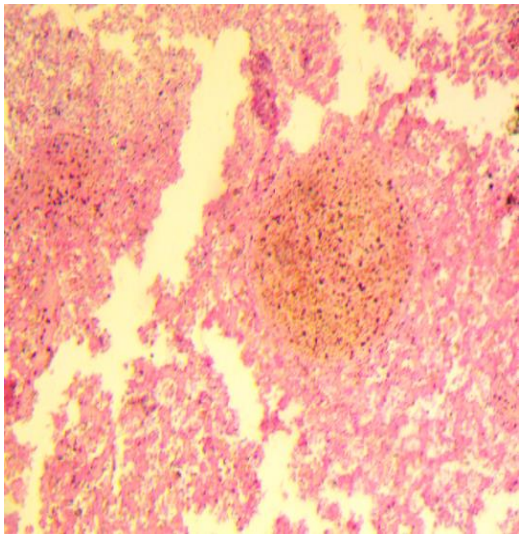

Plate 2: The photomicrograph of liver of turkey poults fed with diet 2 (0.2mg/kg aflatoxin) showing vascular congestion and areas with loss of hepatic cord arrangement (H & E x40)

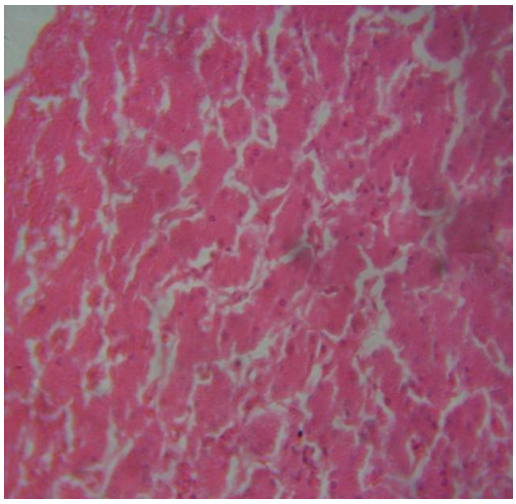

Plate 3: The photomicrograph of liver of turkey poult fed with Diet 3 (0.2mg/kg aflatoxin + 2.0g/kg Nevatox) showing widening of the sinusoids and thinning of cords observed here (H & E x 40)

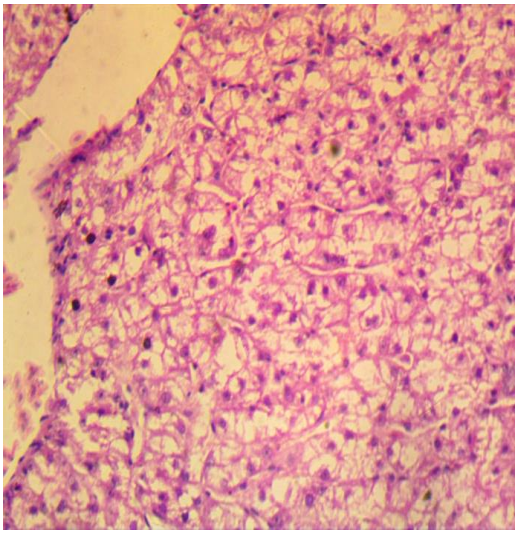

Plate 4: The photomicrograph of liver of turkey poult fed with diet 4 (0.2mg/kg + 4.0g/kg Nevatox). White arrow shows widespread vascular congestion with hepatic degeneration (H & E x40)

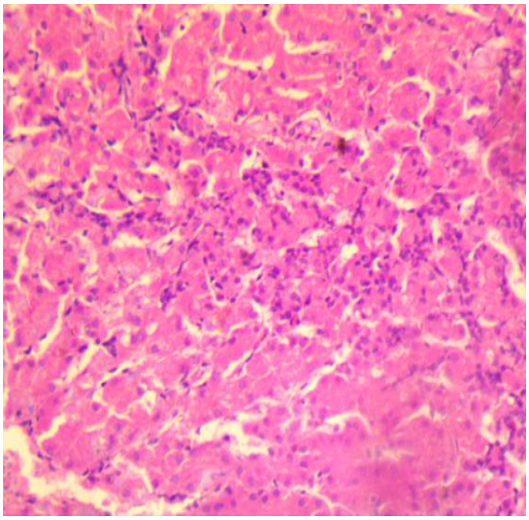

Plate 5: The photomicrograph of Liver of turkey poult fed with diet 5 (0.2mg/kg aflatoxin + 6.0g/kg Nevatox) showing dissociation of hepatic cords, dilated sinusoid (H & E x 40)

The mutagenicity of aflatoxin B1, is considered to arise as a result of the formation of a reactive epoxide at the 8, 9 position of the terminal furan ring and its subsequent covalent binding to nucleic acid (Chrevatidis_*et al*., 2003). Aflatoxin act, after bioactivation in the liver by binding of biological molecules such as essential enzymes, blockage of RNA polymerase and ribosomal translocase (inhibiting protein synthesis) and formation of DNA adducts (Hsieh and Alkinson, 1990). Hence the lesions observed in the liver.

**Conclusion**

It can be concluded from the findings here that 2, 4, 6g/kg of Nevatox binder could not prevent the effect of 0.2mg/kg aflatoxin B1 on the liver. Further studies is recommended to determine if lower concentration of the aflatoxin or higher concentration of the Nevatox binder could make an impact in ameliorating the effect of aflatoxin on liver cancer.

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