Effect of Afla-Toxins B1 on Endocrine Status in Cat fish *(Clarious lazera)*

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Abstract: The influence of dietary aflatoxins on body weight, immunity, and hormonal profile was studied in catfish. The results revealed that, administration of aflatoxins, and aflatoxins plus fax-A-toxin 0.1% in diet for 4 months decrease body weight, IgM, Insulin, Thyroxine however there were elevation in cortisol hormone level. Afla-toxins may induce an immunosuppressive effect on humoral immune response of tilapia Nilotica in *which* was suggested by reduction of immunoglobulin

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Introduction

IgM, is the most important immune factor to neutralize bacteria and render them more succeptible to phagocytosis (Ingram, 1980). It is well known' that in mammals immunoglobulin production is closely related to endocrine status (Berezi, 1989) for example tyroid hormone enhance the production of immunoglobulins (Chen 1980). Cortisol intensify, suppress immunoglobulins production (Pottinger 1985). In teolosts cortisol level markedly increased following stressor exposure and elevated cortisol level results in a significant increase susceptibility to infectious. diseases (Pickering and Pottinger, 1985). The purpose of administration of fax-A-toxin particularly with Aflatoxin to know the effect of fax-A-toxin on Aflatoxin in fish. Many studies concerned the effects of cortisol on IgM production (Anderson et al. (1982).. However there is no previous reports on the effect of. Aflatoxins on serum IgM and endocrine status. Many authors observed the effect of Afla toxins *on* liverdamage. The liver enzymes are changed with observation of malignant tumours (Ostrawski, 1984; and Evmgton et al. 1994).

The present work was under taken to study the effect of *afla* toxins on endocrine status and immunoglobulin M of tilapia fish, this fish was selected because their wide availability edibility in Egypt and their important ecological role m the River Nile .

2. Material and Methods

One hundred and twenty Tilapia Niloticus were used in the present study. Their live body weight averaged of 37.5 gram. The fish were healthy and clinically free from external and internal parasites. They were maintained *in* tanks containing well aireated water at atmospheric temperature for two weeks before the xperiments began. Fish were randomly distributed into four ;groups;, each of 30 fish and 2 control groups, the first group fed Aflatoxin-free ration and used as negative control (C) while the second group (AFC) was fed of Aflatoxins contaminated corn (50 ug toxin/kg ration) and used as positive control. The third group fed aflatoxin- free ration with 0.01% fax-A-toxin. The fourth group fed aflatoxin contaminated corn (50 ug toxin/kg ration) with 0.1% Fax-A-toxin for four months daily. Sources of Aflatoxin is contaminented corn 50 lag toxin/kg ration.

The fish were fed by hand twice daily and feed consumption in all groups was recorded daily. Also the mortality rate and body weight of fish due to Afla-toxins were recorded (Table 1).

Ration:

Ration used during trials contained 16.3% crude protein, 2.5% crude fat and 14% crude fibre, the digestible energy was 26% cal/kg. The diet contained feed additives which included minerals, vitamins and amino acids. Body weight measured every month for four month. Sources of Afla toxins present in corn (50 p.g toxin/kg ration).

Samples:

Serum samples were collected 4 times at one month interval and Sera were frozen at -20 for later analysis. Serum cortisol, IgM, T4, and insulin were determined using kits.

1 - IgM determination:

The serum IgM was measured according to Fuda et al (1991).

1-a. Preparation of antisera:

Antisera for Tilapia was prepared by immunizing rabbits as described by Hara (1976).

Catfish IgM antibody:

The procedure for labeling antibody fragment with enzyme was performed according to the method of Nagae et al (1993).

EIISA assay procedure:

Double antibody sandwich Elisa according to the method of Matsubara et al. (1985) and Nsgae (1993)was used for determination of IgM.

Cortisol was estimated using radio immunoassay technique according to the method of Pickering and Potinger (1983) and Wedmyer (1970).

Serum thyroxine was estimated using radioimmunoassay (RIA) using coat (A) count provide by diagnostic product corporation Los Angelos U.S.A. (Deftoff 1979).

Insulin was determined by RIA according to the method described by Sundly (1991).

Statistical analysis:

The difference between the groups were calculated according to Snedecor and Cochran (1967) by t-test

3. Results

As shown in Table (1-3) and there is a decrease in body weight in aflatoxins and aflatoxins plus fax-A-toxin 0.1% if compared with control groups.

Table (2) showed the influence of aflatoxins and aflatoxins plus fax-A-toxin on IgM. Highly significant decrease of IgM levels was detected in treated groups with afla toxin and fax-A-toxin 0.1%.

Table (3) showed the serum hormonal changes in infected fish treated with Afla Toxin & Fax-A-Toxin. The results revealed decrease level of insulin, and thyroxine while a highly significant elevation of cortisol level was observed.

Table (1) Effect of aflatoxin on body weight of cat fish

Group	1 Month	2 Month	3 Month	4 Month
Aflatoxin	41.8 38.0 34.1	36.1 34.2 30.4	31.7 34.0 30.5	31.1 28.3 31.5
Aflatoxin + 0.1 Fax A toxin	43.0 42.5 35.5	40.3 30.1 41.2	41.2 37.4 31.2	41.5 45.5 32.6
Control	45.2 46.0 36.2	43.4 41.1 41.5	46.7 41.4 46.6	42.5 51.6 46.6
0.1% Fax a toxin	43.6 44.1 36.7	48.0 41.4 47.4	41.0 47.8 51.8	41.2 44.1 52.7

AF= Aflatoxin 50 µg/Kg c number of fish each group=30 body weight 1 gm

Table (2): Effect of aflatoxin in 1gm µgm/ml in cat fish (clarious lazera)

	1 Month	2 Month	3 Month	4 Month
Control	2.86± 0.73	2.45± o.30	2.54± 0.50	2.00± 0.80
Aflatoxin	1.58*± 1.40	0.94*± 0.36	0.98**± 0.50	0.94**± 0.72
Control + Fax A Toxin	1.54± 0.20	2.60± 0.14	2.68± 1.08	2.30± 1.40
A.F + Fax A Toxin	2.05*± 0.54	2.16*± 0.73	1.95**± 0.27	1.76**± 0.30

AF -> Aflatoxin 50 µg/Kg c number of fish each group=30

Table (3): Effect of Aflatoxins on Hormonal profile in cat fish

	Insulin µg/dl				Thyroxine				Cortisol µg/dl			
	1M	2M	3M	4M	1M	2M	3M	4M	1M	2M	3M	4M
Control	13.6 ± 0.31	10.5 ± 2.24	11.00 ± 2.62	13.08 ± 1.70	0.0882 ± 0.05	0.0854 ± 0.077	0.967 ± 0.027	0.950 ± 0.014	0.888 ± 0.16	0.887 ± 0.21	0.921 ±0.34	0.954 ±0.73
Aflatoxin	13.2* ± 0.16	12.01* ± 1.20	14.00 ± 1.27	11.08*	0.0640* ± 0.0330	0.0730* ± 0.022	0.0721* ±0.039	0.0718* ± 0.0549	1.11** ± 0.30	1.42 0.043	1.70** ± 0.027	1.75 ± 0.038
Control + Fax A Toxin	14.1 ± 012	13.50 ± 0.52	13.80 ± 0.23	13.72 ± o.72	0.0942 ± 0.072	0.0988 ±0.440	0.0943 ± 0.24	0.0849 0.074	0.988 0.33	0.942 ± 0.10	0.980 ±0.50	0.962 ± 0.67
A.F. Fa A Toxin 0.1	13.00* ± 0.23	13 ** ± 0.27	12.00* ± 0.20	13.54* ± 0.21	0.0821* 0.069	0.0698* 0.023	0.0764* 0.0023	0.0804** 0.064	1.35* 0.83	1.26* 0.74	1.24* ± 0.86	1.18 ±0.34

* P<0.01 A.F -> Aflatoin ** P<0.05 M -> Month

4. Discussion

IgM level was determined to find out information about fish immune system, which was previously investigated in different species by many authors as Matsubara et al. (1985) and Fuda et al. (1991).

In this work the purified IgM revealed a single preciption in this work reacted against specific polyvalent antiserum to catfish IgM a similar result was obtained by Bagee et al., (1993. They found that chum salmon (IgM) was detected by specific anti (IgM) antibodies.

While the lower limit was 5 mg/ml reported, by Fuda (1991) there is a significant decrease in IgM level in fish with afla toxins, if compared with control groups. Anderson et al. (1982) found a relation between cortisol and (IgM) as when cortisol increased (IgM) decrease.

The significant increase of cortisol level in intoxication with Afla to. groups could be attributed to stress factors and the intoxication have examine response of fish to stress factors e.g. crowding, continous handling, infection John et al., 1994, Barton et al., 1980, Strange, 1978 and Wedemger, 1970,reported that the elevation of cortisol with afla toxins and Fax A 0.1% toxin may attributed to intoxication, and continous handling of fish. These observations emphasizes the extreme care needed during design and *analysis* of experiments, involving the (HPI) axis of teleost fish due to extremly sensitive HPI axis. Similar results were reported by pickering and_Pottinger (1983) .

Serum thyroxine (T4) concentrations in the serum of Tilapia species decreased in the intoxicated groups. It has been shown that intoxication, and chronic stress rin a marked long lasting depression of serum T_4 levels in Tilapia fish (Osborn et al 1978) and Milne and Leatherland, (1980). The response of thyroid gland of telosts fish needs further investigated with particular attention to possible relationship between the H.P.I. axis and pituitary thyroid axis. Milne and Leatherland. (1980), Osborn et al. (1978) and Mooreoud et al. (1977) using histological [r] approach concluded that cortisol reduced thyroidal activity in sock eye salmon. The significant decrease of insulin values may be attributed to aflatoxin which may somehow reduce the metabolic activities in the aflatoxin inttoxicated fishes. The `decrease in body weight was observed, while detectable agrees with Ostrowski[(I)] (1984), Hilton et al. (1987) and Sundly et al. (1991) as they observed a detectable decrease in body weight of duck infected with afla-toxin.

The aim of adminitration of Fax-A-Toxin particularly with Aflatoxin to know if Fax-A-Toxin eleminate Afla-toxins in the body of fish. In the present study Fax A Toxin not affect Afla-toxins as the results indicated that IgM, and endocrine status still not corrected or not return to the normal status in Tilapia fish.

In conclusions afla-toxin reduce of the humoral immune response as detected by decrease of IgM level, body weight and cortisol elevation. Suppress IgM, Thyroxine *(T4)* hormone and insulin levels. Fax-A-toxin has no significant effects on afla toxins.

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