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Acetylcholinesterase activity, total protein concentration in brain regions and blood profile of Yaffa breeder cocks fed fumonisin contaminated diets

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ABSTRACT

The implications of feeding diets contaminated with graded levels of fumonisin B₁ (FB₁), a toxic metabolite of the fungus *Fusarium verticillioides* consumed by farm animals on acetylcholinesterase (AChE) activity and total protein concentrations of the brain, blood profile and serum biochemical parameters of breeder cocks were investigated in a sixteen week feeding trial. Sixty pre-pubertal breeder cocks of about 16 weeks of age were randomly assigned to four diets containing 0.2, 5.2, 10.2 and 15.2 ppm FB₁ constituting diets 1, 2, 3 and 4 respectively. At the end of the feeding trial, blood and serum samples were collected from the cocks and analyzed. Subsequently, the cocks were killed by decapitation, their brains were carefully removed and washed in ice-cold saline. 10mg of brain tissue sample was taken from cerebral cortex, cerebellum, medulla oblongata, hypothalamus, amygdala, pons-varolli and hippocampus for acetylcholinesterase (AChE) activity and total protein concentration determination. Results showed that AChE activity progressively and significantly ($p < 0.05$) decreased in cerebral cortex, medulla oblongata, amygdala, ponsvarolli and hippocampus as the dietary FB₁ levels increased. The dietary FB₁ had no significant effect on the AChE activity, in cerebellum and hypothalamus. Breeder cocks fed diets containing 10.2 and 15.2 ppm FB₁ suffered significantly ($p < 0.05$) reduced concentration of total protein in the cerebral cortex, cerebellum, medulla oblongata, hypothalamus, pons varolli and hippocampus. Erythrocytes, haemoglobin and eosinophils significantly decline while leukocytes, lymphocytes and heterophils significantly increased with increase in dietary FB₁. The serum total protein of cocks fed diets containing 10.2 and 15.2 ppm FB₁ were significantly inferior to those fed other diets whereas the serum AST values significantly increased as the dietary FB₁ increased. This study demonstrated that dietary FB₁ concentration of 10.2 ppm and above is a potential health risk that may adversely affect neurotransmission and alter brain regional neurochemistry in breeder cocks.

Introduction

The presence of mycotoxins and anti-nutritional factors in most of the ingredients used in formulating rations for intensive livestock species, particularly poultry is of great concern to the professionals (Ogunlade and Egbunike, 2013). Fumonisin B₁, a novel mycotoxin produced by the fungus *Fusarium verticillioides* has been reported to be a major contaminant of maize intended for use in poultry rations formulation and human consumption (Shephard *et al.*, 1996).

The importance of maize, a feed ingredient of high caloric value, in formulation of poultry ration cannot be overemphasized. Fumonisin B₁ (FB₁) has been implicated in reproductive disorders in male and female animals (Javed *et al.*, 1993, Bacon *et al.*, 1995, Flynn *et al.*, 1996, Danicke *et al.*, 2004, Ogunlade *et al.*, 2006, Gbore, 2009, Ogunlade, 2015, 2019). It has also been reported that fumonisin in the blood stream has the ability to cross the blood-brain barrier thereby impairing neurotransmission in the brain (Kwon *et al.*, 1997). Swamy *et al.* (2004) implicated fumonisin to have altered the

brain regional neuro chemistry in pigs and broiler chickens chronically fed fumonisin contaminated diets. Acetylcholinesterase is one of the most crucial enzymes for nerve response and function, it is believed to be involved in protein metabolism in the brain (Adejumo and Egbunike, 1988).

With the above in mind, coupled with the dearth of information on the potentials of fumonisin B₁ on the brain and blood chemistry of breeder cocks, this work was undertaken to investigate the implications of feeding diets contaminated with fumonisin B₁ on acetylcholine esterase activity, total protein concentrations in different regions of the brain and blood profile of breeder cocks.

Materials and Methods

Location

This study was conducted at the poultry unit of the Teaching and Research Farm of the University of Ibadan, Nigeria.

Ibadan is located at latitude $7^{\circ} 20^1$ N, longitude $3^{\circ} 50^1$ E and 200m above sea level with an average day time temperature of $24-25^{\circ}$ C and relative humidity of 80-85%.

Experimental Feed Ingredients and Diets

The inocula of toxigenic strain of *Fusarium verticillioides* (MRC 286) were introduced to autoclaved maize grains to produce FB₁ according to the method described by Nelson *et. al.* (1994) at the Mycotoxin Laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The inoculated maize grains were air-dried, ground and quantified for FB₁ using CD-ELISA test kit (Neorgen Corp., U.S.A). Four experimental diets containing 0.2, 5.2, 10.2 and 15.2 ppm FB₁ were formulated by substituting autoclaved, non-cultured maize with the ground cultured maize in various proportions. The diets were isocaloric and isonitrogenous and satisfied the nutritional specifications of breeder cocks (Oluyemi and Robert, 2000). The gross composition of the diets is shown in Table 1.

Animals and Management

Sixty pre-pubertal breeder cocks of about 16weeks of age sourced from a reputable commercial farm in Abeokuta, Ogun State, Nigeria were randomly allotted to the experimental diets after a two-week physiological adjustment period in a completely randomized design such that each experimental diet had 15 breeder cocks replicated thrice with 5 cocks per replicate. The birds were individually housed in previously sanitized cages and were fed their respective diets *ad libitum* for a period of 16 weeks.

Determination of Acetylcholinesterase and Total Protein Concentration

At the end of the feeding trials, all the breeder cocks were starved for 12 hours and thereafter killed by decapitation and the brains were quickly and carefully removed and washed in

ice-cold saline. 10mg of brain tissue sample was taken (using a sensitive digital balance) from cerebellum, amygdala, hippocampus, hypothalamus, cerebral cortex, pons varolli and medulla oblongata regions of the brain with sterilized fine bent forceps and scalpel. Homogenate (1% w/v) of the different regions of the brain was prepared in ice-cold 0.1M phosphate buffer (pH 7). The homogenate was centrifuged for 20min at 6000rpm and the supernatant was used for the assay. (Nayeemunnisaand Suraiya,2009). Activity levels of acetylcholinesterase (AChE) were determined spectrophotometrically by the method of Ellman*et.al.* (1961). The total protein concentration in the brain regions were evaluated as outlined by Bitto*et.al.* (2000).

Determination of haematology and serum biochemical parameters.

Before the breeder cocks were decapitated, blood was collected from the jugular vein of the cocks into two vacutainer tubes, one containing Ethylene diamine tetra acetic acid (EDTA) for haematological parameters and the other without EDTA to allow for clotting and separation of serum which was deep frozen and subsequently used for serum biochemical and enzymological analyses. Red blood cells (RBC), Haemoglobin (Hb), Packed cell volume (PCV), blood constants, white blood cells(WBC), differential counts, serum total protein, globulin, uric acid and aspartate amino transferase(AST) were determined as reported by Ogunlade and Egbunike (2013).

Statistical Analysis

The design of this experiment is complete randomized design. Data obtained were subjected to standard statistical analysis using ANOVA procedure of Statistical Analysis Systems Institute (SAS, 1999) while the treatment means were compared using the Duncan's Multiple Range Test of SAS (1999).

Results

Table 2 shows the acetylcholinesterase activity levels in different regions of the brain of breeder cocks fed fumonisin contaminated diets. AChE activity levels progressively and significantly decreased in cerebral cortex, medulla oblongata,

amygdala, pons-varolli and hippocampus as the dietary levels of FB₁ increased. However, the activity levels of AChE in cerebellum and hypothalamus were not significantly altered by the dietary levels of FB₁.

Table 1: Gross composition (%) of the experimental diets fed to the breeder cocks

Ingredients	Treatments			
	Diet 1 0.2ppmFB ₁	Diet 2 5.2ppmFB ₁	Diet 3 10.2ppmFB ₁	Diet 4 15.2ppmFB ₁
Non-Cultured Maize	40.000	38.26	36.52	34.78
Cultured Maize ^a	-	1.74	3.48	5.22
Wheat Offals	29.20	29.20	29.20	29.20
Soybeans Meal	8.00	8.00	8.00	8.00
Fish Meal	2.00	2.00	2.00	2.00
Palm Kernel Cake	17.00	17.00	17.00	17.00
Bone Meal	2.00	2.00	2.00	2.00
Oyster Shell	1.00	1.00	1.00	1.00
(Nacl) Salt	0.25	0.25	0.25	0.25
Premix ^b	0.25	0.25	0.25	0.25
Methionine	0.10	0.10	0.10	0.10
Lysine	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Analysed Nutrients				
Crude Protein (%)	16.68	16.66	16.64	16.51
Crude Fibre (%)	6.52	6.46	6.40	6.38
Met. Energy (Kcal/Kg	2,561.84	2,515.32	2,472.61	2,441.2

a, Inoculated with *Fasarium verticilloides*

b, To provide per kg of diet; Vit A (8,000IU); Vit D3 (2,000i.u); Vit E (5i.u); Vit K (3.2mg); Choline Chloride (3,000mg); Folic acid (0.5mg); Mn (56mg); I (1mg); Fe (20mg); Cu (10mg); Zn (50mg); Co (1.25mg); Riboflavin (4.2mg); Vit B₁₂ (0.01mg); Pantothenic acid (5mg); Nicotinic acid (20mg); ppm: Part per million (equivalent of mg/kg).

Table 2: Acetylcholinesterase activity levels ($\mu\text{mol/g/min}$) in different regions of the brain of breeder cocks fed fumonisin contaminated diets

Parameters	Treatments				SEM
	Diet 1 0.2ppmFB ₁	Diet 2 5.2ppmFB ₁	Diet 3 10.2ppmFB ₁	Diet 4 15.2ppmFB ₁	
Cerebral cortex	0.94 ^a	0.89 ^a	0.046 ^{ac}	0.33 ^c	0.04
Cerebellum	0.37	0.35	0.34	0.24	0.02
Medulla oblongata	0.65 ^a	0.55 ^{ab}	0.33 ^b	0.24 ^c	0.00
Hypothalamus	0.29	0.29	0.29	0.30	0.01
Amygdala	0.47 ^a	0.45 ^a	0.45 ^a	0.38 ^b	0.02
Pons varolli	0.56 ^a	0.50 ^a	0.44 ^b	0.44 ^a	0.02
Hippocampus	0.32 ^a	0.30 ^a	0.30 ^a	0.21 ^b	0.04

Values shown on the table are means.

SEM: Standard Error of Means

a,b,c: Means differently superscripted across the row are significantly different ($p < 0.05$).

ppm: part per million (equivalent of mg/kg).

Total protein concentration decreased significantly ($p < 0.05$) in cerebral cortex, cerebellum, medulla oblongata, hypothalamus, pons varolli and hippocampus as the levels of FB₁ in the diets

increased. The dietary levels had no significant influence on the total protein concentration of amygdala.

Table 3: The concentration of total protein (g/100ml) in brain regions of breeder cocks fed FB₁ contaminated diets.

Parameters	Treatments				SEM
	Diet 1 0.2ppmFB ₁	Diet 2 5.2ppmFB ₁	Diet 3 10.2ppmFB ₁	Diet 4 15.2ppmFB ₁	
Cerebral cortex	0.52 ^a	0.58 ^a	0.56 ^a	0.39 ^b	0.04
Cerebellum	1.71 ^a	1.66 ^a	1.56 ^b	1.50 ^b	0.13
Medulla oblongata	1.09 ^a	1.01 ^a	0.92 ^b	0.87 ^b	0.14
Hypothalamus	0.70 ^a	0.69 ^a	0.67 ^a	0.33 ^b	0.05
Amygdala	0.78	0.67	0.75	0.61	0.13
Pons varolli	1.34 ^a	1.33 ^a	1.25 ^b	1.19 ^b	0.13
Hippocampus	0.49 ^a	0.46 ^a	0.41 ^a	0.30 ^b	0.14

Values shown on the table are means.

SEM: Standard Error of Means

a,b,c: Means differently superscripted across the row are significantly different ($p < 0.05$).

ppm: part per million (equivalent of mg/kg).

Table 4 shows the haematological values of breeder cocks fed diets contaminated with graded levels of FB₁. Dietary FB₁ resulted in significant decrease ($p < 0.05$) in the erythrocytes, haemoglobin, platelets, eosinophils and a significant increase ($p < 0.05$) in the leukocytes, lymphocytes and heterophil components of the blood of the cocks. Other parameters were not significantly influenced by the dietary treatments. The results of serum biochemical constituents of breeder cocks fed

FB₁ contaminated diets are presented in Table 5. The serum total protein of cocks fed diets 1 and 2 were statistically similar and significantly superior ($p < 0.05$) to those of cocks fed diets 3 and 4. Aspartate amino transferase (AST) activities in the breeder cocks significantly increased with increased in the concentration of dietary FB₁ while albumin, globulin, albumin/globulin ratio and uric acid components of the serum were not significantly altered by the dietary treatments.

Discussion

The variation in the acetylcholinesterase activity levels observed in some of the brain regions monitored in this study may either be due to the inhibition of the enzyme synthesis by the altered cellular environment in the brain or due to a decrease in the rate of enzyme synthesis. This result also revealed that the various brain regions investigated in this study exhibited different responses to dietary FB₁. Adejumo and Egbunike (2001) classified the amygdala and medulla oblongata as AChE high activity regions while the

hypothalamus, hippocampus and pons varolli fall into AChE medium activity regions and low AChE activity regions comprise the cerebral cortex and cerebellum. The decrease in AChE activity level observed in the cerebral cortex, medulla oblongata, amygdala, hippocampus and pons varolli of cocks fed diets 4 (10.2ppm FB₁) and 5 (15.2ppm FB₁) may be a reflection of the type of electrical change in the nervous tissue of the cocks elicited by high concentration of dietary FB₁ because these brain regions are known for quick and short term actions (Adejumo and Egbunike, 2001). These actions are

usually modulated by intense nervous stimulations and muscular contraction. Swammy *et al.* (2004) reported that brain regional neuro chemistry was altered in pigs and broilers chicken chronically fed fumonisin contaminated diets. It is suspected that FB₁ might have inhibited or depressed the AChE activity in these brain regions of the breeder cocks. Similar results of decrease in activity levels of AChE in different regions of the brain of alloxan diabetic rat was reported by Nayeemunnisa and Suraiya (2009).

The lack of significant effect of dietary FB₁ at the various inclusion levels on AChE in the cerebellum and hypothalamus suggest that they are less sensitive to FB₁ than other brain regions. The significantly lower concentrations of total protein in the cerebral cortex, cerebellum, medulla oblongata, hypothalamus, pons varolli and hippocampus of breeder cocks

on the diet with the highest level of FB₁ (15.2ppm) could be related to their corresponding AChE and may be an indication of the interference of FB₁ with neutral mechanisms involved with AChE and protein synthesis and the rate of turnover in these brain regions. Since the enzyme (AChE) is glycoprotein in nature, a low enzyme activity is usually indicative of low metabolism and consequently a low protein turnover rate. Total protein levels in the cerebellum, medulla oblongata and Pons varolli of the cocks used this study were higher than values reported in the brain regions of pigs by Adejumo and Egbunike (2001) and rabbits by Bitto (2008). This disparity may be due to species differences in biochemical characteristics of brain regions (Bitto, 2008).

Table 4: Haematological values of breeder cocks fed diets contaminated with FB₁

Parameters	Treatments				SEM
	Diet 1 0.2ppm FB ₁	Diet 2 5.2ppmFB ₁	Diet 3 10.2ppmFB ₁	Diet 4 15.2ppmFB ₁	
Erythrocytes (×10 ⁶ /mm ³)	3.33 ^a	3.25 ^b	3.24 ^b	3.24 ^b	0.02
Haemoglobin (g/dl)	14.70 ^a	14.55 ^a	14.05 ^a	15.15 ^b	0.19
Mean cell volume (μ ³ μg)	129.25	133.88	131.17	132.66	2.52
MCH (μμg)	44.21	44.77	43.36	43.82	0.47
MCHC (%)	34.20	3.50	33.06	33.06	0.51
Packed cell volume (%)	43.00	43.50	42.50	43.00	0.87
Platelets (×10 ³ /L)	162.00 ^a	151.50 ^a	117.50 ^b	161.00 ^a	9.50
Leucocytes (×10 ³ /mm ³)	16.55 ^b	16.73 ^b	19.42 ^a	19.02 ^a	0.29
Lymphocytes (×10 ³ /mm ³)	11.22 ^b	11.32 ^b	12.60 ^a	12.67 ^a	0.40
Heterophils (×10 ³ /mm ³)	4.42 ^c	4.92 ^{bc}	6.06 ^a	5.59 ^{ab}	0.35
Monocytes (×10 ³ /mm ³)	0.15	0.16	0.19	0.29	0.07
Eosinophils (×10 ³ /mm ³)	0.75 ^a	0.34 ^c	0.58 ^b	0.48 ^{bc}	0.05

Values shown on the table are means.

SEM: Standard Error of Means.

a,b,c: Means differently superscripted across the row are significantly different ($p < 0.05$).

MCH: Mean Corpuscular Haemoglobin.

MCHC: Mean Corpuscular Haemoglobin Concentration.

Table 5: Serum biochemical constituents of breeder cocks fed diets contaminated with FB₁

Parameters	Treatments				SEM
	Diet 1 0.2ppmFB ₁	Diet 2 5.2ppmFB ₁	Diet 3 10.2ppmFB ₁	Diet 4 15.2ppmFB ₁	
Total protein (g/dl)	3.67 ^a	3.60 ^a	3.05 ^b	3.10 ^b	0.15
Albumin (g/dl)	1.78	1.61	1.48	1.43	0.14
Globulin (g/dl)	1.89	1.99	1.58	1.68	0.20
Albumin/globulin	0.95	0.84	1.19	0.86	0.25
AST (i.u/l)	81.38 ^c	82.50 ^c	86.13 ^b	88.63 ^a	0.79
Uric acid	1.34	1.56	1.34	1.40	0.17

Values shown on the table are means.

SEM: Standard Error of Means

a,b,c: Means differently superscripted across the row are significantly different ($p < 0.05$).

ppm: part per million (equivalent of mg/kg).

The significant depression in the erythrocytes and haemoglobins components of cocks on diets 2,3 and suggests that the breeder cocks were anaemic. Maxwell *et al.* (1990) reported that reduction in erythrocytes value of chickens indicates anaemia. Since haemoglobin is associated with transport of oxygen, it is suspected that this function may have been impeded by dietary FB₁. Elevated level of blood circulating leukocytes in the breeder cocks fed diets containing

10.2 and 15.2ppm FB₁ respectively is suspected to be occasioned by the dietary FB₁. The increase in the circulating leukocytes was accompanied by elevated levels of blood lymphocytes. Since lymphocytes and heterophils are associated with immunologic defense, it can be inferred that dietary FB₁ elicited immunologic disorder in the cocks fed diets 3 and 4. The significant reduction in serum total protein of cocks fed diets 3 and 4 implies that total protein metabolism was

impeded by dietary FB₁. Similar result of significant reduction in serum total protein in laying hens fed dietary FB₁ was reported by Ogunlade and Egbunike (2013).

The AST values increased significantly as the dietary FB₁ increased. This is an indication of liver insufficiency or an impairment of the functions of liver by FB₁. This result is consistent with the findings of Ologhobo *et. al.* (1982) who observed an increasing levels of AST in the serum of rats due to toxic substance. The serum total protein and AST obtained from breeder cocks used in this study were below those reported for normal male chickens (Mitruka and Rawnsley, 1977). The variation may be due to differences in breed, age, weight, nutrition and environment.

Conclusion

This study has shown that dietary exposure to FB₁ at a concentration of about 10.2ppm or more for a four-month period may result in significantly reduced acetylcholinesterase activity and total protein concentration in the cerebral cortex, medulla oblongata, Pons varolli and hippocampus regions of the brain. Furthermore, the erythrocytes, haemoglobin, platelets and eosinophil components of the blood as well as total protein contents of the serum were significantly reduced.

Significant increase in the leukocytes, lymphocytes and AST constituents of the blood and serum respectively of the breeder cocks were observed at FB₁ concentration of 10.2ppm and above.

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