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DEPRESSION OF PROTEIN SYNTHESIS IN TILAPIA BY AFLATOXIN

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ABSTRACT

Experimental aflatoxicosis was induced in fingerlings Tilapia nilotica by feeding them with dietary aflatoxin (AF) B_1 at the levels of O; 476.19, 952.38 and 1954.76 µg/kgm of basic diet for a period of 14 weeks. Results indicated that total serum protein was significantly decreased in AF treated groups. The same trend was observed for serum albumin and total globulins at the levels of 952.88 and 1954.70 µ g/kg, wheares at the low level (476.19 µg/kg), the decrease was not significant. Also, the results revealed a significant decrease ($P \leq 0.01$) in the globulin fractions (alpha and Beta), which reached to 61 % and 64 % in the fourth treated group respectively. Meanwhile, the gamma globulin fraction showed significant increase ($P \leq 0.05$) at the level 476.19 µg/kg AF., but significantly decreased at the high doses of AF(B_1). Regarding the enzymatic activities (Serum, choline esterase, lactic dehydroganase, alanine amino transferase and Aspartarte amino transferase), the acetyl choline esterase (ACHE) was significantly decreased in the AF treated groups after the experimental period. On other hand, the activities of lactic dehydrogense (LDH), alanine amino transferase (Al-tase) and aspartase amino transferase (As-tase) were significantly increased in AF treated groups.

INTRODUCTION

The aflatoxin (AF) is a group of mycotoxins produced by ***Aspergillus flavus*** and ***Aspergillus parasiticus*** and is recognized as potent hepatotoxins and carcinogens (Abd El- Hamid et al, 1992; Korshom and Abd El- Hamid 1992). Aflatoxicosis represents one of the most serious diseases of poultry, livestock, producing animals and humans (Edds, et al. 1973), because aflatoxin producing strains of these two types of fungus commonly appear in such agricultural commodities as peanuts, corn, wheat and animal feed-stuffs (Anonymous, 1977). Also, contaminated milk and meat may result from animals ingested contaminated feed (FAO, 1977). The degree of susceptibility varies with species, age, strains, levels of aflatoxin intake and duration of exposure (Korshom and Abd El- Hamid, 1992). The inhibition of protein synthesis in the liver (such as plasma protein and lipoproteins during aflatoxicosis) has been demonstrated (Brown and Abroms, 1965; Tung ***et al.***, 1972, 1975 and Abd El-Hamid, et al. 1992). Aflatoxin has shown to alter the level of serum triacylglycerol, phospholipids and cholesterol by a dose of 0.62 µg/gm (Tung ***et al.***, 1971). The most complete study of aflatoxicosis in chiken (Japnese quail) was documented by Sawhncy ***et al.*** (1973).

However, aflatoxicosis in fish is much less studied.

The objective of the present research was to describe some dose response relationships of experimental aflatoxicosis on total serum protein eletrophoritic pattern and certain enzymes in Tilapia nilotica.

MATERIAL AND METHODS

Experiments were carried on Tilapia nilotica (Oreochromis niloticus) fingerlings weighing about (4.5-4.7 gm) in average, obtained from the Saft Khalid farm at Etay Al-Barood, Behera Governorate. The fish was kept in large tanks with aerated tap-water and left to acclimate to laboratory conditions for about two weeks on the basic diet before the experiment begin. At the start of the experiment healthy fish were weighed and distributed in the experimental aquaria (tank) of (30 x 40 x 100 cm) 120 liters capacity, Eight tanks were (two for each diet) filled with tap water and left for two days before use. There after, about one third of the water volume in each tank was replaced by new

water daily, water temperature was kept thermostatically controlled at 22°C ± 1°C. The formulated basic diet consisted of soyabean meal 35% fish meal 20%, yellow corn 40%, bone meal 2%, fish-oil 2%, vitamins 0.3 and minerals 0.7% was used as control diet Table (1), Rady *et al.* (1992) without the addition of Aflatoxine (AF).

Aflatoxin B_1 was produced by growing Aspergillus parasiticus NRRL 2999 on rice according to the method of Shotwell *et al.* (1966) with the temperature modification, described by West *et al.* (1973). The fermented rice was analysed spectrophotometrically for its aflatoxin content by the method of Nabney and Nesbitt (1965) as modified by Wiseman *et al.* (1976). Weighed amounts of the rice powder were incorporated into the basal diet at the concentration of 476.19, 952.38 and 1954.76 µg/kg of basic diet for 14 weeks. Fish in every tank were fed on experimental diet at the rate of 3 % live body weight. At the end of the experiment; 10 fish from each Jar were taken, and the blood samples were collected from the tail vein.

Acetylcholine esterase (ACHE) was measured according to the method of Ellman *et al.*, 1961 using 0.075 M acetyl thiocholine iodide as substrate and measured at 412 nm.

Glutamic oxaloacetic trans aminase (GOT) and glutamic pyruvic (GPT) were assayed according to Reitman and Frankel (1957) using a commercially available kit from Biomerieux.

Protein was determined according to lowry *et al.* (1951). For preparation of calibration curve serum albumin was used. Lactate dehyrogenase (LDH); EC 1.1.1.27 activity was measured also colorimetrically by the method of Anon (1971). The method is based on reduction of pyruvate by incubation with the enzyme in the presence of reduced nicotinic adenine dinucleotide (NADH). The reaction was arrested by adding dinitrophenyl hydrazine and the colour produced was measured at 510 nm.

Protein fractions were separated electrophoretically according to Grunbaum, (1981) using the Sartophor-syst on cellulose acetate membrane strips SM 12200 - 070 x 154 BBN. Tris- glycine (pH 8.4) served as tank buffer and a full strength of which served as membrane buffer as well. Samples were

electrophoresed for 30 minuts at 350 V and 1-2 mA and stained in Comassive Brilliant blue. Dried strips were quantitated using El Script 2- Hir schmann densitometer at a range from 0.01 to 0.15 absorption units 1 cm. For identifying the different protein fraction the method of (Schlofeldt 1975) was used.

RESULTS AND DISCUSSION

Under aquarium conditions, all the experimental animals survived at all aflatoxin concentrations through the exposure time. All measured biochemical parameters in Tilapia nilotica changed significantly $P \leq 0.01$ after the cronic aflatoxin treatment (Table 1 and 2), compared to the control. presented Data in (Table 1), showed that during aflatoxicosis total serum protein was significantly depressed ($P \leq 0.01$) in all aflatoxin - treated groups, while, albumin and total globulin were significantly depressed only in the third and fourth treated groups, which recieved 952.38 and 1954.76 µg/kg aflatoxin of basic diet respectively. The results revealed a significant decrease ($P \leq 0.01$) in alpha and beta globulin fractions, which reached 61 % and 64 % in the fourth treated group respectively. At the same time, the gamma globulin fraction showed significant increase in the second group, but decreased in the third and fourth groups.

Our findings are in agreement with Brown and Abrams (1965); Tung ***et al.*** (1972); Beers ***et al.***, 1990 and Abd El- Hamid ***et al.*** (1992), who found similar results in chickens treated with aflatoxin in high levels. Such decreases in a aflatoxin - treated groups may be attributed to the lowering of synthetic power of albumin and globulin in the liver. Since, the aflatoxins is a great hepatotoxic agent (Beers ***et al.***, 1990 and Shaaban ***et al.***, 1991). Moreover, aflatoxins interfere with DNA transcripition and subsequently impaired DNA and RNA formation (Shaaban ***et al.***, 1991). The significant decrease in the globulin especially in alpha and beta fractions in infected fish may be due to the non stimulation of the body defensive mechanism to the aflatoxins, which result in the production of more globulin to increase body resistance in a trial to overcome the infection.

Immunological activity of the organsim is indicated by the presence of a high increase in globulin fractions particulary gamma globulin (Kobat and Mayer, 1968). An increase in gamma globulin was noticed in the moderately infected fish than in the highly infected one. The exposure for long period with

Table (1): Mean values of serum protein fractions in Tilapia after dietary feeding of aflatoxin B_1.

Treatment Af/µg/kg diet	Total serum protein g %	Pre-albumin g %	Albumin g %	Total globulin g %	α_1 -glob g %	α_2-globulin g %	B-globutin g %	α- globulin g %	α_1-globulin g %	α_2-globulin g %
Control	5.34 ± 1.21	0.17 ± 0.01	2.84 ± 0.3	2.33 ± 0.20	0.52 ± 0.1	0.60 ± 0.02	0.79 ± 0.04	0.43 ± 0.03	0.25 + 0.02	0.18 ± 0.02
476.19	3.63 ± 0.72	0.09 ± 0.01	2.01 ± 0.3	1.78 ± 0.15	0.38 ± 0.01	0.36 ± 0.02	0.56 ± 0.02	0.48 ± 0.02	o.32 ± 0.03	0.16 ± 0.01
952.38	2.84 ± 0.40	0.08 ± 0.0	1.67 ± 0.02	1.09 ± 0.20	0.20 ± 0.01	0.27 ± 0.0	0.34 ± 0.02	0.28 ± 0.02	0.15 ± 0.01	0.13 ± 0.01
1954.76	1.87 ± 0.20	0.03 ± 0.0	1.27 ± 0.05	0.87 ± 0.10	0.20 ± 0.02	0.23 ± 0.01	0.28 + 0.01	0.16 ± 0.01	0.07 + 0.0	0.09 ± 0.0

Af = Aflatoxin B_1.

Table (2): Effect of aflatoxin B_1 on serum enzyme activities of Tilapia fingerlings.

Treatment Af µg/kg diet	ACHE µM/mg pr/min	LDH I.U./L	AL-T-ase IU./mg protein	As-T-ase IU/mg protein
Control	98.44 ± 14.20	350 ± 33.6	5.85 ± 1.32	23.8 ± 3.08
476.19	86-75 ± 17.80	404 ± 41.70	11.8 ± 2.74	29.10 ± 4.75
952.38	69.80 ± 12.70	465 ± 45.6	17.0 ± 2.81	34.4 ± 6.60
1954.76	50.75 ± 11.30	581 ± 63.41	28.3 ± 2.69	39.50 ± 5.43

ACHE = acetyl choline estrase.
LDH = lactic dehydrogenase.
IU = International unit.
AL-T-ase = Alamin amino transferase.
As-T-ase = Aspordate amino transferase.
Data are presented as means ± S.D.

moderate doses of aflatoxin many give the immuno system a chance to minimize the synthesis of serum choline esterase. The significant increment in the serum lacate dehydrogenase and transaminase enzymes (t) activities are in accordance with the findings of Amer ***et al.*** (1987), who found the same result in broiler chicks. Such increases may be attributed to hepatocellular damage and leakage of cytoplasmic enzymes into serum, since liver and skeletal muscles contain large amounts of these enzynes and aflatoxin reflects serious tissue damage mainly due to necrosis of liver, but other organs may suffer from degenerative changes (Kidney or/and gill) (Nemcsok and Benedeszky, 1990).

Therefore, the measurement of the biochemical parameters in this study paper appear to introduce sensitive biomonitoring indicators to assess exposure of fish to mthropogenic agents in the natural environment.

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