ORIGINAL ARTICLE

Performance and Blood Profile of Turkey Poults Fed With Different Doses of Aflatoxin in the Diets

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ABSTRACT

The effect of dietary aflatoxin on performance and blood profile in turkey was studied in 80 Nicholas turkey poults fed with varying concentration of aflatoxin (Af). In a completely randomized design, the poults were assigned to five experimental groups: T_1, T_2, T_3, T_4 and T_5 comprising 16 poults each. T_1 was given the control diet containing 0ppb Af while T_2, T_3, T_4 and T_5 had 50, 100, 150 and 200ppb Af, respectively in their diets. The feed intake and body weight gain significantly reduced in T_2 to T_5 than in control group. High mortality (6.25 – 87%) was recorded in T_2 to T_5 but none in T_1. Also, the white blood cell (WBC) and monocytes varied significantly ($p < 0.05$). However, the packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), heterophils, lymphocytes, eosinophils, basophils and platelets were not affected ($p>0.05$) by aflatoxin inclusion in the diets. Serum total protein, albumin and globulin were significantly reduced as aflatoxin level increased in the diet. Aspartate amino transferase (AST) on the other hand increased with increased aflatoxin concentration with no trial effect ($p>0.05$) on alanine amino transferase (ALT).

Key words: Aflatoxin, Turkey poults, Blood profile

INTRODUCTION

Aflatoxin is a class of mycotoxin producedmainly by 2 fungi species, *Aspergillus flavus* and *Aspergillus 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. Aflatoxin interacts with the basic metabolic pathways of cell disrupting key enzyme processes including carbohydrate and lipid metabolism and protein synthesis (Cheeke and Shull, 1985). Since 1960, when approximately 100,000 domestic turkey poults died from turkey 'x' disease which was later named aflatoxin, research has been conducted to determine the effects of the toxin on domestic animals (Blount, 1961). Although most animals have been shown to be susceptible, young animals are more susceptible than older animals presumably due to the lack of well developed hepatic enzymatic systems that are required to degrade the toxin (Cheeke and Shull, 1985). Moreover there is tremendous variability in the degree of susceptibility, even among closely related species. For example chickens are quite resistant to the effect of aflatoxin compared with turkeys (Arafa *et al*., 1981), but there is breed variability even among chickens. Similarly bobwhite quail (*Colinus virginianus*) are more susceptible to the effect of aflatoxin than are Japanese quail (*Coturnix japonica*) (Stewart, 1985). A number of studies have been conducted on the actual lethal dose of aflatoxin on turkey but with varying divergent views and results. For example, Giambrone *et al*. (1985) reported an extensive mortality in young domestic turkeys that consumed 400ppb aflatoxin in the diet. Witlock and Wyatt (1981) also found that lower levels of aflatoxin cause blood clotting abnormalities, immune dysfunction and decreased feed conversion. Joffe (1970) concluded that 650ppb is the minimum aflatoxin dose that can significantly reduce the weight gain in turkey poults. Hamilton *et al*. (1972) found that 250ppb is the minimum concentration that can significantly affect body weight gain in turkeys. Given these divergent views on the actual minimum concentration of aflatoxin that can affect the performance of turkeys poults, this study was conducted to investigate the least observable adverse effect level of aflatoxin in turkey or the minimum aflatoxin concentration that can reduce feed intake and body weight of turkey.

MATERIALS AND METHOD

Study Area

The study was conducted at the poultry unit of the Teaching and Research Farm of University of Ibadan, Ibadan Nigeria in the South western part of Nigeria.

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 and quantification of aflatoxin

Aflatoxin was produced from the pure culture of *Aspergillus 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 properautoclaving. 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 he intended purpose. 1mlof tween 20 was added to 1000ml of distil water for the purpose of sticking the spores of the fungi

spores to the maize grains firmly. The tween 20 mixture is then poured gently into each plate containing the fungi while the substrate is stirred gently with a spreader and poured back into the 1000ml jar and mixed vigorously. Five plates of the fungi are poured into 1000ml jar while spore count was done to determine the spore load per ml which was done by multiplying the average count by a factor of 50000. Aflatoxin in the maize grains was produced by inoculating the autoclaved maize with the spore solution as 100ml of spore solution was poured into each 1kg bag of maize and shook vigorously until no visible water is seen in nylon. 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 which is due to high levels of toxin produced from fungus activities on the maize grains. The mouldy maize grains were washed with tween 80 in order to kill the spores prior to drying of the maize grains. The maize grains were sundried on concrete floor in a green house.

Quantification

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.

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 1mg/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:

$$\text{Required amount of contaminated maize (kg)} = \frac{\text{Qty of finished feed (kg)} \times \text{conc. required in finished feed (mg/kg)}}{\text{Conc. of aflatoxin in maize carrier (mg/kg)}}$$

To convert mg/kg to ppb
1mg/kg = 1000ppb
Conc. = concentration

Birds and experimental design

Eighty 1-d-old Nicholas turkey poults were used for the study. The turkey poults were brooded for three weeks and fed with basal diet without aflatoxin. The poults were weighed at the third week and randomly allotted to five dietary treatments as follows: T_1 (Basal diet with no aflatoxin); T_2 (50ppb aflatoxin); T_3 (100ppb aflatoxin); T_4 (150ppb aflatoxin) and T_5 (200ppb aflatoxin). Each dietary treatment had 4 replicates with each of 4 turkey poults in a completely randomized design. The feeding trial lasted for 21 days and routine management practices in terms of medication and vaccination were observed.

Estimation of 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 14 of the feeding trial, blood samples were obtained from the jugular vein of two poults per replicate when mortality figures was below 50%. 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).

Blood samples used for haematology was collected in EDTA bottles to prevent it from clotting. The packed cell volume was determined using the Wintrobe haematocrit method as described by Wintrobe (1933). Haemoglobin was determined by Cyanmethaemoglobin method of Coles (1986). Red blood cell concentration was determined using improved Neubaeur haemocytometer after the appropriate dilution.

Mortality figures obtained from this study were obtained on day 21 of the experiment when it had risen above 50%, hence the need to terminate the experiment.

Data collection and statistical analysis

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

Data analysis

Data collected were analyzed using ANOVA in SAS (SAS, 1999) as completely randomized design and means were separated using Duncan multiple range test of the same software.

RESULTS

The result of performance of birds are presented in Table 2. Feed intake and weight gain of were significantly ($P<0.05$) reduced by aflatoxin levels higher than 50ppb. Feed intake and weight gain of birds were similar in the control and 50ppb aflatoxin diets.

The feed conversion ratio followed a similar trend but the control diet with no aflatoxin and the 50 and 100ppb aflatoxin diets had better feed conversion ratio than the rest diets.

Percentage mortality increased with increased aflatoxin concentration in the diets with values of 0, 6, 18, 56 and 87% for diets containing 0, 50, 100, 150 and 200ppb aflatoxin, respectively.

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

	Diet (ppb aflatoxin)				
Ingredients	0	50	100	150	200
Pure maize	52.40	47.40	42.40	37.40	32.40
Contaminated maize	-	5.00	10.00	15.00	20.00
Soybean meal	40.00	40.00	40.00	40.00	40.00
Fishmeal	5.00	5.00	5.00	5.00	5.00
Di-calcium **p**hosphate	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./Premix	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated nutrients					
Crude Protein (%)	28.44	28.44	28.44	28.44	28.44
Metabolizable Energy (kcal/kg)	3021	3021	3021	3021	3021
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

*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.

Table 2: Performance of turkey poults fed diets containing varying levels of dietary aflatoxin

	Diet (ppb aflatoxin)					
Parameters	0	50	100	150	200	SEM
Initial weight (g/bird)	230.50	232.30	233.30	229.30	232.00	1.04
Final Weight (g/bird)	779.50^{a}	761.50^{a}	643.00^{b}	516.80^{c}	481.50^{c}	29.30
Weight gain (g/bird)	532.80^{a}	529.30^{a}	409.80^{b}	287.50^{c}	249.50^{c}	28.97
Feed intake (g/bird)	1262.30^{a}	1247.00^{a}	982.00^{ab}	915.00^{b}	841.00^{b}	92.43
Feed conversion ratio	2.40^{b}	2.40^{b}	2.40^{b}	3.20^{a}	3.40^{a}	0.19
Mortality (%)	0.00	6.25	18.7	56.25	87.00	

$^{a, b, c}$ Means on the same row with different superscripts are significantly different (P<0.05).

The haematological parameters of turkey poults fed with micro doses of aflatoxin were shown in Table 3. The white blood cells and monocytes out of all haematological parameters assessed showed significant differences in their values but the difference did not follow a particular trend. For example the values of white blood cells in turkeys fed with 0, 50, 100 and 150ppb were similar but lower than those fed 200ppb of aflatoxin. Also values of monocyte for turkeys fed 0, 50, 100 and 200ppb were the same but lower than the value obtained for turkeys fed 150ppb of aflatoxin.

The results of serum biochemistry are shown in Table 4. Serum total protein and albumin were significantly reduced as aflatoxin concentration increased in the diets. The highest value for serum total protein (5.05g/dL) was obtained from turkey poults that fed T_1 (control) while T_4 (150ppb) recorded the lowest value (3.68g/dL). Serum albumin also showed significant reduction in their mean values across the group with turkey poults fed T_1 (control) being the highest (2.35g/dL) while T_3 had the lowest

(1.64g/dL). For the liver function enzyme AST, there was a significantly increases values from (106.38ui/L) in T_1 to 163.33ui/L in T_5. The ALT however did not show any significant difference.

Table 3: Haematological values of poults fed with different doses of dietary aflatoxin

	Diet (ppb aflatoxin)					
Parameters	0	50	100	150	200	SEM
PVC (%)	35.50	39.75	39.00	35.00	37.75	1.03
Haemoglobin (g/dL)	12.30	13.21	13.23	11.88	12.40	1.07
RBC (x 10^6/mm^3)	4.00	4.40	4.28	3.95	4.13	0.37
WBC (x 10^6/mm^3)	26.38^b	25.98^b	29.27^{ab}	26.40^b	32.13^a	2.73
Heterophils (%)	54.87	53.50	52.87	44.17	58.75	7.24
Lymphocytes (%)	43.25	41.88	42.50	50.17	37.25	7.19
Monocytes (%)	1.25^b	2.13^{ab}	2.25^{ab}	2.50^a	1.75^{ab}	0.46
Eosinophil (%)	1.63	2.38	2.25	2.83	1.25	0.76
Basophil (%)	0.25	0.13	0.13	0.33	0.00	0.22
Platelets (x 10^3/L)	263.25	245.12	335.88	263.00	338.75	1.25

[a,b,c] Means on the same row with different superscripts are significantly different (P<0.05). WBC = white blood cells, SEM = standard error of means

Table 4: Serum biochemical values of poults fed with different doses of dietary aflatoxin

	Diet (ppb aflatoxin)					
Parameters	0	50	100	150	200	SEM
Total protein (g/dL)	5.05^a	3.80^b	3.68^{bc}	3.63^{bc}	4.13^{ab}	0.24
Albumin (g/dL)	2.35^a	1.71^b	1.64^b	1.78^b	1.70^b	0.20
Globulin (mg/dL)	2.71^a	2.09^b	2.21^{ab}	1.90^b	2.43^{ab}	0.22
AST (iu/L)	106.38^b	139.75^a	137.75^a	147.50^a	169.33^a	11.97
ALT (iu/L)	31.75	38.63	36.88	40.67	45.00	5.48

[a,b,c] Means on the same row with different superscripts are significantly different (P<0.05). AST = aspartate aminotransferase, ALT = alanine aminotransferase, SEM = standard error of means

DISCUSSION

The decreased feed consumption and weight gain that we observed in this study is commonly seen in all aflatoxin-fed domestic poults (Arafa *et al*., 1981). It has been argued that taste aversion causes the decreased feed consumption and resultant diminished weight gains (Arafa *et al*., 1981). Feed consumption in broilers fed on aflatoxin B_1 was significantly decreased and this is suggestive of reduced appetite during aflatoxicosis as a protection mechanism (Rauber *et al*., 2007). The decreased weight gain observed in this study is similar to reports of 1-day-old (Giambrone *et al*., 1985a; Arafa *et al*., 1981) and 3wk-old (Giambrone *et al*., 1985b) domestic turkey poults fed aflatoxin. Control wild turkey poults weighed more than 400g after aflatoxin-fed group compared with 30% weight differential in domestic poults fed 200ppb aflatoxin (Wenking *et al*., 1994) or 250ppb aflatoxin (Pier and Hedlesten, 1970). Several factors may have contributed to reduced weight gain in turkey poults, such as reduced feed intake, reduction in liver protein synthesis, and decrease in lipid metabolism. In addition, Osborne and Hamilton (1981) investigated the effects of dietary aflatoxins on various digestive enzymes in broiler chickens and found the specific activities of pancreatic amylase, trypsin, and lipase to be approximately one half of the activities observed in control chicks. Also the detrimental effects of Aflatoxin on growth performance in this study agreed with the previous studies of Kubena *et al*. (1993) and Miazzo *et al*. (2000) who reported that feed consumption and body weight gain decreased by 10% and 20% in broiler chicks given 2.5mg/kg of aflatoxin for 3 weeks.

In this study, mortality may have occurred in turkeys fed 50, 100, 150 and 200ppb aflatoxin because of the liver damage associated with necrosis and the interference of the toxin with the immune system of the turkeys. The result obtained in this study is similar to the one obtained by Rauber *et al*. (2007) where they reported 18.7% mortality for turkey poults that were fed with 200ppb. In a study conducted by Giambrone *etal*. (1985b) using 500ppb and 1000ppb of aflatoxin, all the turkeys were dead within 35 days.

The result of haematology from this study showed that there was no significant difference in the values of packed cell

volume, haemoglobin, red blood cells, heterophils, lymphocytes, eosinophils, basophils and platelets for turkey poults that were fed micro doses of aflatoxin This is in agreement with the report of Quist *et al*. (2000) who also found no significant difference in the values of PCV, total WBC, heterophils, basophils, monocytes and lymphocytes for turkey poults that were fed up to 400ppb of aflatoxin. On the contrary, Oguz *et al*. (2003) and Huff *et al*. (1986) have reported the suppressive effects of aflatoxins on haematopiesis and immune responses. Weibking *et al*. (1994) and Tung *et al*. (1975) have also reported decreased values of packed cell volume and haemolytic anaemia in aflatoxin-exposed domestic poults.

Serum parameters are sensitive indicators of toxic effects of aflatoxin on the target organs. The serum biochemical effects are also clearly observed in this study. The significantly reduced serum total protein and albumin in this study were due to the effect of the aflatoxin on protein synthesis (Rosa *et al*., 2001). Also, the reduction in the total serum protein in aflatoxin fed groups could referred to impairment of amino acid transport and mRNA transcription by inhibiting DNA (Kubena *et al*., 1993) and is indicative of impaired protein synthesis (Kubena *et al*., 1989). The main aflatoxin action mechanism is the reduction in the function of liver, primarily inhibition of synthesis of proteins (Hussein and Brasel, 2001). The reduced levels of total protein and albumin are 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, Tejada-Castaneda *et al*., 2008). Quist *et al*. (2000) showed that total protein levels are significantly reduced in turkeys poisoned with 200 and 400ppb of aflatoxin. Witlock and Wyatt (1981) have also found a decrease in total protein levels with aflatoxin contamination equal or higher than 125ppb in turkey.

In the current study, the result for serum enzyme (AST) for turkeys fed with aflatoxin contaminated diets was significantly higher than control and this is indicative of impaired permeability of the liver which is a common occurrence during aflatoxicosis (Duncan andPrasse, 1986). An increase in liver enzyme profile (AST and ALT) in aflatoxicated ration is most likely reflective of liver damage, alternated hepatocyte membrane integrity with leakage of enzymes into the blood (Duncan and Prasse, 1986). The results herein are in accordance with the findings of Aravind *et al*. (2003) and Dehli *et al*. (2009) who reported an increase in AST and ALT activities upon feeding diets contaminated with different doses of aflatoxin. On the contrary, Manegar *et al*. (2010) could not record any significant alteration in liver's enzyme profile upon feeding birds with aflatoxin.

CONCLUSION

Different doses of aflatoxin caused significant changes in performance and blood profile of turkey poults in this study. Aflatoxin concentration up to 100ppb or more is capable of reducing the feed intake, body weight gain and high mortality in turkey poults within a period of 21 days. Turkey poults are highly sensitive to aflatoxin and significant loss in a flock could occur during aflatoxicosis.

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