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PERFORMANCE OF CAT-FISH FED DIFFERENT DOSES OF AFLATOXIN IN THE DIET

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ABSTRACT

Aflatoxin B_1 (AFB1) is a mycotoxin known to frequently contaminate poorly stored foods/feeds. This study was carried out to investigate the performance of parameters of catfish fed 3 different doses of aflatoxins earlier determined from fish feeds. Simulated toxicity test using AFB_1, (1000 µg/ml, 500µg/ml, 250µg/ml) in feeding juvenile cat fish was conducted for 56 days. Catfishes were divided into 4 treatments according to aflatoxin dose (A=control, B=250µg/ml, C=500µg/ml, D=1000µg/ml). At the end of the experiment, body weight and biochemical parameters were evaluated. Results revealed that growth of aflatoxicosed fish was significantly different from control using analysis of variance and Duncan Multiple Range Test at $p< 0.05$. Protein, cholesterol, bilirubin, electrolytes and liver enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) were also significantly different from the control. Histopathological examination of liver sections revealed severe liver damage. There was gross oedema of the hepatocytes with increased inflammatory cells. This is the first report of simulated aflatoxin toxicity obtained from fish feeds sold in Nigeria. It is clear from the results that legislation for the control of aflatoxins in animal feed is now over due because of its attendant economic loss and potential health hazard. Routine surveillance is needed.

Keywords: Aflatoxins, cat-fish, performance, body weight, biochemical parameters

INTRODUCTION

Mycotoxin contamination has had a considerable economic impact on the food grain and livestock industry (Marquardt, 1996; Boudra and Morgavi, 2005). Aflatoxins are one of the most potent toxic substances that occur naturally. These are a group of closely related mycotoxins produced by fungi *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Var and Kabak, 2004). Aflatoxicosis is a disease that can affect many species of fish, and results when feed contaminated with aflatoxins is eaten by the fish (Ashley, 1970).

Aflaxtoxins are common contaminants of oilseed crops such as cottonseed, peanut meal, and corn. Wheat, sunflower, soybean, fish meal, and nutritionally complete feeds can also be contaminated with aflatoxins (Russo and Young, 2007).

Four major aflatoxins (AFB1, AFB2, AFG1 and AFG2) are direct contaminants of grains and finished feeds. Factors that increase the production of aflatoxins in feeds include environmental temperatures above 27^0C, humidity level greater than 62%, and moisture levels in the feeds above 14% (Magan and Lacey, 1988; Whitlow and Hagler Jr., 2002). The extent of contamination will vary with geographical location, feed storage practices

and processing methods. Improper storage is one of the most important factors favouring the growth of aflatoxin producing molds, and it is a major element that can be controlled by the fish producer.

Aflatoxins have received greater attention than any other mycotoxins because of their demonstrated potent carcinogenicity and acute toxicity. After series of experimentation on many animal species like rats, rainbow trout's, aflatoxin especially aflatoxin B1 was confirmed as a potential carcinogen (D'Mello and MacDonald, 1997). Metabolism plays a major role in deciding the degree of toxicity (Kuilman *et al*., 1998). After ingestion, aflatoxin is metabolized by cytochrome P450 group of enzymes in the liver, where it is converted to many products like aflatoxicol, aflatoxin Q1, aflatoxin P1, and aflatoxin M1, depending on the genetic predisposition of the species. Along with the above, another metabolite called aflatoxin 8, 9 epoxide is also formed. The amount of this metabolite decides the species susceptibility as this induce mutations by intercalating in the DNA, by forming an adduct with guanine moiety in the DNA (Guengerich, 2005). This intercalation of epoxide causes a GAT transversion at codon 249 in P53 gene in liver, which may lead to hepatic carcinoma. This was observed in most of the experimental models, and it is presumed that this is the major reason for aflatoxin carcinogencity.

No animal species is resistant to the acute toxic effects of aflatoxins. Animal species respond differently in their susceptibility to the chronic and acute toxicity of aflatoxins. Environmental factors, exposure level, and duration of exposure beside age, health, and nutritional status of diet can influence the toxicity (Gong *et al*., 2003; Turner *et al*., 2003).

Aflatoxins present in contaminated feed are rapidly absorbed in the small intestine, affecting mainly the liver, leading to metabolic disorders. Fat degeneration and proliferation of biliary ducts induce bloody changes generally seen as the increase in hepatic enzyme activity, coagulopathies, and reduction in protein production (Fernandez *et al*., 1995; Rauber *et al*., 2007). Many production parameters can be affected by aflatoxin poisoning, such as Body Weight (BW) gain, feed consumption, plasmatic proteins, cholesterol, and mortality rate in turkey poults as in other avian species (Lanza *et al*., 1980; Giambrone *et al*., 1985; Quist *et al*., 2000).

Rainbow trouts are extremely sensitive to AFB1, while channel catfish are much less responsive. Rainbow trout feed diets containing AFB1 at 0.0004 mg per kg feed (0.4ppb) for 15 months had a 14% chance of developing tumors. Feeding rainbow trout a diet containing AFB1 at 0.2mg per kg feed (20ppb) for 8 months resulted in 58% occurrence of liver tumors, and continued feeding for 12 months resulted in 83% incidence of tumors. Channel catfish, fed a diet containing purified AFB1 at 10mg per kg feed (10,000ppb) for 10 weeks, exhibited decreased growth rates and moderate internal lesions (Jantratail and Lovell, 1990). Rauber *et al*. (2007) fed turkey poults with 7 different doses of aflatoxins and reported that BW and biochemical parameters were significantly affected.

Initial findings associated with aflatoxicosis in fish include pale gills, impaired blood clotting, anemia, poor growth rates or lack of weight gain. Prolonged feeding of low concentrations of AFB1 causes liver tumors,

which appear as pale yellow lesions and which can spread to the kidney. Increases in mortality (higher numbers of dead fish) may also be observed (Tuan *et al*., 2002).

At low cumulative doses in animals, aflatoxin causes poor feed conversion efficiency and growth rates and subsequent economic losses to the farmer.

The growing amount of information about the relation between diet and health has had an impact on the demand for fish. Studies conducted by some authors (Rickertsen, 1996; Ben *et al*., 2001) reported that health information elasticities are significant having a positive effect in fish and poultry and a negative effect on beef and pork. This coupled with the rising cost for beef and the need for self entrepreneurship has led to an increase in fish farming. In addition is the extractive industries and exploitation of natural resources in some areas especially the Niger Delta area of Nigeria making the people to shift from natural to artificial fish farming. The cost of feed as a result is on the rise which is usually the greatest operating cost in aquaculture (Chang *et al*., 2005).

This study was carried out to evaluate the sensitivity of cat-fish to simulated doses of aflatoxins previously quantified in fish feeds sold in South West of Nigeria.

MATERIALS AND METHODS

Mycotoxins

The production of aflatoxins was done by culturing of *A. parasiticus* (NRRL 2999) on maize, following the methodology of Oluwafemi and Taiwo (2004). The aflatoxin extract obtained was quantified using High Performance Liquid Chromatography (HPLC) at the office headquarters of the National Agency for Food, Drug, Administration and Control (NAFDAC).

Simulated-toxicity test using cat-fish

Eight micro fish ponds were constructed with reservoirs. Each pond had ten juvenile fishes that were randomly selected. These fishes were fed with high quality fish feed that had been previously screened for aflatoxins. The average weight of the ten fishes in each pond was taken every week. The protocol for treatment of cat-fish was as follows: 1) control feed without addition of aflatoxins, 2) 250ug/ml, of total aflatoxins, 3) 500ug/g of total aflatoxins, 4) 1000ug/g of total aflatoxins. At the end of the eight weeks feeding trial, the fishes were sacrificed.

Experimental design and statistical Analysis

The experimental design involved 4 treatments (1000, 500, 250, 0 ug/ml AFB1) each having ten fishes that were randomly assigned into 8 ponds. The statistical analysis of the parameters from simulated fishes was carried out using analysis of variance (ANOVA), Duncan Multiple Range Test and least significance difference test.

Necropsies and Pathology Studies

At necropsy, the liver being the target organ was collected in 10% formaldehyde for histopathological analysis

Clinical Biochemical Analysis

Blood samples were collected from the fishes after 56 days of feeding. Samples were centrifuged 30 min after collection, and serum was maintained under $-4°C$ until biochemical testing was performed. Total proteins, albumin, bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, packed cell volume (PCV), potassium, sodium, chloride and bicarbonate ions measurements were performed accord-

ing to the method described by Oluwafemi and Taiwo (2004).

RESULTS AND DISCUSSION

The extent of disease, caused by consumption of aflatoxins, depends upon the age and species of the fish. Fry are more susceptible to aflatoxicosis than adults and some species of fish are more sensitive to aflatoxins than others. Catfish is usually less responsive to aflatoxin as reported by Jantratail and Lovell (1990). This was not the case in this investigation. The initial dose of aflatoxin given to young catfish in this study (2000 to 7000 μg/kg of aflatoxins) led to the death of the fishes as there was 100% mortality. As a result, the doses were reduced to 250, 500, 1000 μg/kg. Body weight of the aflatoxicosed-fishes was monitored for 56 days and results showed that there was positive correlation between body weight and aflatoxin concentration (Table 1). Statistical analysis using ANOVA and Duncan Muptiple Range test showed significant difference at $P<0.05$.Bodyweight of catfish is dose dependent. Studies carried on Nile tilapia (*Orechromis niloticus*) showed reduced growth rates, tissue abnormality when fed with diets containing 1.8mg of AFB1 per kg of feed for 75 days. Tuan *et al.* (2002) tested the effect of varying concentrations (2.5, 10, 100mg AFB1, per kg of feed) on 2.7 gram Nile tilapia and had reduced weight gain and reduced red blood cell counts.

Aflatoxins produce a reduction in bodyweight gain (Rauber *et al*., 2007) and this causes severe economic losses and health problems to humans because of their toxicity and frequency of occurrence in feedstuffs (Miazzo *et al*., 2005). The current study demonstrated the toxicity of the doses adopted in the study which were far lower than the quantified doses of aflatoxins in fish feed sold in South West of Nigeria. Several factors could have contributed to the lower weight gain in the catfishes, such as reduced feed intake, reduction in liver protein synthesis and decrease in lipid metabolism (Rauber *et al.,* 2007).

Results in Table 2 showed significant rise in the levels of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP). Statistical Analysis using Duncan's Multiple Range Test, showed significant difference between the control and the different level of doses of AFB1 with the exception of AST where there was no significant difference between control and doses 500 and 1000μg/ml. The main aflatoxin action mechanism is the reduction on the function of liver, primarily inhibition of the synthesis of protein. AST is an enzyme associated with liver parenchyma cells and it is raised in acute liver damage. This explains why the enzyme was only affected at dose 250μg/ml rather than at 500 and 1000μg/ml. Also, ALP is an enzyme in the cells lining the biliary ducts of the liver, this rises with large bile duct obstruction. ALT is an enzyme present in hepatocytes (liver cells), when a cell is damaged, it leaks this enzyme into the blood. It rises dramatically in acute liver damage (Oluwafemi and Taiwo, 2004).

Lipid metabolism is also affected due to the reduction on enzymes synthesis and activity, mainly in chronic exposure (Hussein and Brasel, 2001). Both hypo and hypercholesterolemia have been associated with liver diseases or liver damage (Campbell and Coles, 1986; Oluwafemi and Taiwo, 2004).

Results in this study (Table 2) showed that total proteins were significantly reduced in catfishes fed especially with 1000 μg/g. This

decrease in total protein is in agreement with findings of other researchers (Oluwafemi and Taiwo, 2004; Rauber *et al.,* 2007). Aflatoxin binds to DNA to form DNA-adduct which will eventually affect protein biosynthesis.

Table 3 shows the results of electrolyte balance and PCV levels of aflatoxicosed catfish compared with control. Sodium and chloride ions were significantly reduced at 1000 μg/ml of aflatoxin in the diet. Also the PCV of fishes fed 1000 μg/ml of aflatoxin was significantly reduced and these findings are in agreement with findings of Tuan *et al.* (2002). Major gross lesions were observed in the liver of catfishes. The histopathology report of the control showed normal hepatocytes with inflammatory cells within the vascular channels (Plate 1) while in plate 2 (fishes fed 10000 μg/ml of aflatoxin), the liver showed oedema of the hepatocytes with increased inflammatory cells. Plate 3 (500 μg/ml aflatoxin) showed oedema of the hepatocytes and the epithelial cells while Plate 4 (250 μg/ml of aflatoxin) showed the liver appearing normal with inflammatory cells seen within the vascular channels. The treatment with the highest dose (1000 μg/ml aflatoxin) revealed liver damage as shown in

Table 1: Body weight of cat-fishes treated with different doses of aflatoxin for a period of 8 weeks

	Doses of AFB1 (μg/ml)			
No of wk.	Control (A)	B(250μg/ml)	C(500μg/ml)	D(1000μg/ml)
1	111.9	86.6	79.8	73.6
2	101.7	89.3	91.0	74.8
3	110.6	92.6	92.4	75.2
4	112.6	92.8	90.5	76.7
5	114.2	90.1	93.7	76.9
6	115.7	90.0	93.1	77.2
7	116.2	89.6	92.2	77.0
8	118.2	89.2	90.6	77.1

Table 2: Total protein and liver enzymes concentration in cat-fish fed different doses of aflatoxins

	Doses of Aflatoxin AFB1			
Traits	Control (A)	B(250μg/ml)	C(500μg/ml)	D (1000μg/ml)
Total Protein	8.80	7.00	7.00	7.10
Albumin	4.10	3.90	4.10	4.40
Total Bilirubin	0.35	0.26	0.36	0.21
Conjugated Bilirubin	0.01	0.04	0.01	0.02
AST (SGOT)	6.00	13.0	9.00	9.00
ALT (SGPT)	9.00	16.00	14.00	13.00
ALK phosphatase	57.00	78.00	86.00	71.00

Table 3: **Electrolytes and PCV values of cat-fish fed different doses of aflatoxins**

	Doses of Aflatoxin AFB1			
Electrolyte	Control (A)	250μg/ml	500μg/ml	1000μg/ml
Sodium	100	102	72	74
Potassium	2.1	2.7	2.4	2.9
Bi carbonate	18.0	16.0	16	16.0
Chloride	92	83	82	57
PCV	20%	21%	21	19%

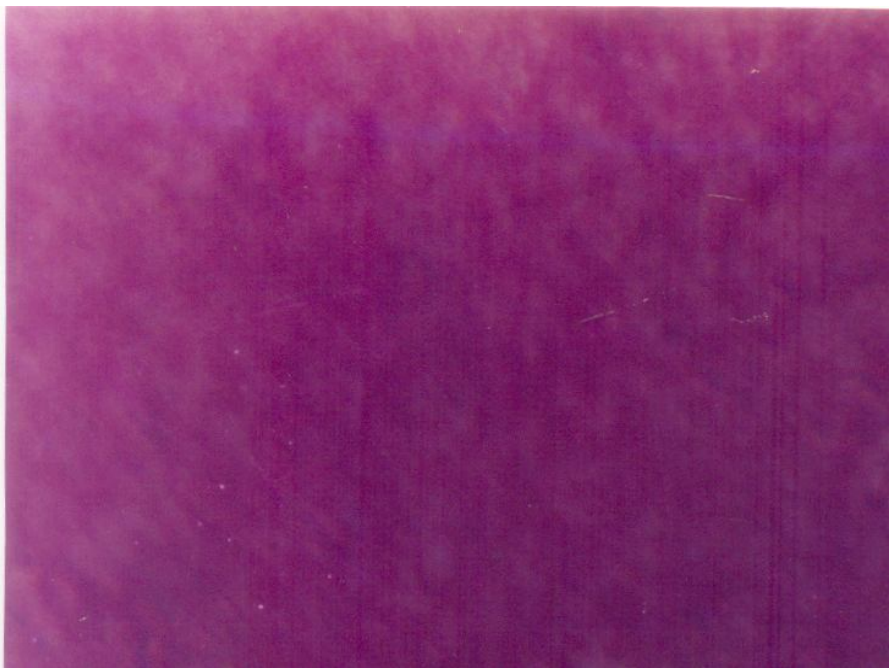

Plate 1: Normal hepatocytes with inflammatory cells within vascular channels of control cat fish

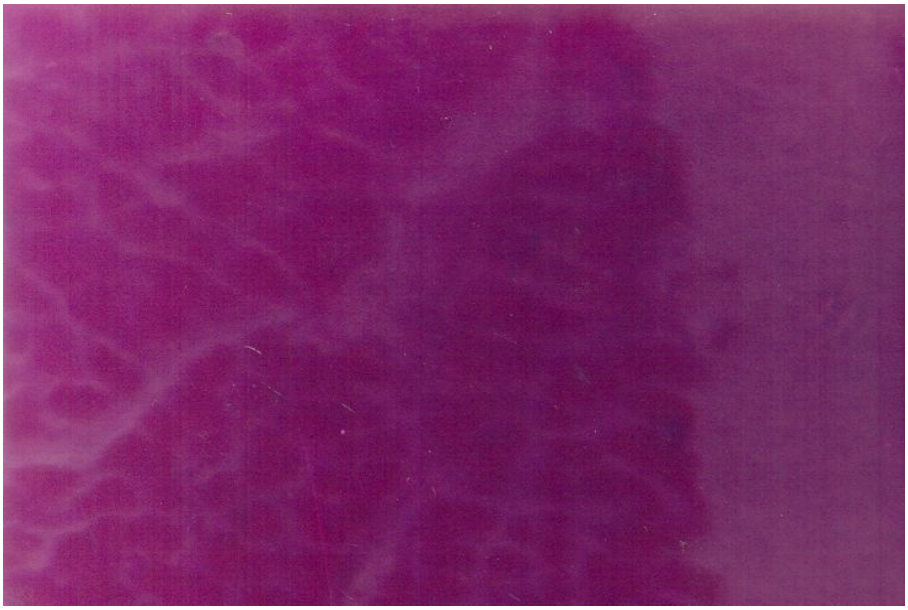

Plate 2: Liver of cat fish treated with 1000 ng / ml aflatoxin showing oedema of hepatocytes

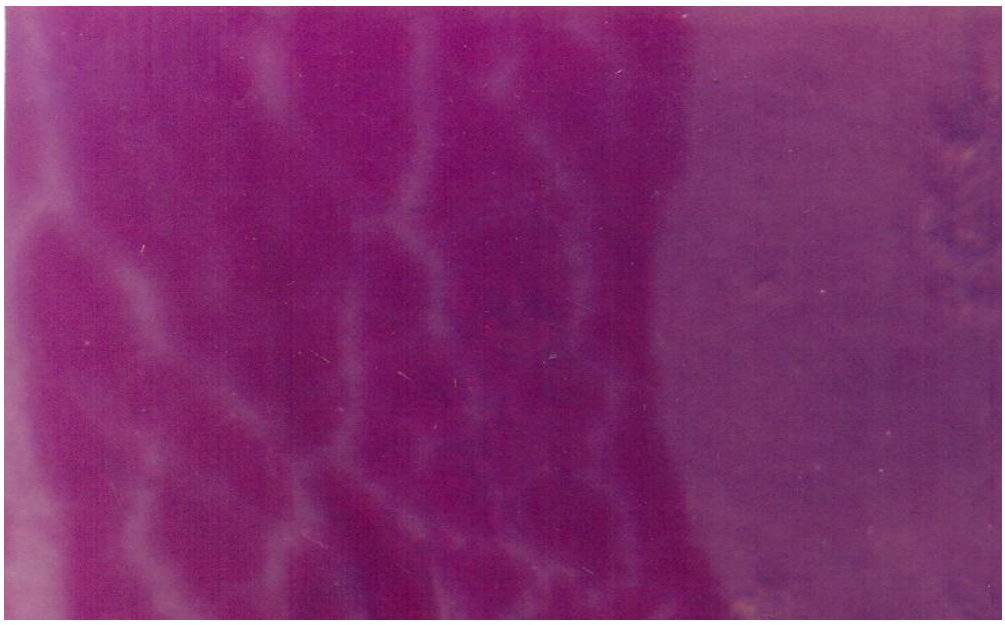

Plate 3: Liver of cat fish treated with 500 ng / ml of aflatoxin showing gross oedema of the hepatocytes

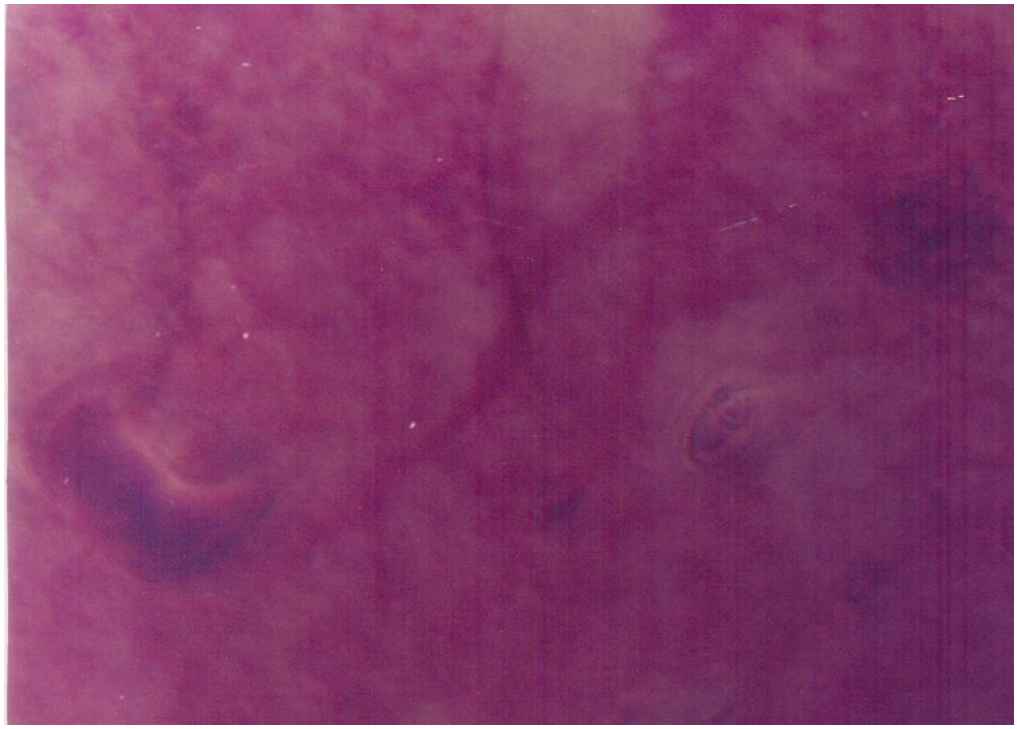

Plate 4: Liver of cat fish fed 250ng / ml of aflatoxin showing normal liver

the liver function test and the histopathological report.

CONCLUSION

Aflatoxins lower production efficiency of cultured fish by reducing growth rates, impairing liver enzymes, and in some cases, causing mortality. Findings show the presence of aflatoxins in doses equal or higher than 250 μg/g affect catfish performance during the evaluated period (1-56 d). This study showed that catfish are sensitive to aflatoxin poisoning and important economic losses can occur mainly in industrial production when microbiological management is not adequately conducted.

Since conditions that favoured toxins production are frequently encountered in the tropic, feeds should be properly stored in a cool dry area on pallets and at least one foot away from any wall. The manufacturer's recommendation regarding shelf life should be followed and locally produced feeds should be checked for aflatoxins levels before they are sold. Feedstuffs causing increased level of aflatoxins such as groundnut should be discouraged in feed formation. Finally, microbiological testing should be carried out in all feed mills so as to reduce economic losses and consequent health hazards.

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