Vet.Med.J.,Giza. Vol.42,No.1.(1994):169-174

SOME INVESTIGATIONS ON AFLATOXICOSIS IN CULTURED COMMON CARP (*CYPRINUS CARPIO L.*)

BY

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(Received 20/1/1994)

SUMMARY

The effect of crude aflatoxins on the common carp (Cyprinus carpio L.) was investigated. The results indicated that common carp exposed to aflatoxin doses intraperitoneally or orally showed non-specific clinical signs. The most commen gross post mortem findings were detected in liver, and spleen of exposed fish. Different haematological changes were also recorded in commen carp in correlation to the aflatoxin doses. Aflatoxins residues, particularly AFB1, and AFG1, could be detected in flesh of exposed fish which could be regarded as human hazards.

INTRODUCTION

Mycotoxins, in particular, aflatoxins represent the most dangerous hepatotoxic and carcinogenic toxins to both fish and man (Wild, 1990). The wide spread biological pollutents in aquatic environment from aflatoxins contaminated feedstuffs, namely grains and dry pellted diets, usually result in sever economic losses in exposed fish (Hueper and Payne 1961; Wales and Sinnhuber, 1966) with serious damage to man and animals consuming such fish (El-Kahdem et. al., 1983).

In Egypt, the semi-intensive, poly-culture fish farms are wide spread. In these fish farming facilities, the Nile tilapia (Oreochromis niloticus) are reared together eith common carp (Cyprinus carpio L.). As Nile tilapia appeared to be highly sensitive to aflatoxins contained rations (Manal, 1993), the aim of this work was to investigate the effect of aflatoxins on some blood parameters of common carp exposed to aflatoxins doses toxic to Nile tilapia as well as measuring the aflatoxins residues in flesh of these fish.

MATERIALS AND METHODS

1. Fish:

A total number of 110 clinically normal commen carp (Cprinus carpio L.) with 100-120g. average body weight and 18-22 cm total length were used. These fish were kept in full glass aquaria supplied with dechlorinated tap water at 20°C to be used in the experimental intraperitoneal and oral aflatoxicosis.

2. Asperigellus flavus fungus (A. flavus):

Highly and multitoxin-producing A. flavus standard strain (M-93) used in this study was kindly supplied from Mycotoxin Central Laboratory of Food and Dairy technology Department, National Research Center, Dokki.

3. Aflatoxins:

a- Crude aflatoxins (AFB1 -, AFB2 - AFG1 and AFG2 at ratio of 0.6. 0.2 0.6 and 0.2mg/ml chcl3) respectivley were prepared from standard Aspergillus flavus (M-93) in Yeast Extract Sucrose (YES) medium according to Davis et al., (1965). The prepared and purified crude aflatoxins were assayed and standardized according to A. O. A. C. (1980).

b- Standard aflatoxins (AFB1 - AFB2 - AFB1 and AFG2) used for measurement of crude aflatoxins on YES medium and assays of aflatoxins residues in fish flesh were obtained from Sigma Chemical

Company (Sigma Aldim Corporation, Missouri, U.S.A.).

4- Intraperitoneal (I/P) aflatoxicosis of commcn carp:

A total number of 70 commen carp of 100 - 120g, average body weight were grouped into 7 groups of 10 fish each. Each fish in the first 6 groups received a dose of 0.2, 0.4, 0.6, 0.8, 1.0 and 4 mg/kg body weight (b.w.) of crude afaltoxin dissolved in 0.2 ml corn oil as injected intraperitoneal dose respectively. Fish in the 7th group were left as control receiving the com oil only without aflatoxins. The fish in all groups were observed for 10 days during which clinical signs, post mortem findings and mortalities were recorded.

5. Oral aflatoxicosis of Commcn Carp.:

A total number of 40 commcn carp of 100-120g. average body weight were grouped into 4 groups of 10 fish each. The fishes in the first 3 groups were feed on pelleted calculated diets (as 3% of their body weight/day) to which 0.25,0.5 and 1.0 mg/kg. b.w. of crude aflatoxins respectively were added daily for 10 successive days. These doses are equivalent to half, the same and double the LD-50 calculated dose of crude aflatoxin to Nile tilapia (Oreochromis niloticus) (Manal, 1993). Clinical and postmortem findings as well as mortalities were recorded during the 10 days observation time.

6. Haematological examination:

Blood samples were collected from all survival and moribund commen carp in both intraperitoneal and oral aflatoxicosis experiments at the end of observation time. Different haematological parameters namely, haemoglobin concentration (HB), packed cell volume (PCV) values, erythrocyte (RBC) and leucocyte (WBC) counts and differential leucocytic counts were carried out according to Hesser (1960).

7. Estimation of aflatoxin residues in Cammea Carp Flesh:

Samples of fleshs from treated and control common carp in both intraperitoneal and oral aflatoxicosis experiments were used for estimation of aflatoxin residues using Thin Layer Chromatography (TLC) technique (Stubblefield and Shorwell, 1981).

8. Statistical analysis:

Data obtained in this study were statistically analysed using statview 512 program accoring to Statview 512*(1986).

RESULTS

Table (1):Mortality Percent of Commcn Carp Inoculated Intraperitoneally (I/P) and Recieved Orally Different Doses of Aflatoxins.

Mortalities (Mr) / Observation time in days	I/P Aflatoxicosis doses mg/k b. w.						Oral aflatoxicosis doses mg/k b. w.			Control
	0.2	0.4	0.6	0.8	1.0	4.0	0.25	0.50	1.00	
1	--	--	--	--	1	1	--	--	--	--
2	--	--	--	--	--	2	--	--	--	--
3	--	--	--	--	--	1	--	--	--	--
4	--	--	--	--	--	--	--	--	--	--
5	--	--	--	--	--	--	--	--	--	--
6	--	--	--	--	--	--	--	--	--	--
7	--	--	--	--	--	--	--	--	--	--
8	--	--	--	--	--	--	--	--	--	--
9	--	--	--	--	--	--	--	--	--	--
10	--	--	--	--	--	--	--	--	--	--
Total fish death Mr. percent	0/10 0	0/10 0	0/10 0	0/10 0	1/10 10	4/10 40	0/10 0	0/10 0	0/10 0	0/10 0

Table (2) : Haematological Values of Common CarpInoculated I/P and Received Orally Different Doses of aflatoxins

Aflatoxin doses / * Haematolog. Ical Parameters	I/P Aflatoxicosis doses mg/k b. w.						Control	Oral aflatoxicosis doses mg/k b. w.			Control
	0.2	0.4	0.6	0.8	1.0	4.0		0.25	0.5	1.0	
HB Cancentration	7.9 ± 0.4	7.4± 0.4	6.5± 0.2	6.7± 0.4	7± 0.7	5±* 0.1	8.7±0.6	7.2± 0.2	6.8±* 0.3	6.7±* 0.4	7.9±0.6
PCV Value	37.3 ± 1.7	37.7± 2.7	32.2± 0.4	31.5± 2.2	34± 5.1	19.8±* 0.4	37.9±1.6	33.7± 2.3	31.6± 2.1	3.51± 2.9	37.7±1.5
RBCs Count	1.3± 0.07	1.4± 0.15	1.2± 0.09	1.1± 0.11	1.2± 0.14	0.79±* 0.01	1.5±0.14	1.4± 0.07	1.3± 0.07	1.2±* 0.01	1.4±0.13
WBCs count	13±* 2	11±* 2	13±* 2	11±* 2	10±* 1	9±* 0.8	20±3	19± 4	13± 2	16± 1	20±3
Lamphocytes %	58± 13**	65±**	54±** 3	94± 3	79± 6	71± 6	95±2	96± 2	79± 8	86± 1	95±2
Neutrophils %	42±** 13	40±** 4	45±** 3	35± 2	28± 5	27±* 7	7±1	3± 1	20± 7	14±* 3	7±2

+ : Means (± SE) of haematological parameters
* : Significant difference between control and aflatoxicated fishes (P < 0.05)
** : Significant difference between control and aflatoxicated fishes (P<0.01)

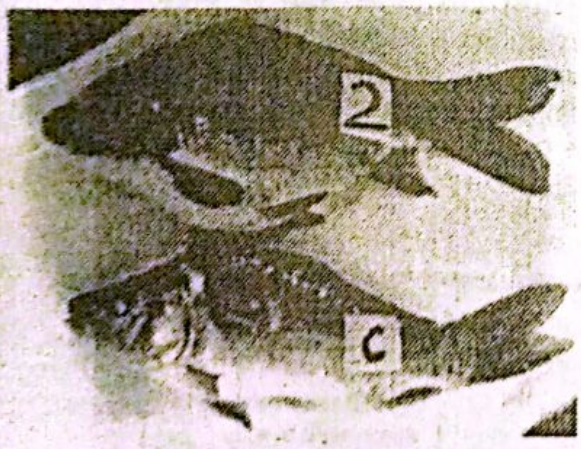

Fig. 1: Fish species: Commo carp Clinical signs: Abdominal distension (2) i intraperitoneally aflatoxicated fish in comparison to control fish (c).

1- Clinical signs and post-mort mfindings:

The moribund intraperitoneally inoculated fishes showed sluggish swimming behaviour and increased opercular movements. Abdominal distension was a prominant signs in some fishes (Fig. 1). No clinical alternations detected among orally aflatoxicated fishes. The gross post-mortum lesions in common carp inoculated intraperitoneally with different doses of aflatoxins and these received different doses of aflatoxins in pelleted diet were almost similar in the form of pale, brownish to yellowish liver with enlargement in spleen (Fig. 2). The mortalities in those fishes received 0.25, 0.5 and 1.0 mg crude aflatoxins/kg. b.w. daily for 10 days which represent the half, equal to and double the LD50 dose of Nile tilapia (O.niloticus) were completely absent, while those fish inoculated intraperitoneally with different aflatoxins doses revealed different mortalities in some groups. The results of mortality percent are to be seen in table1.

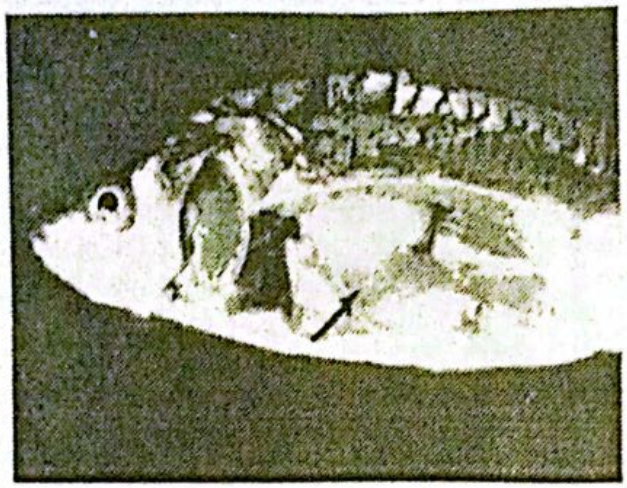

Fig. 2: Fish species : Common carp Lesion: Pale Yellowish enlarged liver after intraperitoneal and oral aflatoxicosis

2- Haematological parameters:

Different haematological parameters of common carp in both intraperitoneal and oral aflatoxicosis experiments were estimated. The results of intraperitoneal aflatoxicosis indicated a significant decrease in HB, PCV and RBCs of common carp inoculated intraperitoneally with 4 mg/kg. b. w. of crude aflatoxins only. While the WBCs were significantly decreased in all fish groups. The results of differential leucocytic count indicated significant decrease in both lymphocytes and neutrophils in groups inoculated with 0.2, 0.4 and 0.6mg/kg b. w. crude aflatoxins. The haematological values in common carp received oral aflatoxins showed significant decrease in HB concentrations, RBCs count and lymphocytes percent and significant increase in neutrophils percent in fishes orally aflatoxicated with lmg/kg. b. w./day. While, fishes aflatoxicated with 0.5mg/kg. b.w./day showed significant decrease in HB concentrations and lymphocytes percent only. The haematological parameteres of fishes fed aflatoxin contaminated diet with 0.25 mg/kg. b. w./day appeared nearly normal. The results of haematological examinations are shown in table (2).

3- Examination of experimental common carp flesh to aflatoxins residues:

Different flesh samples of common carp from different groups as well as controls in both intraperitoneal and oral aflatoxicosis experiments were analysed for aflatoxins residues. The results of this analysis are to be seen in (Tab. 3).

Table (3): Afaltoxins Residues in Flesh of Common Carp Inoculated I/P and Received Orally Different Doses of Aflatoxins

Aflatoxin doses / Aflatoxin Fractions	I/P Aflatoxicosis doses mg/k b. w.						Oral aflatoxicosis doses mg/k b. w.			Control
	0.2	0.4	0.6	0.8	1.0	4.0	0.25	0.50	1.00	
AFB_1	--	--	--	--	0.15	4.44	--	1.91	3.06	--
AFB_2	--	--	--	--	--	--	--	--	--	--
AFG_1	--	--	--	--	0.12	4.03	--	1.78	2.65	--
AFG_2	--	--	--	--	--	--	--	--	--	--

DISCUSSION

In this study, the effect of crude aflatoxins (AFB_1 AFB_2, AFG1 and AFG_2) was invesitigated in common carp (Cyprinus L.). The results of intraperitoneal aflatoxicosis with different doses of crude aflatoxins 0.2, 0.4, 0.6, 0.8, 1.0 and 4.0 mg/kg. b. w. of fish revealed non-specific clinical signs in the form of sluggish swimming behaviour, respiratory distress, and abdominal distension in some cases. (Fig. 1). The gross post-mortem findings revelaed pale yellowish to brownish friable liver and enlargement of spleen, with 10% and 40% mortalities in fish groups inoculated with lmg and 4mg/kg. b. w. respectivley. Similar signs and lesions were also recorded by Halver (1968) in rainbow trout; Jantrarotai et al., (1990) in channel catfish and Manal (1993) in ile tilapia (Oreochromis niloticus).

As commen carp are reared with Nile tilapia in polyculture fish farms in Egypt, and as common carp feed exclusively on dry pelleted rations, so half, the same and double LD_{50} oral aflatoxins doses of Nile tilapia (0.5mg/kg. b.w.) (Manal, 1993) were given in food daily for 10 days to investigate the effect of such oral aflatoxicosis under natural culturing conditions in commen carp. The results of clinical and post-mortm findings revelaed a more or less milder effect than in intraperitoneal aflatoxicosis with complete absence of mortalities within the 10 days observation time. This could suggest the high resistance of common carp species than Nile tilapia species to aflatoxicosis under the same cultural circumstances.

Regarding the results of haematological examinations. all common carp in both intraperitoneal and oral aflatoxicosis experiments showed significant decrease in haemologin value, packed cell volume and red blood cells count especially in groups exposed to high intraperitoneal (4.0mg/kg. b. w.) and oral (lmg/kg. b. w.) doses. The white blood cell count showed significant decrease in both lymphocytes and neutrophils percent, while in fish group received lmg/kg. b. w./ day for 10 days crude aflatoxins, there were significant decrease in lymphocytes and significant increase in neutrophils. (Tab. 2). These results were recorded to great extend also by Manal (1993) in Nile tilapia fish and Jantrarotai et . al., (1990) in channel catfish. This decreas in blood parameters could be attributed to the sever damage of the common carp haemopiotic organs, namely liver, slpeen and head kidney by the highly hepatotoxic and carcingoenic aflatoxins (Wild, 1990).

Concerning the aflatoxins residues in the flesh of common carp. only AFB1 and AFG1 could be detected in both intraperitoneal and oral aflatoxicosis experiments, the amounts of which were correlated to the aflatoxins dose (Tab. 3). The presence of these aflatoxins were also reported by Manal, 1993 in Nile tilapia. The absence of AFB2 and AFG2 residues in common carp flesh could be due to their very small ratio in the crude aflatoxins used or their presence in very small amounts beyond their detection by the TLC method.

In conclusion, these results indicated that in polyculture fish farming system, the common carp fish are more resistant to aflatoxins in comparison to Nile tilapia. Also spotlight should be given on the public health risk in consuming fish fed on aflatoxicosis contaminated diets with aflatoxins residues in their flesh.

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