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PERFORMANCE AND TISSUE RESIDUE OF TILAPIAS FED DIETARY AFLATOXIN

BY

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INTRODUCTION

Sarotherodon niloticus is a native African fish and has been reared in ponds, since ancient Egyptian times. Murals and engravings dating from 1400 B.C. show the fish held in ornamental ponds (**Hepher and Pruginn, 1981**). The tiiapia species have become increasingly important in fish culture especially in warm climates. Diets can negativly influencs the healthy growth of fish either by nutrient deficiences, imbalances, introducing infective agents or toxins to fish. The mould Asperigllus flavus grows well on many feedstuffs and under appropriats temperature and humidity produce a very potent carcinogen of latoxin (**Friedman and Shibko, 1972**). Although the effect of feeeding on feeds contained aflatoxin to livestock are well documented (**Allcrart, 1969**), much less is known about these effects on fish. Rainbow trout is the most sensetive animal to aflatoxin known, showing a significant incidence of hepatoma at dietary level as low as o.5 ppb (**Ashley et al., 1965**). Contaminated aflatoxin B_1 (AFB_1) was reported as the cause of trout liver cancer (**Halver, 1967**), also **Halver (1969)** found that, prolonged feeding of AFB_1 at concentration as low as 0.4µg/kg diet, caused liver neoplasma, necrosis of hypatocytes and degenerative changes in pancreatic tissues and kidney of rainbow trout. Concerning channel catfish, **Ashley (1966) and Friedman and Shibko (1972)** reported that, catfish fed crude aflatoxin in diets ranging from o to 100 mg/kg body weight showed a relatively low response, while **Jantrorotar and Loveil (1990)** found in channel catfish fed 10, 000ug/kg feed for 10 weeks, had shown a slgnificant decrease in growth rate haematocrite, hemogloblin concentration, erythrocyt count and necrotic hepatocytes but not when fish fed the concentration of 2154µg/kg or less. In carp fish, at low dietary level (2ppb) AFB_1, had no effact on body weight, health physiology (**Svobodova and Piskac, 1980**).

The priincible target organ in aflatoxicosis is the liver. The brood range of biological effect of aflatoxin proberly related to their reaction with cell nucleoprotein and nuclic acid and the ultimate effect of these reaction on protein synthsis and cellular integrity (**Patterson, 1976**). Evidance from several devoloping counteries showing an increase in incidence in aflatoxin content of the diet points to aflatoxin as a cause of human liver cancer.

This study was planned to provide more information on the potential risk involved the feeding tilapia species on aflatoxin contaminated feed. The objectives were to evaluate the effect of feeding of 50, 100 and 200 ppb aflatoxin on tilapias performance throughout 10 weeks feeding experiment. Moreover it is worth to detect aflatoxin residue in fish tissues.

MATERIAL AND METHODS

Table (1): Composition of the experimental ingredients "%".

Ingredients	Moisture	CP	EE	Ash
SBM	11.0	44.0	1.3	6.0
Fish meal	8.0	65.0	6.7	16.2
Wheat bran	11.0	15.0	1.4	7.0
Yellow corn	10.5	8.8	3.0	1.3

Diet formulation: Fish meal, SBM, wheat bran and yellow corn wasdone were the ingredients used for diet formulation. The actua chemical analysis of these ingre dients (Table, 1) according t A.O.A.C. (1975) Diet ingregient were analyzed to ensure freedu of AFB_1 before they were through ly mixed, pelleted and used i feeding trails.

Experimental diets: Contami

Table (2): Experimental diets in different treatments.

Ingradients (%)	Control diet	Aflatoxin level (ppb)		
		50	100	200
SBM	28	28	28	28
Fish meal	20	20	20	20
Wheat bran	20	20	20	20
Yellow corn	28	27	25.08	24.2
Contaminated y.corn	--	1.0	2.92	3.8
Mineral mixture	2.0	2.0	2.0	2.0
Vitamin mixture	2.0	2.0	2.0	2.0
Total	100	100	100	100
CALCULATED ANALYSIS %				
CP	30.8	30.8	30.8	30.8
EE	2.8	2.8	2.8	2.8
Ash	6.7	6.7	6.7	6.7
CF	4.7	4.7	4.7	4.7
Ca	1.24	1.24	1.24	1.24
P	1.13	1.13	1.13	1.13
ME Kcal /Kg	3359	3359	3359	3359
Cal /protein ratio	109	109	109	109

* Mineral mix. was prepared by mixing 50% dicalcium phosphate (25% Ca., 8% P)+ 25% Ramecal trace element premix+ 25% NaCl. Each Kg contains Ca 25 g , P 90 g ,Fe 25000mg , Cu 2000 mg , Mn 6000 mg ,Se 100 mg , Zn 40000 mg ,NaCl 5000 mg.
* Each Kg contains : Vit.A 4000000 IU , Vit.D 8000 IU , Vit.E. 10000mg Vit.B_1 2000 mg , Vit.B_2 10000 mg ,Vit.B_6 1000 mg , Vit.B_{12} 3 mg , Vit.C 10 mg , Folic acid 500 mg , Pantothianic acid 500 mg , Vit.K 1000mg and nicotine amide 5000 mg.

nated yellow corn was used in fo mulating the experimental diet contaied 5200µg/aflatoxin/kg wa prepared as described by El-Banu (1987). This corn used to formula 3 experimental diets containe 50,100 and 200 ppb aflatox (Table, 2).

Laboratory conditions: Twel 120-L glass aquaria equipped wi an air pump, maintained at 28±2 by using electric heaters 70 wa whils PH throughout the expe mental period was 7.5 ± 0.4 whi are the optimal recommended

tilapia species were used. Tap water was used and treated by anti-chlor reagent according to Boyd (1979).

Fish: Tilapia fingerlings were obtained from El-Abassa Fish Hatchery. They were devided into 4 groups each of 3 replicate with 10 fingerlings. The average initial body weight was 5.9 ± 0.22 g, while the initial standerd lenght was 6.04 ± 0.08 cm.

Feeding regime: Fish were fed twice daily at a rate of 3% of body weight (Marek, 1975), which was adjusted in accordance to body weight change during 10 weeks experimental period. Each aquarium was drained and thourghly cleaned weekly.

Performance Parameters: All fish of each group were weighed weekly, also standerd length was measured, while weight gain and feed/gain were calculated. At the end of experimental period all alive fish in each group were killed, whole body was dried, ground and prepared to chemical analysis according to A.O.A.C. (1975). Three fish of each group were killed and examined internally for signs of aflatoxicosis. Histological examination was done by taking speciements from gills and liver from 3 fish of each group and fixed in 10% formaline solution, dehydrated, cleaned and embeded in paraffine wax blocks then sectioned at 5 micron, stained by haematoxlin easein stain (Carlton et al., 1967). Detection of aflatoxin residue: After 7 weeks feeding trails, 2 fish from each reatment also 3 fish from each treatment at the end of 10 weeks experimental period were taken to detect the presence of aflatoxin residue according to the method of A.O.A.C. (1975).

RESULTS AND DISCUSSION

Aflatoxins are potent hepatotoxins and also potent carcinogenic. In general, young animals of any species are more susceptable to acute toxic effect of afletoxin then older animals of the same species (Edd, 1973).

Aflatoxicosis and tilapia performance:

Effect of AFB_1 on tilapia performance found in table (3). Concerning weight gain, fish fed the control diet was the best (5.46g) throughout the 10 weeks experimental period. Fish fed either 50 or 200 ppb AFB_1 showed nearly simillar body weight gain (5.16 and 5.18g respectively. Diet contained 100 ppb AFB1 showed the lowest weight gain (4.64g). About fish lenght, fish fed the control diet showed the highest increase in length (0.75 cm/fish). A simillar increase in length was found in

both groups fed 50 or 200 ppb AFB_1 (054 and 0.51 cm/fish respectively). The least increase in fish length was in group fed 100 ppb of AFB_1. Growth rate (GR) was simillar (0.9) in all groups except that fed 100 ppb of AFB_1 it was only 0.8 . Regarding the mortality, only fish fed the highest level of AFB_1 (200ppb) showed

Table (3): Effect of aflatoxin on tilapia performance.

	Control diet	Aflatoxin level ppb		
		50	100	200
Initial weight (g/fish)	5.96 1.36	5.6 1.08	6.09 0.98	6.03 1.16
Final weight (g/fish)	11.42 1.49	10.77 0.89	10.70 0.61	11.2 2.3
Weight gain (g/fish)	5.64^a	5.16^a	4.64^b	5.18^a
Initial length (cm/fish)	6.04 0.81	5.93 0.9	6.13 0.72	6.06 0.05
Final length (cm/fish)	6.79 0.99	6.47 1.02	6.56 0.77	6.57 0.81
Increase in length (cm/fish)	0.75^a	0.54^a	0.43^b	0.51^a
Mortality %	0.0	0.0	0.0	16.7
Growth rate	0.89	0.9	0.79	0.86

Each mean represents 10 fish/treatment. a&b means with different superscripts are significantly diferent at (P<0.05)

16.7% mortality. There is no available literature dealing with the effect of aflatoxin on tilapia performance. The adverse effect that have been shown in fish fed the diet contained AFB_1 at level of 100 ppb regarding tilapia performance that was not clear in fish fed the level of 200 ppb of AFB_1, may be related to the high mortality rate of 16.7% in the later group, so fish of higher body weight may can overcome the adverse effect of AFB_1 than fish of smaller body weight.

Effect of aflatoxin on Feed/ gain:

Calculated feed/gain in different

Table (4): Effec of aflatoxin on gain in body mass,feed feed consumption and feed convertion inexperimental groups (average of 3 replications with 10 fish each)

	Control diet	Aflatoxin level ppb		
		50	100	200
Gain in body mass (g/group)	163.8	154.8	139.2	99.35
Feed consumption (g/group)	447.0	412.2	426.5	356.95
Feed/gain (g/g)	2.73	2.66	3.05	3.59

groups was found in table (4). Data showed that fish fed the control diet and that fed the lowest AFB_1 level had a similiar values (2.75 and 2.66 respectivly) while feed/ gain was increased with increase the dose of AFB_1 (3.05 and 3.59 respectivly) in fish fed 100 and

Table (5):Effect of aflatoxin on whole body consumption of tilapia fish.

%	Control diet	Aflatoxin level ppb			initial
		50	100	200	
Dry matter	25.54 1.05	25.73 0.56	25.51 2.46	25.18 0.23	20.78 0.9
CP	61.37 1.42	62.83 0.74	61.83 0.1	63.53 0.18	62.94 0.51
EE	26.1 0.13	21.18 0.62	26.4 0.19	25.85 0.81	18.0 1.2
Ash	13.5 0.4	15.6 0.9	15.5 0.7	16.9 1.0	12.2 0.8

200ppb AFB_1. At the end of 10 weeks experimental period, the weight of fish fed the highest level of AFB_1 was 20% less than the fish fed the control diet. In carp, **Svabodova et al.** (1982) reported that neither concentration of AFB_1 (20 or 200µg/kg feed) affected feed intake of increase the mortality.

Effect of AFB_1 on fish body composition:

The data concerning fish body composition is found in table (5). All values concerning moisture, CP, EE and ash% were within the normal values of tilapia body composition.

Aflatoxin residue in fish body:

The resutls in table (6) showed that the amount of AFB_1 residues in fish body were directly related to both the level of AFB_1 and the duration of feeding period. The greatest amount of AFB_1 residue (31.5 ng/g) was detected in fish fed 200 ppb AFB_1 for 10 weeks. It can be observed that, with as minimum as 50 ppb AFB_1 in tilapia diet, the tissue residue was high (13.4ng/g), therfore the calculated feed/tissue ratio for this level was 3.7 which is cosiderably very narrow when compered with that reported for laying hens. For example, **Stoloff (1977)** who reported that afnlatoxin have been detected in the eggs of

Table (6): Aflatoxin residue and feed/tissue in whole fish body (g/g).

	AFB_1 residue after		Feed/tissue after	
	7 weeks	10 weeks	7 weeks	10 weeks
Control diet	Nil	Nil	Nil	Nil
50 ppb AFB_1	6.53	13.4	7.67	3.7
100 ppb AFB_1	13.10	26.71	7.63	3.7
200 ppb AFB_1	17.82	31.50	11.22	6.34

laying hen recieving in their rations 2200ug/kg of AFB_1 in ration will show up to 1ug/kg of AFB_1 in eggs. An intersting point to recognized is the identical effect that produced by two levels of AFB_1 (50 and 100ppb) on the feed/tissue ratio (3.7). Regarding duration of AFB_1 feeding, it is noticed that up to 7 werms of feeding, a positive relatichsip between the level of AFB_1 and the fesidue found in fish tissue, However, AFB_1 feeding for 10 weeks demonstrated duplication of the amount or residue that found in the same group after 7 weeks feeding period. This effect emphe-

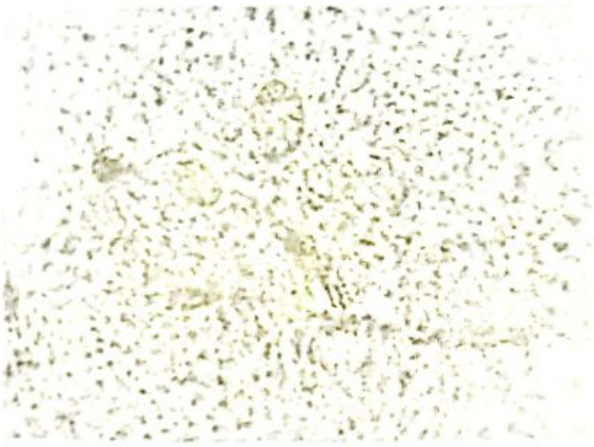

Fig.(1) : Liver of fish fed 50 ppb AFB1 showing vacculation of hepatocytes with necrotic depris with the bile canaliculi

sised the cumulative effect or AFB_1 in tissue which was supported by feed tissue ratio.

Pathological effect of AFB_1:

Liver of fish that fed 50ppb AFB1 after 7 weeks feeding period revealed vacular degeneration of hepatocytes, slight activation of epithalial linning the bile duct with the presence of necrotic debris

within the bile ccanliculi (Fig. 1) together with slight congestion of central vain. At end of loweeks feeding period we found the same picture, moreover there were foccal coagulative necrosis and areas of intravascular haemolysis. Gills showed telangectazia or the fine blood capelleries of the secondry lameliae togther with proliferation or the epithelial linning of the secondary lamellae.

Liver of fish fed 100ppb AFB_1; 7 weeks feeding period, showed proliferative changes of the epithelial linning the bile duct, leucocytic infelteration in the portal areas, focal areas of congestion and activation of melanine carrying cells. After 10 weeks feeding period found the same picture was noticed in addition to dissolution of cytopllasm of hypatocytes together with marked appearance of vascuhar cnannels (sunusoids) Channels takes rays appearance. Gills showed slight proliferative changes in the epethelial linning of the lameilae with severe congestion or the main branchial blood vessels (Fig. 2).

Fish fed the highest level of AFB_1 200 ppb after 7 weeks feeding period, their liver showed foccal areas of vaccular degeneration leucocytic infilteration in the portal areas, the vaculation of hepatocytes was more pronounced and bile duct prolir eration was very clear. After 10 weeks feeding, the same pictu was noticed in addition to int vascullar haemolysis associted

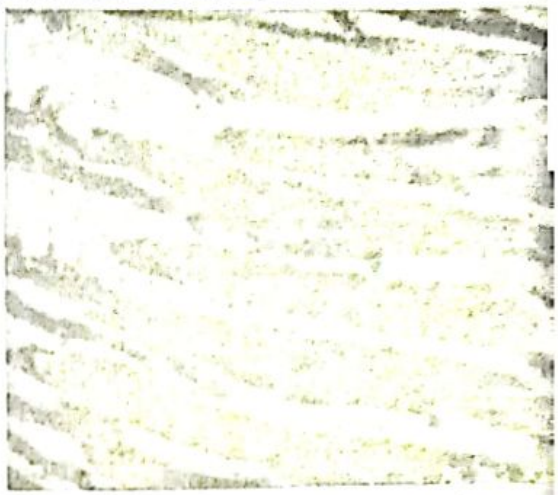

Fig.(2): Gills showing congestion of the ma branchial blood vessels

. with foccal areas of necrosis Gills showed prolferative change in the epithelial linning with sever congestion of the main branche blood vessels. These findings indi cate that the gills showed no spe cific redction while in liver there i a negative effect which is related t both AFB_1 level in the diet an feeding period which agree witl the findings dealing with perfor mance and residue found in fisl tissue.

The most important aim of th food control organization in an country is the protection of th consumers from health hazard posed by feeds containing patho genic microorganisms of their. tox ins, in amount sufficent to caus disease or toxicosis. From thi work, it can be concluded that, tilapia fed as low as 50ppb of AFB_1,

were normal in appearance and perrormance althnough they contain high amount of AFB1 residue in their body that hazard humen health. further studies, on levels below 50ppb AFB_1, are needed to detect the critical level that produce a residue in fish body.

SUMMARY

In 10 weeks feeding period, tilapia fingerlings were fed on diets contained 0.50, 100 and 100ppb of aflatoxin B_1 (AFB_1). Fish fed on diet contained 50ppb of AFB_1 showed littel or no effect on fish perrormance, but the negative effect was increased by both level of AFB_1 and the duration of feeding period. Tese levels of AFB_1 showed no effect on fish body composition. Histopathological examination revealed an adverse effect on liver, not in gills, that reilated to both AFB_1 level and duration of feeding. Even with as low as 50ppb of AFB_1, its residue was detected in fish body in higher amount than that reported in other species. AFB_1 residue showed a commulative effect related to level of AFB1 and feeding period.

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