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FIX-A-TOX IN AQUACULRUTE
II- MONITORING THE PREVENTIVE EFFECT OF FIX-A- TOX AGAINST AFLATOXICOSIS IN CULTURED OREOCHROMIS NILOTICUS

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SUMMARY

The effect of the recently used sequestering agent "Fix-A-Tox" was monitored in preventing aflatoxicosis probelm among cultured Oreochromis niloticus (O.niloticus) in Egypt. The supplementation of different levels of Fix-A-Tox to fish fed on control and crude aflatoxins contaminated diets for 6 months indicated a noticeable changes in the body weight development, mortalities and serum biochemical constituents. The histopathological examinatin revealed some alterations particularly in liver of fish received Fix-A-Tox either alone or in combination with crude aflatoxins . Moreover, the residual analysis in the fish liver revealed a non-efficient effect of Fix-A-Tox in preventing aflatoxicosis among cultured O.niloticus.

INTRODUCTION

Aflatoxins are produced in feeds and feed components, in fact prior to, during and after harvest, during storage, processing, handling and transportation (Goldblat and Stoloff, 1983). These compounds exhibit many different adverse effects to the health of fish and as a consequence a hazard to the health of human beings. Determination of aflatoxins contamination in feed stuffs is difficult and involves high costs. There is no safe level of aflatoxins in fish feed. It is generally considered that aflatoxins level below 2u/kg diet can be fed to fish with minimal risk (Svobodova and Piskac, 1980). However, the expression of toxicity of aflatoxins can be altered by various factors as fish species and environmental stresses (Srour, 1992 and Curtis et al., 1995). Moreover, tilapias have considerably very narrow feed/ tissue ratio for aflatoxins when

compared with that reported for other species (El-Banna et al., 1992). Since contamination by aflatoxins can be completely avoided and serious damage to man and animals can't be awaited, a variety of physical, chemical and biological methods for aflatoxins inactivation have been applied (Bol, 1990; Abdel Hamid, 1993 and Abdel Hamid et al., 1994a and 1995a). A more economical and practical solution would be the addition of sequestering agent which bind aflatoxins and decrease their bioavailability. Vivo investigations, have shown that activated charcoal, hydrated sodium calcium aluminosilicate (HSCAS), sodium bentonite and Volclay, NF-BC reduce the toxicity of aflatoxins (Lindermann et al., 1990; Bonna et al., 1991 and Voss et al.,1992). Fix-A-Tox, another sequestering agent, overcomed the deleterious effects of aflatoxins in growing birds (Rawia, 1994) when used as a dietary supplement. Fix-A-Tox was the proposed aflatoxins adsorbent agent in the El-Zawia fish farm in Kafr El-Sheikh governorate where there was several lots of aflatoxins contaminated dry pelleted feeds (20-52 ug crude aflatoxins/ kg diet)... Unfortunately, literatures dealt with the amelioration of aflatoxicosis in fish by Fix-Axtox are almost lacked. Therefore, this study was planned to monitor the effect of Fix-A-Tox supplementation in preventing chronic form of aflatoxicosis in cultured O.niloticus..

MATERIAL AND METHODS

1- Fix-A-Tox:

In this study, Fix-A-Tox, the product from Werfft Chemie, Vienna, Austeria was used . It is grey, odourless and tasteless powder. It added to feed at different concentrations recommended by the producer

2. Asperigellus parasiticus (A.parasiticus) fungu A highly and multi-toxins produci A.parasiticus standard strain (NRRL-29 obtained from American Culture Collecti was used for contaminating the ground yell corn, the ingredient of the experimental diets

3.Experimental diets:

Fish meal, ground yellow corn, wheat bran a soy bean meal were the ingtedients used in di formulation. One basal diet was formulat (El-Banna, 1991) and served as contro Contaminated yellow corn and/ or Fix-A-T at the level of 0.1% or 0.3% were added (Tab 1). The toxins concentration was adjusted be 100ug crude aflatoxins (AFBI, 18.7, AFB 31.4, AFGI, 32.5 and AFG2, 17.4 ug)/ kg fee The concentration of crude aflatoxins in t corn and the formulated diets was estimat by high performance liquid chromatograph (HPLC) analysis according to the method A.O.A.C. (1995).

4. Fish:

A total number of 360 clinically norma O.niloticus. with average body weig 35.78-36.95g/ fish and average total leng 10-11.5 cm/ fish were used. These fish we held in eighteen full glass aquaria (40 X 60 100cm), equipped with an air supply a declorinated tap water (Inness, 1966) in a ra of 20 fish/aquarium . Water temperature w maintained at 26±1.0°C and pH was adjusted

Table 1:

Diet composition and experimental design

Fish group	Aquarium No.	Fish/ Aquarium	Diet	Aflatoxin ug/kg diet	Fix-A-Tox g/kg diet	Schedule of necropsy in months and number of sacrificed *O. niloticus*				
						1	2	3	4	5	6
I	1	20	Crude aflatoxins contaminated diet (ACD)	100	0	1	3	1	3	1	all
	2	20				1	3	1	3	1	survivors
	3	20				1	3	1	3	1	
II	4	20	Crude aflatoxins + 0.1% Fix-A-Tox diet(ACD + 0.1% F)	100	1.0	1	3	1	3	1	all
	5	20				1	3	1	3	1	survivors
	6	20				1	3	1	3	1	
III	7	20	Crude aflatoxins + 0.3% Fix-A-Tox diet(ACD + 0.3% F)	100	3.0	1	3	1	3	1	all
	8	20				1	3	1	3	1	survivors
	9	20				1	3	1	3	1	
IV	10	20	Control uncontaminated diet (Cont.)	0	0	1	3	1	3	1	all
	11	20				1	3	1	3	1	survivors
	12	20				1	3	1	3	1	
V	13	20	Control + 0.1% Fix-A-Tox diet (Cont. + 0.1% F)	0	1.0	1	3	1	3	1	all
	14	20				1	3	1	3	1	survivors
	15	20				1	3	1	3	1	
VI	16	20	Control + 0.3% Fix-A-Tox diet (Cont. + 0.3% F)	0	3.0	1	3	1	3	1	all
	17	20				1	3	1	3	1	survivors
	18	20				1	3	1	3	1	

7.5±0.4 throughout the experimental period . Fish were acclimatized to laboratory conditions 2 weeks before the experiment, during which they fed the control diet.

5. Experimental design:

The experimental fish were grouped into 6 groups receiving different treatments for 6 months as illustrated in table (1):

* Fifh in group I were fed crude aflatoxins-contaminated diet (ACD).
* Fish in group II and III were fed crude aflatoxins-contaminated diet supplemented with 0.1% Fix-A-Tox (ACD-0.1% F) and 0.3% Fix-A-Tox (ACD-0.3%F) respectively.
* Fixh in group IV were fed control uncontaminated diet (Cont.).
* Fish in groups V and VI were fed control diet supplemented with 0.1% Fix-A-Tox (Cont: 0.1% F) and 0.3% Fix-A-Tox (Cont. 0.3% F).

All of these forementioned treatments were carried out in 3 replicates (Table 1). Fish were fed twice daily at a rate 0f 30% of their fortnightly checked body weight. Throughout the experiment, clinical abnormalities and mortallitis were determined . Different numbers of fish were sacrificed at different period (Table 1) for detection of post-mortem changes, histopathological alterations, serum biochhemical variables and aflatoxins residues in fish liver.

6. Histopathological examinations:

Tissue specimens from liver and intestine of different fish groups fixed in 10% buffered neutral formalin. The fixed specimens were dehydrated, blocked and sectioned at 4µ thickness and stained with Haematoxyline and Eosin (H&E) as described by Hibiya (1982).

7. Serum biochemical analysis:

Blood samples were individually drawn from the vessels of the experimental fish and the serum separated for assay of:

* Total bilirubin by using total bilirubin K reagent set, Pointe Scientific Inc.., Lincoln Park, Michigan according to the method of Strumfjord (1973).
* Serum alanine and asparate amino transferase (All and AST) by using transaminases kits of Bio-Merieux according to the methods of Reitman and Frankel (1957) and King (1965) respectively.
* Serum total protein by using total protein "Biuret kit" of Pointe Scientific Inc., Lincoln Park, Michigan according to the method of Herny (1968).
* Albumin by using albumin kit reagent set Pointe Scientific Inc., Lincolin Park, Michigan according to the method of Dauman et al (1971).
* Globulins as the difference between total protein and albumin.

8. Aflatoxins residues estimation in fish liver:

Liver of the experimental O.niloticus. in the 6 groups were used for estimation aflatoxins residues using HPLC analysis according to the method of A.O.A.C. (1995)

9. Statistical analysis: The obtained data were statistically analysed according to

procedures reported by Snedecor and Cochran (1980). Treatments means were compared by the least significant difference test (L.S.D.) at the 5% level of probability.

RESULTS

1. Growth rate:

From the 4th week post-treatment and up, the body weights of both fish fed aflatoxins contaminated diet with and without Fix-A-Tox and those fed control diet with Fix-A-Tox were significantly less than that of fish fed the control diet (Table 2). At the end of the experiment, the reduction percent in the live weights of the fish in the groups No.I,II,III, V and VI were 33.89%, 24./44%, 17.41%, 9.92% and 11% respectively. Fix-A-Tox supplementation to ACD resulted in a numerical increase in the body weights as compared to ACD, which is positively correlated with Fix-A-Tox level after four weeks. However, the increase in body weight became significant after feeding ACD - 0.3% F. for 12 weeks and after feeding ACD - 0.1% F. for 20 weeks. The improvement percent in the live weights of these fish were 14.3% and 24.93% respectively.

2. Mortality percent:

The highest mortality percent (26.67%) was detected in fish fed ACD, while the lowest one (0%) was in those fed control uncontaminated diet (Table 3). Supplementation of 0.1% and 0.3% Fix-A-Tox to the ACD reduced the mortalities to 15% and 6.67% respectively, while their addition to the control uncontaminated diet increased the mortalities to 1.67% and 3.34% respectively (Table 3).

3. Clinical signs and lesions:

The clinical examination of the experimental fish revealed the prlesence of slight abdominal distention and skin darkening as the only signs in some fish fed on ACD with and without 0.1% Fix-A-Tox after 4 months from the beginning of the treatments. No clinical signs were noticed among fish fed control diet with and without Fix-A-Tox and those fed ACD with 0.3% Fix-A-Tox. The postmortem investigation, revealed pale brown liver with congested patches (Fig.1) in some fish fed the ACD only or with 0.1% Fix-A-Tox. Liver of other fish showed whitish patches alterated with haemorrhagic foci (Fig.2). Liver atrophy was detected in few cases at the 5th month of the experiment. Distended gall bladder with brownish bile was also noticed.

4. Histopathological findings:

The histopahological changes produced by aflatoxins in experimental O.niloticus. were mainly pronounced in the liver. The hepatic parenchyma during the 1st three months of the experiment revealed ballooning degeneration with scattered foci of necrosis. Single, large esinophilic hyaline bodies might be seen inside the vacuolated hepatocytes. The hepatic blood vessels were almost congested and the portal vessels were specially dilated. From the 4th month of the experiment , the hepatocytes became hypertrophied with enlarged nucleus and nucleuolus and there was increase in

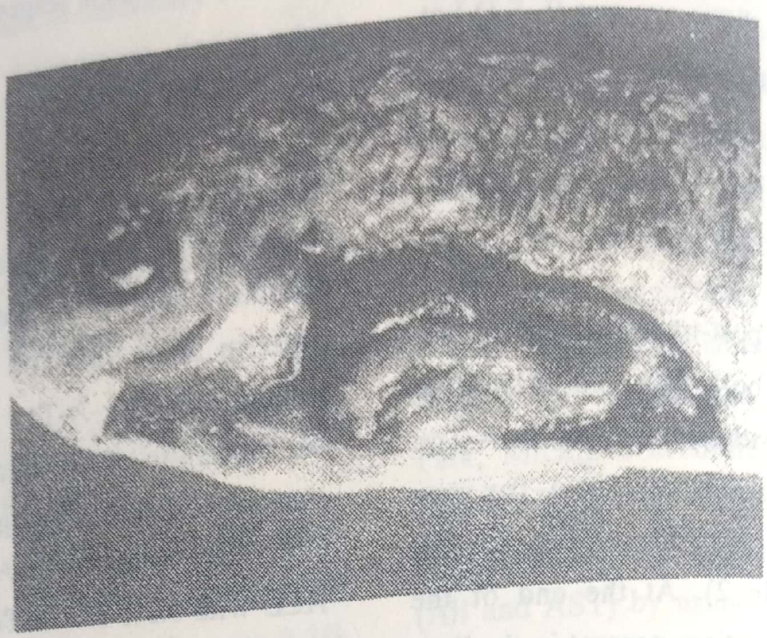

Fig. (1): Fish species:: O.niloticus
Lesion : Pale brown liver with congested patches

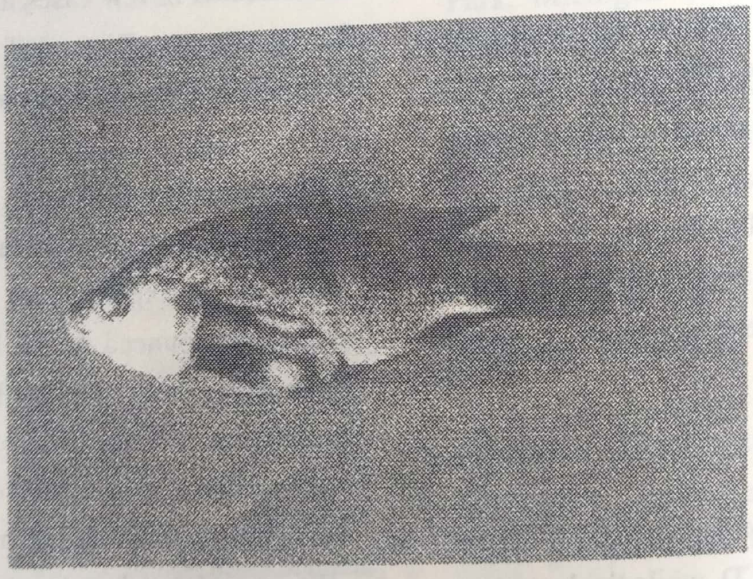

Fig. (2): Fish species:: O.niloticus
Lesion : Skin darkening with whitish patches alternated with haemorrhagic foci in the liver. Distended gall bladder is also se.

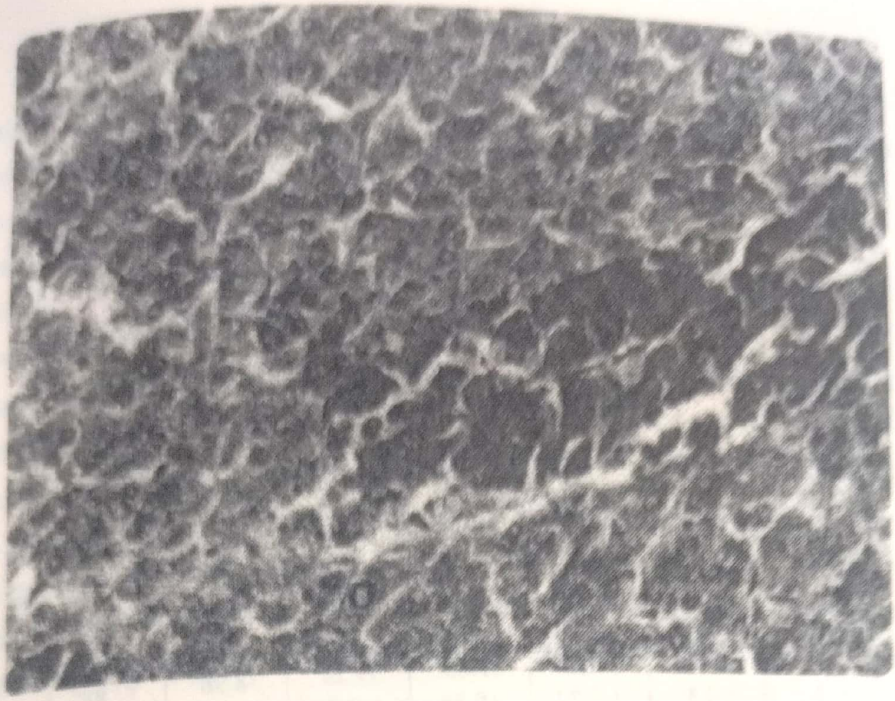

Fig. (3): Liver showing nuclear hypertrophy and marked pleomorphism. Note the activation of MMCs in the portal area (H &E. X 400).

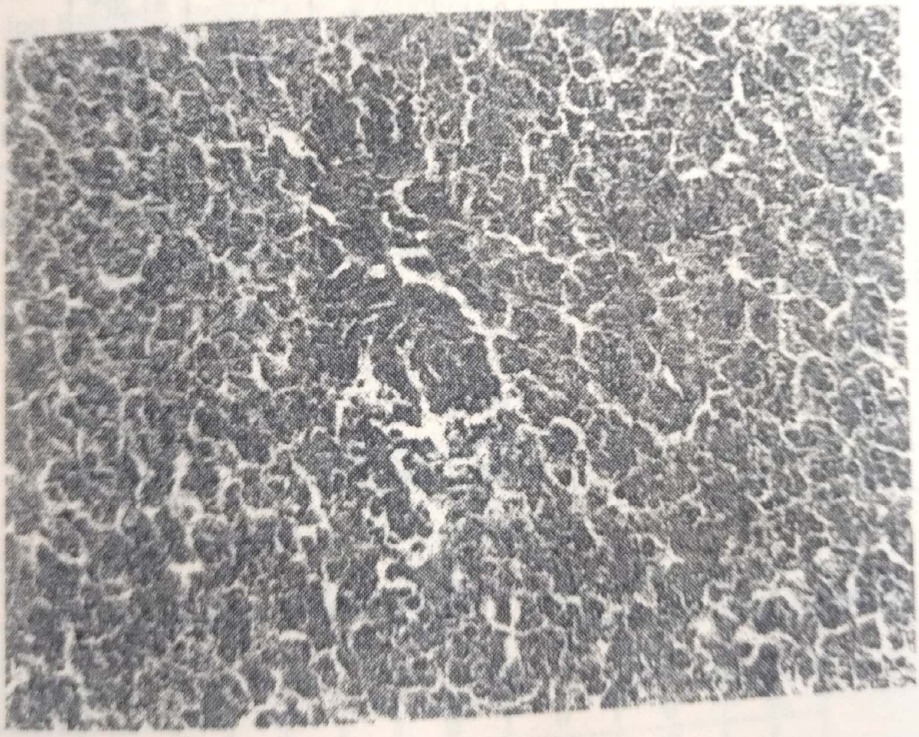

Fig. (4): Liver of fish. Note the focal pancereatic necrosis invaded with mononuclear cells (H & E. X 400).

Table (2):

Mean values ± Standard error of body weights of *O. niloticus* fed on control or crude aflatoxins contaminated diet with and without Fix-A-Tox

TIME IN WEEKS	FISH GROUPS					
	I	II	III	IV	V	VI
0	36.81^{a} ± 0.83	35.78^{a} ±0.72	36.28^{a} ± 0.74	36.24^{a} ± 0.67	36.63^{a} ± 0.82	36.95^{a} ± 0.85
2	37.09^{a} ± 0.82	36.25^{a} ± 0.72	36.12^{a} ± 0.76	37.90^{a} ± 0.72	36.88^{a} ± 0.71	36.05^{a} ± 0.87
4	37.25^{a} ± 0.89	37.33^{a} ± 0.77	37.65^{a} ±0.86	39.20^{a} ± 0.92	38.98^{a} ± 0.93	37.88^{a} ± 0.77
6	37.21^{a} ± 0.73	37.5^{a} ± 0.61	38.5^{ac} ± 0.78	43.56^{b} ± 0.70	39.90^{c} ± 0.49	37.21^{a} ± 0.70
8	37.48^{a} ± 0.72	37.85^{a} ± 0.48	39.03^{a} ± 0.80	44.18^{b} ± 0.74	41.38^{c} ± 0.62	39.58^{ac} ± 0.72
10	36.63^{a} ± 0.68	38.38^{ac} ± 0.66	39.55^{cd} ± 0.61	45.13^{b} ± 0.74	41.73^{e} ± 0.59	41.20^{de} ± 0.80
12	38.33^{a} ± 0.68	40.05^{ac} ±0.81	40.53^{ac} ± 0.70	45.32^{b} ± 0.74	43.68^{bd} ± 0.66	42.08^{cd} ± 0.79
14	39.20^{a} ± 0.78	40.20^{a} ± 0.71	43.63^{c} ± 0.52	48.78^{b} ± 0.75	44.00^{c} ± 0.82	43.38^{c} ± 0.68
16	38.83^{a} ± 0.48	40.64^{ac} ± 0.63	42.13^{cd} ± 0.77	48.94^{b} ± 0.61	45.07^{e} ± 0.79	43.83^{de} ± 0.54
18	39.39^{a} ± 0.76	40.53^{a} ± 0.73	43.33^{c} ± 0.72	49.36^{b} ± 0.75	46.88^{d} ± 0.63	45.05^{cd} ± 0.79
20	39.63^{a} ± 0.68	41.65^{a} ± 0.76	44.08^{c} ± 0.77	50.78^{b} ± 0.47	47.28^{d} ± 0.77	46.23^{d} ±0.71
22	37.91^{a} ± 0.50	42.13^{c} ± 0.75	44.80^{d} ± 0.58	52.73^{b} ± 0.71	48.45^{e} ± 0.72	47.25^{e} ± 0.76
24	36.79^{a} ± 0.63	42.05^{c} ± 0.79	45.96^{d} ± 0.56	55.65^{b} ± 0.65	50.13^{e} ± 0.74	49.53^{e} ± 0.74

* a - e Values in the same row with the different superscripts are significantly differed at $p< 0.05$.

binucleated cells. Moreover hepatic dissociation were noticed with the presence of fibroblasts between hepatocytes. Some bile ducts showed active proliferation of their epithelial lining. At the end of experiment , the hepatocytes loss the normal architecture , their nuclei became enlarged, hyperchromatic and showed marked pleomorphism (Fig.3). Activation of Melano-macrophage centers (MMCs) were common near the portal area. The pancreatic acini appeared necroticzed and was invaded with mononuclear cells and melanomacrophages (Fig.4). In fish fed ACD with 0.1% Fix-A-Tox, the hepatic lesions were improved and the liver exhibited mild vaccular degeneration as shown in some control cases which might progressed to small focal necrosis. The bile duct s showed hyperplastic activation of their epithelial lining and narrowing of its lumen (Fig.5). The liver of some fish fed ACD with 0.3% Fix-A-Tox showed vaccular degeneration, but the hepatic nuclei attained an enlarged size with margination of chromatin (Fig.6) and increased binucleated cells. The liver of fish fed control diet with 0.1% and 0.3% Fix-A-Tox suffered from severe and diffuse vaccular degeneration associated with nuclear pyknosis or lysis (Fig.7) and activation of MMCs. especially at the portal areas. The intestine in fish fed ACD showed necrosis and exfoliation of enterocytes (Fig.8) with atrophy and fusion of the intestinal villi. Otherwise the other groups didn't show significant enteric lesions than control.

5. Serum biochemical findings:

Statistical analysis of serum biochemical parameters, namely the Total bilirubin, ALT, AST, Total protein, Albumin and Globulin indicated a variability in their values in O.niloticus. groups as compared with those fed on control diet. The serum biochemical results are to be seen in table (4).

6. Aflatoxins residues:

From table (5), it is clear that, the amount of crude aflatoxins resides in fish liver were positively correlated to the feeding duration.

Table (3) :

Mortality percent in *O. niloticus* fed control or crude aflatoxins contaminated diet with and without Fix-A-Tox

Time in months	FISH GROUPS					
	I	II	III	IV	V	VI
1	2	2	1	0	0	0
2	1	1	0	0	0	0
3	1	0	0	0	0	0
4	3	1	0	0	0	0
5	5	3	1	0	0	1
6	4	2	2	0	1	1
Total No. of dead fish	16/60	9/60	4/60	0/60	1/60	2/60
Mortality percent	26.67	15.00	6.67	0	1.67	3.34

Fig. (5): Liver showing active proliferation of the bile ducts epithelial lining (H & E X 62.5).

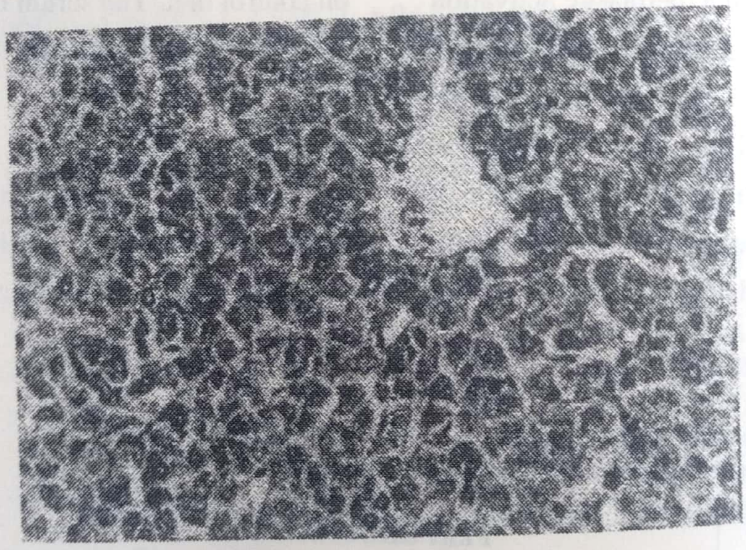

Fig. (6): Liver of fish. Note the hypertrophy of some nuclei with focal necrosis (H & E X400).

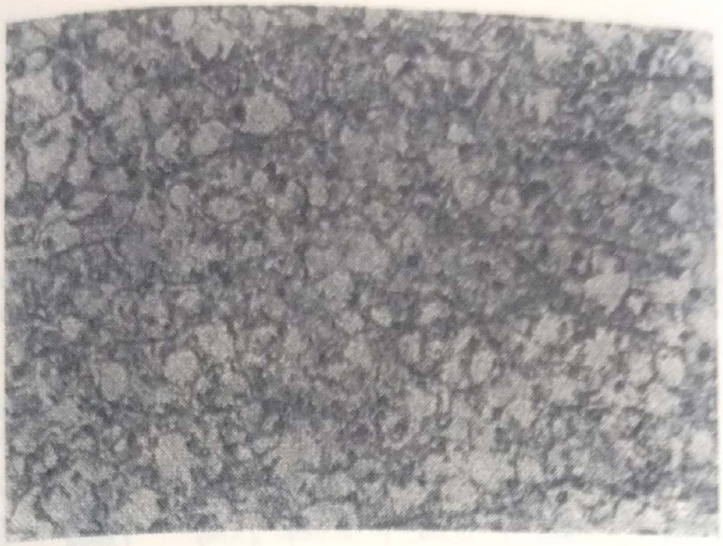

Fig. (7): Liver showing extensive vaccular degenration, karyopyknosis and karyolysis are prominent (H & E X 400).

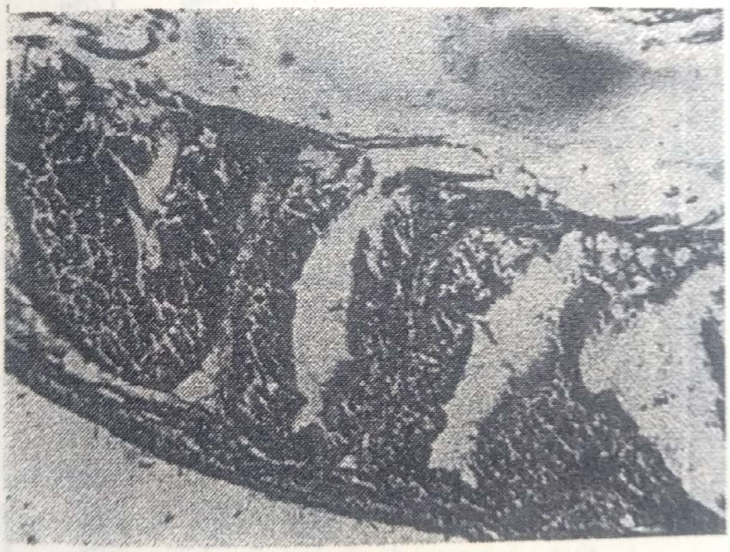

Fig. (8): Intestine showing necrosis and exfoliation of eneterocytes (H & E X 62.5).

Table (4) :

Mean values ± SE of serum biochemical parameters in *O. niloticus* fed on control or crude aflatoxin contaminated diet with and without Fix-A-Tox.

Feeding period/ months	Fish group	Total Bilirubin (mg/dl)	ALT (u/ml)	AST (u/ml)	Serum protein (g/dl) Total protein	Albumin	Globulin
2	I	0.66d ±0.10	11.27a ±1.72	38.33d ±2.11	2.98a ± 0.79	1.06d ±0.02	1.95a ±0.79
	II	0.60cd ±0.09	10.33a ±1.58	35.67cd ±2.07	3.00a ±0.70	1.31bcd ±0.23	1.68a ±0.48
	III	0.44bcd ±0.06	8.87a ±1.35	33.671cd ±2.01	3.07a ±0.53	1.27bd ±0.28	1.80a ±0.27
	IV	0.17a ±0.03	7.90a ±1.21	20.83a ±1.83	4.03a ±0.57	2.94a ±0.33	1.10a ±0.28
	V	0.30ab ±0.06	8.10a ±1.24	26.50ab ±1.91	3.60a ±0.55	1.91bc ±0.34	1.69a ±0.21
	VI	0.41bc ±0.06	11.50a ±1.76	30.0bc ±1.96	3.21a ±0.36	2.09c ±0.24	1.12a ±0.21
4	I	0.68c ±0.10	12.57a ±1.93	33.0a ±2.03	2.50b ±0.39	1.061b ±0.02	1.49a ±0.33
	II	0.69c ±0.11	11.17a ±1.70	32.92c ±2.03	3.03ab ±0.57	1.31bcd ±0.23	1.69a ±0.39
	III	0.47bc ±0.07	10.50a 1.60	32.17c ±1.99	3.10ab ±0.24	1.27bd ±0 28	1.50a ±0.24
	IV	0.21a ±0.03	8.60a ±1.31	23.67a ±1.83	4.23a ±0.05	2.964a ±0.33	1.27a ±0.28
	V	0.30ab ±0.05	8.63a ±1.32	25.66ab ±1.92	3.44ab ±0.49	1.91bc ±0.34	1.41a ±0.28
	VI	0.35ab ±0.05	9.87a ±1.51	30.67bc ±1.97	3.39ab ±0.46	1.88cd ±0.19	1.76a ±0.24
6	I	0.83d ±0.03	15.73c ±2.84	34.55b ±2.0	2.53b ±0.50	1.06b ±0.12	1.47a ±0.39
	II	0.58c ±0.05	12.67bc ±1.93	31.50ab ±2.01	2.75b ±0.42	1.57bc ±0.18	1.43a ±0.10
	III	0.49bc ±0.06	10.57abc ±1.61	31.00ab ±3.53	3.00b ±0.27	1.56bc ±0.27	1.19a ±0.20
	IV	0.32a ±0.02	5.33a ±2.05	25.17a ±3.53	4.09a ±0.40	2.75a ±0.30	2.02a ±0.67
	V	0.41ab ±0.04	8.83ab ±1.35	24.67a ±2.38	3.30ab ±0.05	2.12ac ±0.17	1.18a ±0.12
	VI	0.44ab ±0.05	9.20ab ±1.41	25.42a ±2.08	3.03b ±0.05	1.94c ±0.31	1.09a ±0.27

* a -d Values in the same column with the different superscripts significantly differed at $P < 0.05$.

Table 5 :

Aflatoxin residues in tissues of *O. niloticus* fed control or crude aflatoxin contaminated diet with and without Fix-A-Tox.

Aflatoxin residues (ng/g)*	Time in months								
	2			4			6		
	I L	II L	III L	I L	II L	III L	I L	II L	III L
AFB1	0.76	0.608	0,319	4.68	3.37	1.966	6.2438	4.267	2.732
AFB2	0.5	0.39	0.221	3.64	2.55	1.456	8.697	6.62	4.119
AFG1	0.9	0.675	0. 396	2.996	2.25	1.288	3.359	2.454	0.988
AFG2	----	----	----	----	----	----	----	----	----
Total	2.16	1.673	0.936	11.316	8.17	4.71	18.294	13.332	7.839

* No Aflatoxin residues could be detected in tissues of fish fed on control diet with and without Fix-A-Tox.

L. : Liver
AFB1 : Aflatoxin B1
AFG1 : Aflatoxin G1
AFB2 : Aflatoxin B2
AFG2 : Aflatoxin G2

The greatest amounts were detected in the liver (18.294ug/g) of fish fed ACD for 6 months. Fix-A-Tox supplementation caused a decrease in aflatoxins residues in the liver of the experimental fish as its level increased (Table 5).

DISCUSSION

Aflatoxicosis is one of the most important problem facing the intensive animal and fish production and human health as well . The different trials to overcome this problem enforced us to study the role of Fix-A-Tox, which is used in poultry industry, in preventing chronic aflatoxicosis in cultured O.niloticus.. For this aim, O.niloticus. fed control or crude aflatoxins contaminated diet (ACD) (100ug/kg. b.w.) with and without Fix-A-Tox (0.1% and 0.3%) for 6 months.

Concerning the body weight development, the body weights of both fish fed ACD with and without Fix-A-Tox and those fed control diet with Fix-A-Tox were significantly decreased than that of fish fed the control diet at 4 weeks and up (Table 2). At the end of the study, the depression percent were 33.89%; 24.44%, 17.41%; 9.92% and 11% respectively. Growth suppression in chronic aflatoxicated fish was repeatedly recorded by Halver et al., (1966); Lee et al., (1978); Jantrarotai et al., (1990); El-Banna et al., (1992); Srour (1992); Manal (1993) and Chavez-Sanchez et al., (1994). Svobodova and Piscak (1980) and Svobodva et al. (1982) did not observe any effect of the dietary aflatoxin on growth rate of carp when fed 2,20 and 200 ug aflatoxin /kg diet. Failure of Fix-A-Tox to pro
fish from aflatoxin-induced growth depress
effect also reported by Mahmoud et al., (199
duckling and Abd El Hamid and Mahmoud (19
in quails Moreover, the obtained res
supported those of Abdel Hamid and Mahm
(1996) that fix-A-Tox addition to control
lowered the body weight . However, it
noticed that Fix-A-Tox was able to alleviate
growth depressing effects of aflatoxicosis
O.niloticus. as reported by Rawia (1994)
fowels.

Regarding the mortality percent, the highest
(26.67%) was reported in fish group No.1 (Ta
3). Similar mortalities were also reported
El-Banna et al., (1992); Srour (1992) and Ma
(1993) in O.niloticus. fed on dietary aflatox
while Chavez Sanchez et al., (1994) a
Mahmoud and Mokhbatly (1997) could not det
any mortalities in Nile tilapia fed on aflatoxi
contaminated diet. On the other hand, the addit
of 0.1% and 0.3% Fix-A-Tox reduced
mortality percent to 15% and 6.67% in fish grou
II and III as reported by Rawia (1994) in chic
but incresed the mortality percent to 1.67%
3.34% in fish groups V and VI in opposite
findings of Rawia (1994). These variations
mortalities could be attributed to the immu
differences of fish and poultry as well as
environmental factors, health status of fish and
aflatoxin dose and type.

Concerning the clinical signs and leisons w
common non specific signs with lesio
concentrated to liver of fish fed ACD (Sro
1992; Manal, 1993 and Chavez-Sanchez, 199

with and without 0.1% Fix-A-Tox. On the other hand, the fish received 0.3% Fix-A-Tox with their diet did not have any abnormal clinical signs . Similarly, To these findings came Rawia (1994) in her experiment with Fix-A-Tox in chickens.

The histopathological findings were concentrated in the liver experimental fish fed ACD. The hepato-pathological changes produced by afaltoxins were also reported by many authors (El-Banna, 1992; Manal, 1993; Chavez-Sanchez, 1994; Mohamed and Mokhbatly, 1977). These findings were supported by the significant elevation of total serum bilirubin, ALT, AST and decrease total protein and albumin (Aletor et al., 1981; Sova et al., 1982. Chattopadhyay et al., 1985; Petkove et al., 1985; Manning et al., 1990 and Stanley et al., 1993).

The addition of Fix-A-Tox could alleviate the pathological changes of aflatoxicosis in the liver of fish. It was appeared to be proportional with the level of Fix-A-Tox in the given diet and corresponded with the results of serum biochemical tests which showed decrease of serum ALT and AST values . On the other hand, the Fix-A-Tox failed to retmove the effect of aflatoxins on the total billirubin values. These results were also obtained by Kubena et al., (1992) and Rawia (1994). The increase of serum total biliriubin in the groups treated by Fix-A-Tox might be attributed to the cholestasis resulted from the hyperplasia of the bile ducts epithelial linning in addition to the mild hepatocytic degeneration recorded histopathologically.

The supplementation of Fix-A-Tox to the afaltoxins free control diet significantly decrease the total protein and albumin. This finding corresponded with the hepatopathological lesions recorded in these groups although it disagreed with those of Rawia (1994) in her study on chickens .

The current work showed that the supplementation of Fix-A-Tox to fish diet could prevent the pathological alteration of the intestinal mucosa of the experimental fish. These findings are similar to those of Jantrarotai (1990).

The residual analysis of fish group I,II, and III revealed the presence of aflatoxins residues in their livers (Table 5). These residues were directly related to the duration of feeding period. The addition of different concentrations of Fix-A-Tox could decrease the liver aflatoxins residues (Table 5), where negative correlation between the aflatoxins residues and dietary Fix-A-Tox was detected . This could be attributed to the adsorption of aflatoxins on Fix-A-Tox which becomes unavailable for gastero-intestinal absorption (Dalvi and Mc Gowan, 1984).

In conclusion, the results of this study revealed the non-efficient effect of Fix-A-Tox in preventing aflatoxicosis in cultured O.niloticus.. ALso the use of this chemical could only decrease the drastic harmful effect of aflatoxins and at the same its combination to the aflatoxins-free control diet could initiate some harmful effects on fish health. Also, further studies on this product shold be required to clarify the probability of its absorption through fish gills.

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