

## RESPONSE OF BROILER CHICKENS FED AFLATOXIN MAIZE-BASED DIETS SUPPLEMENTED WITH VARIOUS LEVELS OF MYCOTOXIN BINDER

Leye ALAYANDE<sup>1</sup>, Gideon Shaibu BAWA<sup>2</sup>, Nuhu Bello RANO<sup>3\*</sup>, Samson Olabanji OGUNDIPE<sup>2</sup>

<sup>1</sup>Hybrid Feeds Nigeria Limited, Kaduna, Nigeria

<sup>2</sup>Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria

<sup>3</sup>Department of Animal Science, Bayero University, PMB 3011, Kano, Nigeria

### ABSTRACT

The efficacy of varying levels of mycotoxin binder supplementation on growth performance, carcass characteristics and serum metabolites of broiler chickens fed aflatoxin maize-based diets was assessed. A total of four hundred and thirty-two (432), one-day-old Hubbard Cobb hybrid broilers were used for the experiment. The chicks were equally weighed and allotted to 12 dietary treatments in a 3 x 4 factorial arrangement of an activated clay toxin binder at three different inclusion levels (0 g.kg<sup>-1</sup>, 0.1 g.kg<sup>-1</sup> and 0.2 g.kg<sup>-1</sup>) of feed challenged with 4 levels of aflatoxin B, (0, 40, 60 and 80 ppb). Thirty-six chicks were allocated to each treatment group and replicated three times with 12 chicks per replicate in deep litter for eight weeks. At the end of the feeding trial, the birds were starved overnight prior to blood collection, three birds per treatment were selected on the basis of average pen weight. Blood samples were collected for serum and haematological analysis. Data collected were subjected to One-way analysis of variance (ANOVA) using SAS software. Results revealed significant ( $p < 0.05$ ) differences in the total protein (TP) content across the dietary treatments. Interaction between aflatoxin and binder levels showed a significant ( $p < 0.05$ ) effect only on the Alanine amino transferase (ALT) of the birds. Addition of mycotoxin binder (clay type) to aflatoxin contaminated diets was able to ameliorate the effect of aflatoxin on birds regardless of the level at which it was supplemented. Inclusion of 1 % binder in the diets is therefore recommended and feed ingredients for feed formulations be always subjected to test for aflatoxins.

**Key words:** aflatoxin; broiler chicken; serum biochemistry; internal organs

### INTRODUCTION

Mycotoxins are a group of secondary fungal metabolites notably by *Aspergillus flavus* and *A. parasiticus* that are commonly found in naturally contaminated food and feed and are most toxic to animal health, and they can cause considerable financial losses to the animal industries (Wu and Munkvold, 2008; da Rocha *et al.*, 2014). The contamination of feed with mycotoxins is one of the world's most serious concerns (Tapingkae *et al.*, 2022). To minimize the harmful effects of mycotoxin contamination, several approaches have been investigated in order to

degrade, destroy, inactivate and remove mycotoxins from contaminated feeds using physical, chemical or biological methods (Peng *et al.*, 2018; Elliott *et al.*, 2020). The usual route of mycotoxin exposure is ingestion as food or feed, however, dermal and inhalation also may be important routes of exposure. Direct effect of mycotoxins ranges from acute disease where severe conditions of altered health may exist prior to death as a result of exposure to the toxin, while indirect exposure of humans to mycotoxins likely occurs when toxic residues or metabolites that persist in milk, egg or edible tissues are consumed (CAST, 2003). The main mycotoxins of interest in poultry

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**\*Correspondence:** E-mail: nbrano.asc@buk.edu.ng  
Nuhu Bello Rano; Department of Animal Science,  
Bayero University, PMB 3011, Kano, Nigeria  
Tel. +234 (0) 8093524481

Received: August 25, 2022

Accepted: March 21, 2023



<https://doi.org/10.36547/sjas.822>

feedstuffs are aflatoxins, mostly inclusive of 4 major forms: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2; Yang *et al.*, 2012). In the face of various challenges limiting the achievement of broiler production in the country, the disease outbreaks in flocks presents dangers with various outcomes (Musa *et al.*, 2012), most of which affects the animals when their immune response is low. Mycotoxins contamination of grains is more widespread particularly in developing countries due to improper agricultural management practices (pre-harvest and post-harvest). Therefore, there is need to study various means of eliminating or reducing to barest minimum, the level of toxicity of livestock feeds which are mostly compounded with contaminated grains using mycotoxin binders. There are several feed additives used in diets to detoxify or remove mycotoxin as well as reducing their negative effects on animals. Mycotoxin binders or absorbents are substances that bind and immobilize the mycotoxins in the gastrointestinal tract of the animal thereby reducing the bioavailability of the toxins. Aflatoxin contamination can occur in a wide variety of feedstuffs including maize, sorghum, millet, wheat, peanuts, soya, rice, cottonseed and various derivative products made from these primary feedstuffs (Busby and Wogan, 1979; Ewuola *et al.*, 2014). A peculiar problem with respect to their occurrence in compound feed is the ability of the animal to assimilate high amount into their system. This may result in decreased animal performance and even death. The presence of the toxin in animals' system can be further complicated when it is metabolized into animal products such as eggs, meat and milk for human consumption (Iheanacho *et al.*, 2014). Therefore, aflatoxins are a major concern because they are human hepatocarcinogens and are considered to play an important role in the high incidence of human hepatocellular carcinoma in certain areas of the world (CAST, 2003). Aflatoxins can cause liver disease in animals they are also carcinogenic with Aflatoxins been the most potent carcinogen (Wogan, 1992; Kumar *et al.*, 2012). Susceptibility varies with breed, species, age, doses, length of exposure and nutritional status, Aflatoxins may cause decreased production (milk, eggs weight gain etc.) and are immunosuppressive, carcinogenic and mutagenic (Smith, 2020). Additionally, the growth of fungi and therefore, the production of mycotoxins are limited by the use of propionic acid or ammonium-isobutyrate, which can be used for a post-harvest

treatment. Absorbents (toxin binder) will reduce or eliminate the effects of Aflatoxins, but can only be applied in animal feeds (Nazarizadeh and Pourreza, 2019). This study therefore aimed to determine the extent to which mycotoxins binder can ameliorate the effect of aflatoxin in broiler diets and consequently the response of the chickens.

## MATERIALS AND METHODS

The experiment was carried out at the FeedTech Research Farm, Kakau Poultry Production Unit, Kaduna, Nigeria. It is located at latitude 10.5264" N and longitude 7.4388" E elevation of 704 m above sea level in the Northern Guinea Savannah Zone of Nigeria. The annual rainfall ranges between 617 and 1365 mm with an average of 1041 mm between July and September (Ovimap, 2015). The temperature varies between 26 °C and 35 °C. The humidity during harmattan period is 21 and 27 % during the wet season.

### Source of ingredients for feeding trial

Clean maize grains were purchased from the local market in Kaduna metropolis and sorted. Damaged, colored and bad kernel maize were removed and disposed. Pure culture of *Aspergillus flavus* was obtained at the International Institute for Tropical Agriculture (IITA, Ibadan). Some feed ingredients used for feed compounding were obtained from feed ingredient suppliers in Kaduna while activated clay-based mycotoxin binder and other micro-nutrients were purchased from Feedtech Limited, Kaduna.

### Processing of test ingredients

#### Culturing and inoculation of maize grains

Maize grain served as the aflatoxin carrier. The maize grains used for this experiment were obtained and inoculated with toxigenic strain *A. flavus* predominant in Nigeria. The culturing and inoculation were done at the Motherhens Farm Laboratory Unit, Kaduna State. Pure culture of *A. flavus* was prepared at IITA Ibadan in which sorghum was used as the carrier using method described by Wicklow *et al.* (1988).

#### Aflatoxin quantification

Aflatoxins were quantified using scanning densitometer, CAMAG TLC scanner 3 with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland). Thin

layer chromatography (TLC), also known as flatbed chromatography or planar chromatography was used as the separation technique in Aflatoxin analysis. Since 1990, it has been considered the AOAC (1995) official method and the method of choice to identify and quantitative aflatoxin at levels as low as  $1 \mu\text{g.g}^{-1}$ .

#### Toxin binder inclusion

An activated clay based mycotoxin binder (Toxibond®) was approximately measured and first mixed with maize before being incorporated in the diets by a manual mixer.

#### Toxibond® specifications

The activated clay-based mycotoxin binder was purchased from Feedtech Limited, Kaduna. The origin of the Toxibond (V-Number V20508) was South Africa supplied by BITEK INDUSTRIES (PTY) Ltd. The active ingredients are; Formic acid  $110 \text{ mg.kg}^{-1}$ , Propionic acid  $4290 \text{ mg.kg}^{-1}$ , Sorbic acid  $> 5000 \text{ mg.kg}^{-1}$ , Clay mineral  $> 5000 \text{ mg.kg}^{-1}$ .

#### Experimental diets

Four isonitrogenous and isocaloric diets were formulated according to the Nigeria Institute of Animal Science (NIAS) optimized procedures. The diets were formulated and manufactured to meet the nutrient requirement of the birds. Diet 1 (Control) contained

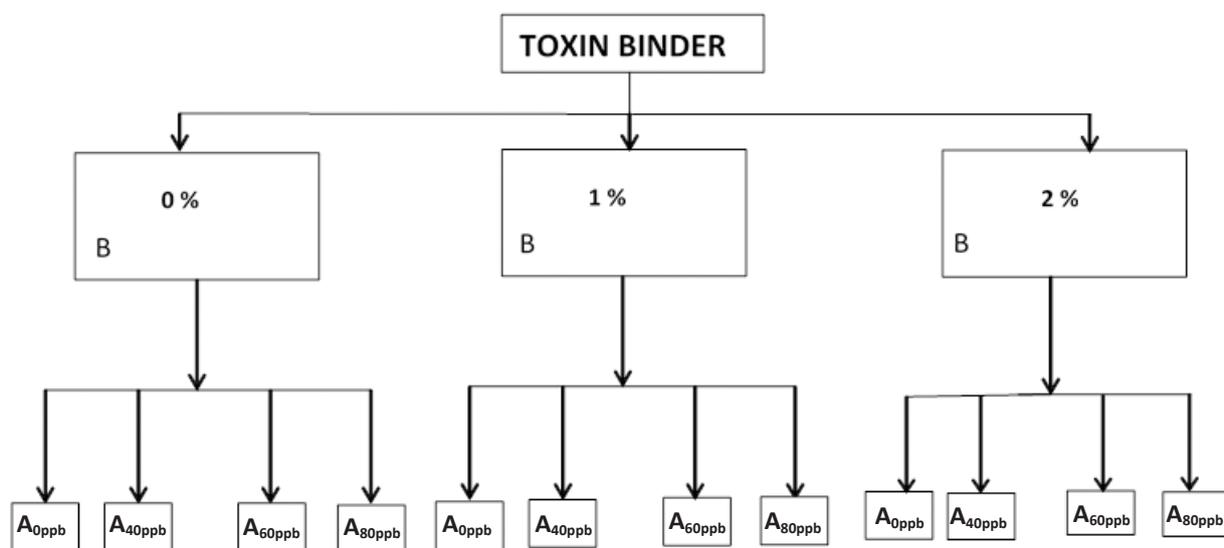
natural maize grains without aflatoxin contamination. Then a stock of grains was used in substitution for the uninfected maize grains to vary the concentrations of aflatoxin for diets 2, 3 and 4. The experimental birds were fed the starter diets for the first four weeks and later fed the finisher diets at the finisher stage (4–8 weeks). The diets were identified upon the aflatoxin levels as described in Table 1. Ingredients composition of the broiler starter and finisher diets are presented in Tables 2 and 3.

#### Experimental design and birds management

A total of four hundred and thirty-two (432), one-day-old Hubbard Cobb hybrid, broilers were used for the experiment. The chicks were equally weighed and allotted to 12 dietary treatments in a  $3 \times 4$  factorial arrangement of an activated clay toxin binder at three

**Table 1. Description of dietary treatments that were used in the broiler study**

Diet	Aflatoxin inclusion (ppb)
1	No (0 Control)
2	Yes (40)
3	Yes (60)
4	Yes (80)



A: varying levels of aflatoxin in the feed (0 ppb, 40 ppb, 60 ppb, 80 ppb); B: clay binder with inclusion levels (0.1 and 2 %)

**Figure 1. Experimental layout**

**Table 2: Ingredient composition of broiler starter diet**

Ingredients	Treatments														
	T1 (0 ppb)			T2 (20 ppb)			T3 (40 ppb)			T4 (80 ppb)					
	0 %	1 %	2 %	0 %	1 %	2 %	0 %	1 %	2 %	0 %	1 %	2 %			
Untreated Maize	57.00	57.00	57.00	35.30	35.30	35.30	20.80	20.80	20.80	20.80	20.80	20.80	6.30	6.30	6.30
Aflatoxin treated Maize	0.00	0.00	0.00	21.57	21.57	21.57	36.20	36.20	36.20	36.20	36.20	36.20	50.70	50.70	50.70
Soya Bean Meal	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00	33.00
Maize offal	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15	5.15
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
DCP	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

DCP = Dicalcium phosphate. \*Premix<sup>1</sup> vitamin premix (Bio-Organics) supplied per kg of feed: Vit. A 5,000 I.U.; D<sub>3</sub> 3,500 I.U.; Vit.K 2.5 mg; B<sub>1</sub> 2 mg; B<sub>2</sub> 6 mg; B<sub>6</sub> 4 mg; Niacin 40 mg; B<sub>12</sub> 0.02 mg; Pantothenic acid 10 mg; Folic acid 1 mg; Biotin 88 mg; Choline chloride 0.5 mg; Anti-oxidant 0.125 mg; Manganese 0.096 mg; Iron 0.24 mg; Copper 6 x 10<sup>3</sup> mg; Iodine 1.4 x 10<sup>3</sup> mg; Selenium 0.240 mg; Cobalt 0.240 mg. Calculated Analysis: Crude Protein = 21.7 %, Metabolizable energy = 2,994.54 Kcal.kg<sup>-1</sup>, Ether extract = 4.8 %, Crude fibre = 4.4 %, Calcium = 1.2 %, Available Phosphorus = 0.45 %, % Lysine, % Methionine.

**Table 3: Ingredient composition of broiler finisher diet (kg)**

Ingredients	Treatments														
	T1 (0 ppb)			T2 (40 ppb)			T3 (60 ppb)			T4 (80 ppb)					
	0 %	1 %	2 %	0 %	1 %	2 %	0 %	1 %	2 %	0 %	1 %	2 %			
Untreated maize	57.00	57.00	57.00	35.30	35.30	35.30	20.80	20.80	20.80	20.80	20.80	20.80	6.30	6.30	6.30
Aflatoxin treated maize	0.00	0.00	0.00	21.57	21.57	21.57	36.20	36.20	36.20	36.20	36.20	36.20	50.70	50.70	50.70
Soya bean meal	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00	27.00
Maize offal	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15	11.15
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
DCP	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

DCP = Dicalcium phosphate. \*Premix<sup>1</sup> vitamin premix (Bio-Organics) supplied per kg of feed: Vit. A 5,000 I.U.; D<sub>3</sub> 3,500 I.U.; Vit.K 2.5 mg; B<sub>1</sub> 2 mg; B<sub>2</sub> 6 mg; B<sub>6</sub> 4 mg; Niacin 40 mg; B<sub>12</sub> 0.02 mg; Pantothenic acid 10 mg; Folic acid 1 mg; Biotin 88 mg; Choline chloride 0.5 mg; Anti-oxidant 0.125 mg; Manganese 0.096 mg; Iron 0.24 mg; Copper 6 x 10<sup>3</sup> mg; Iodine 1.4 x 10<sup>3</sup> mg; Selenium 0.240 mg; Cobalt 0.240 mg. Calculated Analysis: Crude Protein = 19.5 %, Metabolizable energy = 3,037.80 Kcal.kg<sup>-1</sup>, Ether extract = 5.2 %, Crude fibre = 4.5 %, Calcium = 1.2 %, Available Phosphorus = 0.45 %, % Lysine, % Methionine.

different inclusion levels (0 g.kg<sup>-1</sup>, 0.1 g.kg<sup>-1</sup> and 0.2 g.kg<sup>-1</sup>) of feed challenged with 4 levels of aflatoxin B, (0, 40, 60 and 80 ppb). Thirty-six (36) chicks were assigned to each treatment group replicated three times with 12 chicks per replicate in deep litter house. The trial lasted for eight weeks comprising of the starter phase (0–4 weeks) and finisher phase (5–8 weeks). The layout of the experimental design is shown in Figure 1. Prior to the arrival of the experimental birds; the experimental pens were washed and disinfected with Morigad® and finally sprayed with formalin. Daily routine management practices were strictly adhered to and fresh water was provided *ad-libitum* throughout the period of the experiment and were also subjected to standard broiler management.

Data was collected for a period of 8 weeks in which the following parameters were measured:

**Initial Live weight:** The weight of the birds at the beginning of the experiment. This was performed using single pan electronic balance (Setra® BL-310S).

**Final Live weight:** The weight of the birds at the end of the experiment. This was performed using triple beam balance (Camry® Dial Spring Scale).

Feed intake/bird/day (g):  $\frac{\text{Quantity of feed given-leftover}}{\text{(Number of birds x 28 days)}}$

Daily live weight gain/bird (g):  $\frac{\text{(Final live weight-initial weight)}}{\text{(Number of birds x 28 days)}}$

Feed conversion ratio:  $\frac{\text{(Quantity of feed consumed)}}{\text{(Weight gained)}}$

**Water intake:** Water intake was determined by measuring and subtracting the left over from the total offered daily. The difference in volume represented values for daily water consumption per replicate. These daily values were summed up for a 7-day period and the mean calculated.

**Mortality** was monitored and recorded as when it occurred throughout the feeding trial. The recorded data were then expressed as percentages.

#### Proximate analysis of experimental diets

The proximate analyses of the experimental diets were determined at the Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria according to the procedures described by AOAC (1995). The result obtained corroborated with the results of duplicate samples analysed using Near Infra-red machine at Hybrid Feed Limited.

#### Determination of serum biochemical indices and internal organs characteristics

At the end of the feeding trial (8 weeks), the birds were starved overnight prior to blood collection and three birds per treatment were selected on the basis of average pen weight. Blood samples were collected through the wing web vein for serum biochemical analysis. The samples were placed into sterilised glass tubes without ethylene diamine tetra acetate (EDTA) for serum biochemical study. Serum total protein was determined by Biuret method (Reinhold, 1953). Albumin was determined using Bromocresol green (BCG) method as described by McGinlay and Payne (1988). Aspartate amino transferase (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP) activities were determined using spectrophotometric methods as described by Abei (2004). The birds were then killed by slaughter method and the bled weight of each bird was recorded before evisceration. The carcasses were eviscerated and dissected according to the methods by Jones *et al.* (1984). The internal organs (liver, heart, spleen, lungs, bursa of fabricius, small intestine and large intestine) were removed and weighed and expressed as percentage of live weight.

#### Statistical analysis

Data collected were entered in an excel spreadsheet and calculations were performed to determine the averages of values recorded for organ weights. The collected data were subjected to One-way analysis of variance (ANOVA) using The General Linear Model of SAS (2008) analytical software to study the effect of aflatoxin levels, binder inclusion levels and their interaction on digestive organs characteristics as well as serum biochemical indices for significance at  $p < 0.05$  for the total period. Where the effect of a factor was significant for more than 2 treatments, means of individual treatments were compared for significance ( $p < 0.05$ ) using the Duncan's option of the same software.

## RESULTS

#### Growth performance

The effects of toxin binder levels on growth indices of broiler finisher chickens were as shown in Table 4. Final weight, Total weight, Average daily gain, Total feed intake and Average daily feed intake significantly differ ( $P < 0.05$ ) in broiler chickens fed varying

**Table 4. Effect of mycotoxin binder levels on the performance characteristics of broiler chickens at 8 weeks**

Parameter	Binder Levels (%)			SEM	LOS
	0	1	2		
Initial live weight (g/bird)	292.68	284.69	304.69	25.98	NS
Final live weight (g/bird)	1035.13 <sup>b</sup>	1059.36 <sup>b</sup>	1064.21 <sup>a</sup>	32.46	*
Average feed intake (g/bird/day)	98.15 <sup>b</sup>	100.94 <sup>ab</sup>	103.18 <sup>a</sup>	1.48	*
Total feed intake (g/bird)	2748.18 <sup>b</sup>	2826.32 <sup>ab</sup>	2889.04 <sup>a</sup>	198.75	*
Average daily gain (g/bird/day)	26.48 <sup>b</sup>	28.01 <sup>a</sup>	27.11 <sup>ab</sup>	1.97	*
Total live weight gain (g/bird)	742.45 <sup>b</sup>	775.47 <sup>a</sup>	759.52 <sup>ab</sup>	64.68	*
Feed conversion ratio	3.28	3.00	3.22	0.09	NS
Water intake (ml/bird)	488.61	466.39	499.72	50.43	NS

<sup>a,b</sup> Means along the same row with different letter superscripts differ significantly ( $p < 0.05$ ), SEM: Standard Error of Means, LOS: Level of Significance, NS: Not Significant.

levels of mycotoxin binder inclusion (0 %, 1 and 2 %). Total feed intake and Average daily feed intake of birds fed on 2% mycotoxin binder supplemented diet were significantly higher than those fed on other dietary mycotoxin binder levels. Feed intake increased gradually with increasing mycotoxin binder level but no significant difference was observed between birds on 0 and 1 % mycotoxin binder level. Total weight gain and Average daily gain of birds were also slightly but not significantly ( $P > 0.05$ ) higher for birds fed 2 % mycotoxin binder level than those fed on 0 % mycotoxin binder diets. The least average daily weight gain/bird was obtained in birds fed 0 % mycotoxin binder inclusion level. The FCR and water intake of birds were not significantly affected ( $P > 0.05$ ) by mycotoxin binder levels. However, 1 % binder level inclusion was numerically the best amongst the treatments.

The interaction effects between various aflatoxin levels and binder levels on the growth performance of broiler finisher chickens are presented in Table 5. Significant interactions were observed for Feed Intake and average daily weight gain, and FCR of the birds between aflatoxin levels and binder inclusion levels. Higher average daily weight gain was observed in birds fed diet 1 (0 ppb at 1 % binder inclusion level) and lowest average daily weight gain value was obtained in birds fed diet 4 (80 ppb at 0 % mycotoxin binder level). High mortality rates were observed in birds fed diets 2, 3 and 4 irrespective of binder supplementation. However, mortality rate reduced with slightly increasing level of binder supplementation. Highest percentage mortality was observed in birds fed diet 4 (80 ppb) at 0 % mycotoxin binder inclusion level. Economic

evaluation of the different diets at the time of the study revealed feed cost (Naira per kg diet) was numerically slightly low (₦ 277.33/kg) in diet 2 (40 ppb) compared to diets 1, 3 and 4.

#### Serum biochemistry

Effect of aflatoxin levels on serum metabolites of broiler starter chicks at 4 weeks of age is presented in Table 6. No Significant ( $p > 0.05$ ) differences were observed in Aspartate transferase (AST), Alanine transferase (ALT), Alkaline phosphatase (ALP) and the albumin indices of the birds fed the various diets. However, there were significant ( $p < 0.05$ ) differences in the total protein (TP) content across the dietary treatments where birds fed diet T1 (control) presented the highest ( $p < 0.05$ ) value followed by birds on diet T4, while birds on diet T3 had the least value.

The effect of dietary levels of aflatoxin on serum metabolites of broiler finisher chickens at 8 weeks of age is presented in Table 7. There were significant ( $p < 0.05$ ) differences across the dietary treatments for ALT values which was significantly higher for birds fed diet T4 followed by birds on the control diet T1 (0 ppb). There were no significant differences observed for the other parameters measured.

The effect of various mycotoxin binder levels on serum metabolites of broilers at 8 weeks of age is presented in Table 8. There were no significant ( $p > 0.05$ ) differences in AST, TP and ALP of birds across dietary treatments. However, serum alanine amino transferase was significantly ( $p < 0.05$ ) influenced by the dietary levels of mycotoxin binder. Birds on mycotoxin binder level of 2 % presented the highest

**Table 5: Interaction effect between aflatoxin levels and binder levels on performance characteristics of broiler chickens at 8 weeks**

Parameters	0 ppb						40 ppb						60 ppb						80 ppb						SEM			P value		
	0%		2%		1%		0%		1%		2%		0%		1%		2%		0%		1%		2%		A	B	A x B			
	0%	1%	2%	1%	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%											
IW (g/b)	313.70	316.31	329.52	319.62	305.30	332.02	316.46	301.96	330.46	314.63	303.10	327.57	29.15	0.992	0.141	0.999														
FW (g/b)	1163.13	1253.94	1206.74	1095.72	1023.12	1148.12	1060.03	1081.83	1207.94	987.58	1004.00	1109.03	70.04	0.121	0.006	0.961														
ADFI (g/b/d)	100.191	102.870	104.615	99.023	98.048	104.230	96.701	102.854	101.854	96.694	99.991	101.999	2.991	0.684	0.780	0.936														
TFI (g/b)	2805.32	2880.36	2929.36	2772.64	2745.34	2918.44	2707.61	2879.80	2851.82	2707.32	2799.72	2855.72	127.76	0.008	0.014	0.050														
ADG (g/b/d)	30.34	33.49	31.33	27.72	25.64	29.15	26.56	27.85	27.77	24.03	25.00	27.91	2.97	0.003	0.012	0.002														
TWG (g/b)	849.44	937.63	877.22	776.18	717.82	816.10	743.57	779.87	777.48	672.95	700.91	781.46	59.89	0.001	0.013	0.001														
FCR	2.727	2.625	3.150	3.233	3.017	3.175	3.433	2.758	3.358	3.691	3.608	3.183	0.148	0.024	0.710	0.013														
Mortality (%)	2.75	--	--	16.70	8.30	8.30	41.70	33.30	25.00	55.50	50.00	41.70																		
FC (₺/kg)	277.65	277.65	277.65	277.33	277.33	277.33	277.65	277.65	277.65	277.65	277.65	277.65																		

IW = Initial weight, FW = Final weight, TWG = Total weight gain, ADG = Average daily gain, TFI = Total feed intake, ADFI = Average daily feed intake, FCR = Feed conversion ratio, SEM = Standard error of means, FC = Feed cost.

value. Interaction effects between varying levels of dietary aflatoxin with binder supplementation at different levels on serum metabolites are presented in Table 9. Interaction between aflatoxin and binder levels showed a significant ( $p < 0.05$ ) effect only on the ALT of the birds. We observed that for every level of aflatoxin, there was an increase in the value of ALT especially at higher mycotoxin binder level. There were also no significant ( $p > 0.05$ ) interaction effect recorded for ALP, Albumin and TP. However, AST increased with increasing levels of binder supplementation only for T1.

### Organ weight

Effect exerted by varied aflatoxin levels on internal organs characteristics of broiler chickens is shown in Table 10. There were no significant ( $p > 0.05$ ) differences in all the parameters measured except for liver weight, spleen weight and bursa of fibricius weight. The main effect of varied binder levels on internal organ parameters of the broiler chickens is shown in Table 11. No significant ( $p > 0.05$ ) differences were observed between the varied binder levels in all the parameters measured except for % liver weight and small intestine length.

The interaction between varied Aflatoxin levels and mycotoxin binder levels on internal organs of broilers is shown in Table 12. The interaction shows significant ( $p < 0.05$ ) effect in the small and large intestines length of birds fed experimental diets while no significant ( $p > 0.05$ ) differences were observed on the other internal organs measured.

## DISCUSSION

Growth performance indices in this study revealed that inclusion of mycotoxin binder at 1% resulted in improved final live weight and daily weight gain. Feed conversion ratio also improved significantly with inclusion of toxin binder. The present report is not in agreement with the report of Girish and Devegowda (2004) who did not detect any differences in body weight and feed conversion ratio between broilers fed non-contaminated feeds containing toxin binder and without toxin binder. It however agreed with the report of Basalan *et al.* (2006) and Nadziakiewicz *et al.* (2019) who indicated that clay type toxin binders may change the cation binding capacity of the feeds and therefore feed and water consumption may change.

**Table 6. Effect of aflatoxin levels on serum metabolites of broiler chickens at 4 weeks**

Parameter	Aflatoxin Level (ppb)				SEM	LOS
	0	40	60	80		
AST (IU/L)	121.33	92.89	71.67	113.67	21.64	NS
ALT (IU/L)	44.89	87.00	42.67	101.56	367.80	NS
ALP (IU/L)	290.89	299.78	227.78	210.56	91.38	NS
Albumin (g/l)	31.89	31.00	32.89	33.00	2.11	NS
Total Protein (g/l)	56.11 <sup>a</sup>	45.44 <sup>bc</sup>	43.11 <sup>c</sup>	53.78 <sup>ab</sup>	2.53	*

NS: Not Significant, AST: Aspartate transferase, ALT: Alanine transferase, ALP: Alkaline Phosphatase.

<sup>a,b,c</sup> Means along the same row with different superscripts differ significantly ( $p < 0.05$ ).

**Table 7. Effect of aflatoxin levels on serum metabolites of broiler chickens at 8 weeks**

Parameter	Aflatoxin Level (ppb)				SEM	LOS
	0	40	60	80		
AST (IU/L)	201.33	207.56	219.89	278.44	33.13	NS
ALT (IU/L)	121.67 <sup>ab</sup>	106.11 <sup>b</sup>	113.44 <sup>b</sup>	178.78 <sup>a</sup>	27.22	*
ALP (IU/L)	130.00	141.11	172.11	160.22	14.05	NS
Albumin (g/l)	41.22	42.00	30.00	28.00	6.89	NS
Total Protein (g/l)	48.22	48.89	49.56	50.33	2.70	NS

AST: Aspartate transferase, ALT: Alanine transferase, ALP: Alkaline Phosphatase, LOS: Level of Significance, NS: Not significant.

<sup>a,b</sup> Means along the same row with different superscripts differ significantly ( $p < 0.05$ ).

**Table 8. Effect of mycotoxin binder levels on serum metabolites of broiler chickens at 8 weeks**

Parameter	Binder Levels (%)			SEM	LOS
	0	1	2		
AST (IU/L)	193.83	255.92	230.67	27.84	NS
ALT (IU/L)	82.25 <sup>b</sup>	135.67 <sup>a</sup>	172.08 <sup>a</sup>	21.37	*
ALP (IU/L)	149.67	160.92	142.00	12.74	NS
Albumin (g/l)	27.00	38.08	41.25	6.12	NS
Total Protein (g/l)	52.58	46.17	49.00	2.17	NS

AST: Aspartate transferase, ALT: Alanine transferase, ALP: Alkaline Phosphatase.

<sup>a,b</sup> Means along the same row with different superscripts differ significantly ( $p < 0.05$ ).

Final weight is a reflection of the breed and type of experimental diets fed to the birds, for example in the literature, the final live weight of broiler chicken of between 1.3 and 1.5 kg are considered standard (Oluyemi and Roberts, 2011).

High mortality was observed across treatments except for the birds fed diets containing no aflatoxin with or without binder supplementations. The percent mortality observed irrespective of binder supplementations

in diets 2, 3 and 4 clearly suggests the failure of the toxin binder to ameliorate the effect of aflatoxin. It can be deduced based on the findings that one of the very good signs of aflatoxicosis is heavy mortality which is in agreement with the work of Giambrone *et al.* (1985) who reported that turkeys that received 0.5 and 1.0 mg.kg<sup>-1</sup> of aflatoxins during 35 days of feeding were all dead. Heavy mortality may occur during aflatoxicosis for the following reasons:

**Table 9: Interaction effect between aflatoxin and mycotoxin binders levels on serum metabolites of broilers at 8 weeks**

Parameters	Binder Levels (%)										SEM	A	B	A x B	
	0	1	2	0	1	2	0	1	2	0					
AST (IU/L)	128.33	224.67	251.00	170.67	232.00	220.00	150.33	299.00	219.33	326.00	241.33	45.11	0.450	0.400	0.641
ALT (IU/L)	75.00	92.33	197.67	82.67	65.67	170.00	109.67	86.67	144.00	61.67	176.67	31.65	0.079	0.006	0.010
ALP (IU/L)	154.00	119.33	116.67	118.67	153.00	151.67	168.00	203.67	144.67	158.00	167.67	23.13	0.211	0.585	0.655
ALB (g/l)	31.33	58.67	33.67	27.33	31.33	67.33	29.33	28.00	34.33	20.00	34.33	9.22	0.502	0.330	0.472
Total Protein (g/l)	51.00	50.33	50.33	52.33	47.67	47.67	51.33	44.00	53.33	55.67	45.67	4.29	0.957	0.194	0.802

<sup>a,b,c</sup> Means along the same row with different superscripts differs significantly ( $p < 0.05$ ).

AST: Aspartate transferase, ALT: Alanine transferase, ALP: Alkaline Phosphatase, ALB: Albumin, TP: Total Protein.

During acute aflatoxicosis, aflatoxin B1 is hydrolysed to 8,9 epoxide and then to 2,3 – dihydrodiol which is the most carcinogenic form of toxin. This results in quick onset of necrosis of the liver cells and eventual death of the animal (TDRI, 1984).

During acute aflatoxicosis, there is interference of the toxin with the immune system of the chickens thereby reducing their resistance to other infections. Aflatoxin induces immuno suppression and increases susceptibility of toxicated birds to bacterial viral and parasitic infections. Immuno suppression caused by aflatoxin B1 has been demonstrated in chickens and turkeys as well as in laboratory animals (Sharma, 1993; Li *et al.*, 2022).

Haematological and serum biochemical indices are an index and a reflection of the effects of dietary treatment on the animal in terms of the type, quality and amounts of the feed ingested and whether the nutrients were available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola and Egbunike, 2008; Smith, 2020). It has been reported that serum biochemical alterations are better observed during chronic aflatoxicosis, even before major clinical signs appear (Oguz and Kurtoglu, 2000). This may probably be the reason why aflatoxin did not alter the values of serum parameters significantly in this study. Although there were no significant differences in the mean values of serum biochemical parameters for broiler chickens fed with high levels of aflatoxin with or without binder supplementation in this study, the trend observed in serum total protein showed a numerical increase in the values for the control group compared with the groups treated with aflatoxin. The numerically reduced serum protei values may probably be indicative of toxic effect of aflatoxin on hepatic and renal tissues. This was similar to the report of Kubena *et al.* (1993) and Solis-Cruz *et al.* (2019) who concluded that the reduced levels of total protein and albumin are indicative of the toxic effect of aflatoxin B1 on hepatic and renal tissues. Also, Kubena *et al.* (1998) reported that reduction in total protein in aflatoxin fed groups could referred to impairment of amino acid transport and RNA transcription by inhibiting DNA.

A marked differences were observed between the periods of exposure to aflatoxin as values obtained at starter phase is lower compared to values obtained at finisher phase. Mycotoxin binder supplementation at various levels in the diets did not have a significant ameliorative effect on the aflatoxin. This corroborate the study of Cole (1986) who stated that serum

enzymes have their principal function within the cell and increased level of serum enzymes are often a reflection of cellular destruction or disease and when these enzymes are present in large quantities in the liver of the animal (Contreras-Zentella and Hernández-Muñoz, 2016), it is increased in serum when cellular degeneration or destruction occurs in this organ. An increase in liver enzyme profile during aflatoxicosis is most likely reflective of liver tissue damage, alternated hepatocyte membrane integrity with leakage of enzymes into the blood (Dalvi and McGowan, 1984; Cattley and Cullen, 2013). This was also in accord with the work of Hung *et al.* (1989) who stated that ALP is a membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce dearrangement in transport of metabolites. An increase of these enzyme activities in the extracellular fluid or serum is a sensitive indicator of even minor cellular damage in which cellular enzymes are released from the cells into the blood stream, which in-turn indicate stress-based tissue impairment. The serum ALP at 4 weeks was relatively higher and this shows that there is much effect of aflatoxin at the starter phase on broiler chickens.

Liver is considered the target organ for aflatoxin B1 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 (Quist *et al.*, 2000; Miazzo *et al.*, 2005; Bailey *et al.*, 2006; Pasha *et al.*, 2007). Studies in chickens indicate that the liver is the organ most sensitive to the effect of aflatoxin (Huff *et al.*, 1986) while increment in liver size has been reported to be implicative of an inflammatory response (Xie *et al.*, 2000). Similarly, Ortatatli *et al.* (2005) reported an increase in the absolute weight of liver of birds fed on diet containing aflatoxin indicating hepato and nephrotoxicity of aflatoxin. During aflatoxicosis, synthesis of lipid in the liver is severely impaired due to depressed activities of fatty acid synthetase and microsomal enzyme system responsible for fatty acid elongation and lipid transport from liver is severely decreased (Gaines *et al.*, 1987; Dianzani, 2020). Hence, accumulation of liver lipid with depletion of normal fat reserves in the carcass (Wyatt, 1991). Inclusion of binder across the treatment diets showed a reduced liver weight which agreed with the work of Weibking *et al.* (1994) who reported that separate addition of HSCAS and turmeric powder to aflatoxin B1 diet prevented significantly the increasing

organ weight in turkeys that consumed aflatoxin diet alone. Kubena *et al.* (1993), Ledoux *et al.* (1999) and Gowda *et al.* (2008) showed that an increase in organ weight caused by aflatoxin B1 in broiler chickens could be counteracted by addition of HSCAS to the diet. Also, Sehu *et al.* (2007) demonstrated microscopically that addition of HSCAS to quail feed partially decreased fat deposition caused by the aflatoxin in the liver and consequently reduced the liver's weight.

Birds fed with 80 ppb level ppb level also had the highest gizzard weight. This could be attributed to the amount of work required of the muscular wall of the organ to comminute the feed particles (Johnson and McNab, 1983; Adeniji, 2005). Fasuyi (2007) also reported that gizzard are known to naturally increase in size to accommodate the introduction of bulkiness in diets. Their reports however corroborates with the present findings.

The significantly reduced weight of bursa of fabricius observed in birds on aflatoxin treatments might be due to regressive action of aflatoxin on lymphoid organ. This is in support of the work of Kubena *et al.* (1990) who reported a reduced weight of bursa of fabricius in aflatoxin treated birds and they attributed this to depression in immune system. The reduction in size of bursa of fabricius might be due to necrosis and cellular depletion by the mycotoxins (Hoerr *et al.*, 1981; Ekhlas, 2012). Mycotoxin exert potentiated depressing effects on bursa and thymus weight when fed in combination than in isolation, suggesting additive toxic effect among the lymphoid organs (thymus and bursa) (Raju and Devegowda, 2002; Kumar and Patra, 2017).

## CONCLUSION

The inclusion of mycotoxin binder has been reported to be beneficial and widely accepted in the prevention of aflatoxicosis as low inclusion level is required and it is easy to manage. From the present study, it was concluded that addition of mycotoxin binder (clay type) to aflatoxin contaminated diets was able to ameliorate the effect of aflatoxin on birds regardless of the level at which it was supplemented. Addition of mycotoxin binder to broiler diets shows optimum efficacy of the binder at 1% level as improved performance rate was obtained at this level of inclusion.

## ACKNOWLEDGEMENTS

The authors are grateful for technical support from the Mother-Hens Farm Laboratory Unit in Kaduna, Nigeria.

## AUTHOR CONTRIBUTIONS

Conceptualization: Alayande, L., Bawa, G. Sh.

Methodology: Alayande, L., Bawa, G. Sh.

Investigation: Alayande, L.

Data curation: Alayande, L., Ogundipe, S. O.

Writing-original draft preparation: Rano, N. B.

Writing-review and editing: Rano, N. B.

Project administration: Alayande, L., Rano, N. B.

All authors have read and agreed to the published version of the manuscript.

## INFORMED CONSENT STATEMENT

Not applicable.

## DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

## CONFLICTS OF INTEREST

There is no conflict of interest with any individual or organization regarding the materials discussed in this manuscript.

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