Enzyme profile and haematology as indices of morbidity in broilers fed dietary aflatoxin

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Abstract: An experiment was conducted to evaluate the toxic potentials of aflatoxin contaminated feed on vital organs and tissues in broilers using various 'marker' like enzymes, kidney function indices and haematological parameters. Fifteen birds were randomly distributed on three dietary treatments comprising of five birds per treatment. Treatment A (control) received a diet containing less than 20ppb, treatment B (90ppb) and treatment C (180ppb) aflatoxin level respectively throughout the 5-week study period. Using analysis of variance (ANOVA) and Duncan's Multiple Range Test, the result showed that the concentration of Liver alkaline phosphatase and liver alanine transaminase and aspartate transaminase reduced progressively with an increase in dietary aflatoxin concentration. However, there was an increase in serum alkaline phosphatase and alanine transaminase as the toxin load increased in the feed.. The overall results of the haematological parameters indicate that the birds are not affected by the varying aflatoxin levels.
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INTRODUCTION

Mycotoxins are a group of extremely toxic and biologically active substances. Among them are the aflatoxins (AF) which are produced consistently by strains of by the fungi *Aspergillus flavus* and *A. parasiticus* (Wilson and Payne, 1994). A. flavus is the most common contaminant of many grains used in human and animal nutrition (Abarca *et al*., 1994). AFs have been detected in the pre-harvest, post-harvest, transport, storage and after processing and packing of grains. Under appropriate humidity and temperature conditions, A. flavus produces four toxins: aflatoxin B1 (AFB1) and three compounds with similar structures (AFB2, AFG1, and AFG2). AFB1 is considered to be one of the most potent hepatotoxins and well-known hepatocarcinogens (Wilson and Payne, 1994). Aflatoxins are the most common contaminants in the feed of domesticated animals, including birds (Jindal *et al*., 1993). One report on contamination of grains with aflatoxins showed 77% was due to B1 while the rest were contaminated with other aflatoxin types (Wilson and Payne, 1994). Some important characteristics of these toxins are their capacity for bioconcentration and bioaccumulation as well as their great stability in different biotic and abiotic environments (Penla and Duran – de Bazua, 1990). Aflatoxins are potent carcinogens and cause growth depression (Umesh *et al*., 1990) and reduced disease resistance in poultry, other livestocks (Giambrone *et al.,* 1978). The Aflatoxin contamination of feed stuff has been reported to range from 10-1500 ppb in commercially used feed ingredients and 34-115 ppb in mixed feed samples (Devegowda *et al*., 1993). The high level of contamination though not resulting in better utilization of available ingredients and severe outbreaks of aflatoxicosis causes heavy economic loss in terms of health and production. The toxicity with aflatoxin followed by contamination of feed with fungi in chickens is characterized by mortality, listlessness, anorexia, decreased growth rates, negative feed conversions, fatty liver, decreased egg production, poor pigmentation and increased susceptibility to other diseases (Arafa *et al*., 1981; Doerr *et al*., 1983) and cause of economic losses in broiler production. Furthermore, a small amount of aflatoxin and its metabolites can be found in several edible tissues (Micco *et al*., 1998) and risks for public health. The toxin may also serve as an antimicrobial(Praveena and Padmini, 2011).

The aim of this study was to evaluate the toxic potentials of aflatoxin contaminated feed on vital organs and tissues in broilers with particular reference to enzymes, kidney function indices and haematological parameters.

MATERIALS AND METHODS:

The experimental diets used were formulated in the Biochemistry and Nutrition unit of the Fountain University Osogbo, Osun state, where the study was

carried out. Common to all of the formulations were 23% crude protein (CP), 0.6% methionine, and 1.2% lysine. Three diets were prepared.(Table 1) Control diet contained 20%ppb aflatoxins level. Diets 2 and 3 (experimental) had 90 and 180% respectively as shown in table 1. The aflatoxin assay was carried out by the ELISA method using AgraQuant test kit

A total of 15 day-old broiler chicks (Arbor Acre strain, CHI Ltd, Ajanla Farms, Ibadan) were wing banded, weighed and randomly allocated to the 3 dietary treatments.. The birds were housed in a well illuminated and ventilated poultry house. Feed and water were provided *ad libitum*. They were vaccinated against Newcastle disease virus (lasota vaccine) at day 8 of age, and against Infectious Bursal Disease (IBD) virus at day 10 of age via drinking water. Second Newcastle disease virus vaccine (booster vaccine) was given at the 16th day. There were 5 birds for each treatment

Serology and preparations of tissue homogenate:

Blood samples were collected from the five birds in each treatment through the jugular vein at the end of the experiment. Serum was separated by centrifugation (8,000 rpm for 5 minutes) and was kept frozen until needed. The liver and kidney were removed, cleaned, weighed and were homogenized in 0.25M ice - cold sucrose solution which was later frozen till required.

Haematological study:

Blood samples were collected using EDTA treated bottles from five chicks per treatment through the jugular vein at the 35th day (last day) of the experiment and was analysed for the following haematological parameters : white blood cell, red blood cell, lymphocyte, platelets (Mitruka and Rawnsley 1981), so as to assess the health status of the birds .

Chemical analysis / Statistical analysis :

The aflatoxins level in the feed was determined using Enzyme linked immunoassay kit according to the methods of AgraQuant test kit.

The group mean (n=5) + S.D (Standard Deviation) was subjected to analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT).

Table 1. Gross composition (g/100gDM) of the experimental diets.

	DIET A	DIET B	DIET C
Crude protein (%)	23	23	23
Methionine (%)	0.6	0.6	0.6
Lysine (%)	1.2	1.2	1.2
Afflatoxins level	<20% ppb	90% ppb	180% ppb
Ingredients			
Maize	36.00	36.00	36.00
Soybean meal	41.00	41.00	41.00
Wheat Offal	13.00	13.00	13.00
Palm oil	5.00	5.00	5.00
Dicalcium phosphate	2.14	2.14	2.14
Limestone	2.09	2.09	2.09
Vitamin-mineral premix	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Met	0.27	0.27	0.27
Lysine	-	-	-
Total	100.00	100.00	100.00

CP-crude protein **Premix supplied the following information kg of diet: Vitamin A (12,500,000 I.U), Vit D3 (2,500,000 I.U), Vit E (40,000mg) Vitamin K3 (2,000mg), Vit B, (3,000mg), Vit B2 (5,500mg), Naicin (55,000mg), calcium panthothenate (11,500mg) Vit B6 (5000mg) Vit B12 (25mg), choline chloride (500, 000mg), folic acid (1,000mg), Biotin (80mg), Mn (120,000,mg), Fe (100,000mg), Zn (80,000mg), Cu (8,500mg), I (1,500mg) Co (300mg), Se (120mg)*

RESULTS AND DISCUSSION:

At 20ppb, there was a significant reduction in alkaline phosphatase activity in the liver from 36.630±1.722 to 28.992±1.376(Table 2) in birds fed with 180ppb aflatoxin based diet while there was a corresponding significant increase in serum and kidney. The cellular enzyme increased in the serum. This means that the enzymes could have leaked into extracellular fluids as a result of loss of membrane components , the loss in ALP activity in liver may adversely affect the transfer of metabolite or required ions across the cell membrane, which may result in insufficient ions and metabolite to renal cells. This was earlier suggested by Akanji *et al*; (1993).

Table2: Results of Enzyme studies of birds fed varying levels of aflatoxins based diets

		Dietary treatments	
Parameters	<20ppb	90ppb	180ppb
Serum Alkaline Phosphatase	3.202±0.170[a]	3.752±0.206[ab]	11.80±2.138[b]
Kidney Alkaline Phosphatase	34.260±0.477[a]	39.300±2.217[a]	69.760±6.492[b]
Liver Alkaline Phosphatase	36.630±1.722[a]	36.528±1.818[a]	28.992±1.376[b]
Serum Alanine Transaminase	1.328±0.122[a]	4.540±0.493[b]	4.880±0.432[b]
Kidney Alanine Transaminase	0.316±0.150[a]	1.580±0.912[b]	3.640±0.344[c]
Liver Alanine Transaminase	1.926±0.161[a]	1.738±0.152[b]	1.232±0.188[bc]
Serum Aspartate Transaminase	76.064±4.174[a]	32.722±1.221[b]	23.646±1.036[c]
Liver Aspartate Transaminase	68.572±6.510[a]	38.294±4.352[b]	34.958±3.744[b]
Serum Creatinin	4894.4±73.531[a]	5046±57.678[b]	5094.8±63.291[b]
Serum urea	95.964±4.704[a]	107.280±18.821[b]	142.920±16.463[c]

[*abc] means within the rows with different superscripts are significantly different. ($p<0.05$)

Table3: Haematological Parameters of birds fed varying levels of aflatoxins based diets

		Dietary Treatments	
Parameters	<20ppb	90ppb	180ppb
Red blood cell	2.66±0.198[a]	2.59±0.193[a]	2.32±0.197[ab]
White blood cell	257.80±22.538	245.26±17.296	264.82±11.389
Haemoglobin	7.84±1.435[a]	10.46±0.826[b]	11.00±0.803[b]
Haematocrit	36.66±2.978	34.44±2.131	35.40±2.691
MCV	140.38±5.126	139.56±4.250	136.96±0.508
MCH	38.68±4.101	41.56±1.201	41.92±1.184
MCHC	29.08±1.648	29.80±1.336	30.58±0.909
Platelets	0.80±0.837	1.20±0.837	1.20±0.837
Lymphocytes	95.58±0.581	95.48±2.701	95.17±2.273
RDW	16.46±1.643	16.56±0.546	17.28±1.737

[*ab] means within the rows with different superscripts are significantly different. ($p<0.05$)

The aminotransfererases occupy a central position in amino acid metabolism and are active in both the cytoplasm and mitochondria of cells where they linked protein metabolism to carbohydrates metabolism (Rafelson *et al*., 1980). They are present in the liver, heart, kidney, skeletal muscle and other tissues. (Tietz, 1987). Both enzymes are 'markers' of liver damage caused by exposure to chemicals (Nelson and Cox, 2000) with alanine transaminase been more liver specific (Tietz, 1987). Aspartate transaminase levels are elevated when there is liver damage, leading to possible heart attack. (Tietz, 1987). Increase in serum enzyme may also be due to cell proliferation, increase cell turnover, or reduced clearance from plasma (Mayne, 2005).

The loss in alanine transaminase activity in liver from 1.926±0.161 in birds fed less than 20ppb to 1.232±0.188 in birds fed 180ppb aflatoxin based diet is consistent with its increase in the serum and the kidney. It may be due to leakage of this enzyme into extra cellular fluid caused by altered endothelial permeability (Wroblewski and La Due, 1955; 1956) leading to escape of abnormal quantities of the enzyme into the extracellular space. The loss in alanine transaminase activity would adversely affect the liver since pyruvate is a source of carbon for glucose synthesis and the

enzyme is also involved in deamination of alanine to pyruvate, providing amino groups for the urea cycle.

The consistent decrease in activity observed in the serum and liver aspartate transaminase in birds fed from less than 20ppb to180ppb aflatoxin based diet could be due to inhibition of the enzyme activity, inactivation of the enzyme *in situ* or depletion of important molecules required for their activities.

Kidney Functions Indices:

The kidney excretes urea and also reabsorbs electrolytes back into the blood thereby regulating their excretion. Filtration occurs in the glomeruli and reabsorption occurs at the renal tubules (Mayne, 2005). As glomerular function deteriorates, substances normally cleared by the kidneys accumulate in the plasma, e.g. urea and creatinine. Urea is formed in the liver from amino acids and is excreted by the kidneys. Creatinine is mostly derived from endogenous sources by tissue creatine breakdown and its concentration in blood is related to body mass (Mayne, 2005). The significant increase in serum Creatinine concentration from 4894.4±73.531 in birds fed less than 20ppb to 5094.8±63.291 in birds fed 180ppb aflatoxin based diet revealed a low glomerular filtration rate caused by impaired glomerular function.

Concentration of serum urea increases significantly from 95.964±4.704 in birds on 20ppbb to 142.920±16.463in birds on 180ppb aflatoxin based diet possibly due to the fact that its rate of production exceeds rate of clearance.

Haematological Parameters:

Marked difference among the treatments was noticed on the red blood cells and haemoglobin levels(Table 3) Red blood cell count, haemoglobin and mean corpuscular haemoglobin concentration are all indices of red blood cell and their reduction can indicate anaemia just as an increase can indicate increased rate of erythropoiesis (Ganong, 2001). Platelets activate the blood clotting mechanism (Pasternak, 1979) and function mainly in the formation of mechanical plugs during the normal haemostatic response to vascular injury (Hoffbrand *et al*., 2004). Haematological analyses(Table 3) did not also show a marked departure from the normal which indicated that the birds were not affected by the varying aflatoxin levels, even though there was a likely compromise in the rate of oxygen transport, which is a major function of rbc. In an experiment with chicken and ducks, Ostwoski-Meissner,(1984) earlier reported no significant reduction in body weight .Haematology, body weight gain may therefore be linked in avian aflatoxicology

Conclusion:

The results obtained in this study showed that birds could still survive at less than 20ppb and 90ppb in most cases but not at 180ppb aflatoxin level. This report could not confirm that consumption of chicken poults fed above 90ppb aflatoxin based diet could be injurious to the health even when Gregory *et al*, (1983) earlier reported that muscles (not of the liver) tissues of chicken fed up to 500ppb aflatoxin B1may not be a source of serious danger to a human consumer of such birds' infected muscles

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References

1 Abarca, M.L., Bragulat, M.R., Castella, G., Cabanes, F.J. 1944.Mycoflora and aflatoxin-producing strains in animal mixed feeds. *J. Food Prot. 57, 256-258.*

2 Akanji, M.A., Olagoke, O.A. and Oloyede, O.B. (1993) Effect of chronic consumption of metebisulphite on the integrity of the rat kidney cellular system. Toxicol. 81: 173- 179.

3 Arafa AS, Bloomer RJ, Wilson HR, Simpson CF, and Harms RH (1981) Susceptibility of various poultry species to dietary aflatoxin. British Poultry Science 22: 431–436.

4 Cortés G, Carvajal M, Méndez-Ramírez I, Avila-González E, Chilpa-Galván N, Castillo-Urueta P, Flores CM.2010 Identification and quantification of aflatoxins and aflatoxicol from poultry feed and their recovery in poultry litter Poult Sci. 89(5):993-1001

5 Devegowda, G. and B.I.R. Aravind, 1993. Survey of aflatoxin B1, contamination of feedstuffs. Proc. 6th Animal Nutrition Research Workers Conference, Bhubaneshwar, pp: 194.

6 Doerr JA, Huff WE, Wabeck CJ, Chaloupka GW, May JD, and Merkerley JW (1983) Effects of low-level chronic aflatoxicosis in broiler chickens. Poultry Science 62: 1971–1977.

7 Ganong, W.F. 2001. Review of Medical physiology. 20th ed. Lange Medical Books/mcgraw Hill medical publishing Division, London pp 414 – 417.

8 Giambrone, J.J., M. Partadivedja, C.S. Edison and S.H. Klevan, 1978. Interaction of aflatoxin with infectious bursal disease virus infection in young chickens. Avian Dis., 22: 431-439.

9 Gregory J F; S.L. Goldstein, and G.T. Edds (1983) Metabolite distribution and rate of residue clearance in Turkeys fed a diet containing aflatoxin B_1. Food and Chemical Toxicology 21 (4), 463–467

10 Hoffbrand, A.V., Pettit, J.E. and Moss, P.A.H. 2004. Essential Haematology, 4th ed. Blackwell science Asia Ply Ltd. Victoria, Australlia.
11 Jindal, N., Mahipal, S.K., Mahagan, N.K., 1993. Occurence of aflatoxin in compound poultry feeds in Haryana and effect of different storage conditions on its production. *Indian J. Animal Sci 63, 71-73*
12 Mayne, P.D. 2005 Clinical Chemistry in Diagnosis and Treatment. 6th Ed. Lloyd—Luke (Medical books) Ltd.
13 Micco C, Miraglia M, Onori R, et al. (1998) Long term administration of low doses of mycotoxins to poultry: Residues of aflatoxin B1 and its metabolites in broilers and laying hens. Food Additives & Contaminants 5: 303–308.
14 Mitruka, B.M. and Rawnsley, H.M. 1981. Clinical, Biochemical and Hematological reference values in normal experimental animals and normal humans, 2nd edition, Masson Pub., New York.413 pp.
15 Nelson, D.L and Cox, M.M. (2000) Lehninger Principles of Biochemistry. 3rd edition Worth Publishers
16 Ostrowski-Meissner H T (1984) Effect of contamination of diets with aflatoxins on growing ducks and chickens Tropical Animal Health and Production
15 (3), 161-168,
17 Praveena Y S N and Padmini, P C (2011) Antibacterial activities of mycotoxins from newly isolated filamentous fungi. Int J. Plant, Anim Env Sci.1(1)8-12
18 Rafelson, M.E., Hayashi, J.D and Beckoro-Vainy, A. (1980) Basic Biochemistry 4th edition. Macmillan Publishing Co. Inc. New York.
19 Snyder, D.B., Marquardt, W.W., Mallinson, E.T. and Savage P.K. (1984). Linked immunosorbent asay.III. Simultaneous measurements of antibody titers to infectious bronchitis, infectious bursal disease, and New-castle disease viruses in a single serum dilution. Avian Dis. 28: 12-24.
20 Tietz, N.W. 1987 Fundamentals of Clinical Chemistry 3rd Edition W.B. Sunders Company Philadephia. P.391
21 Umesh, D., G. Devegowda and B.S. Barmase, 1990. Reducing the adverse effects of aflatoxin through nutrition in broiler chickens XIII Annual Poultry Conference and Symposium, Abst. PMHE L pp: 50-136.
22 Wilson, D.M., Payne, G.A. 1994. Factors affecting Aspergillus Flavus group infection and aflatoxin contamination of crops. In: Eaton, D.L. Groopman, J.D. (Eds.). The toxicology of Aflatoxins. Academic Press inc, San Diego, pp123-145
23 Wroblewski,F. And La Due, J.S. (1955) Serum glutamate – oxaloacetate transaminase as an index of liver cell injury, a Preliminary Report. Am. Int. Med. 43: 345
24 Wroblewski,F. And La Due, J.S. (1956) Serum glutamate – pyruvate transaminase in cardiac and hepatic disease. *Proc. Soc. Exp. Med. 91 : 569-571*

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