

PLASMA PROTEINS PROFILE IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) AND COMMON CARP (*CYPRINUS CARPIO*) EXPOSED TO OCHRATOXIN- A AND TREATED WITH SOME ANTITOXINS

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Abstract

This study was carried out to investigate the ability of minimum level (0.5%) (Tri star, Mycoband and Mould Stop Super) to resist the toxicity of Ochratoxin A (7mg O.A/ Kg diet). The growth parameters and protein fractionation in *Oreochromis niloticus* and *Cyprinus carpio* were recorded. Five groups of apparently healthy fish with mean body weight of 50-60 g for *Oreochromis niloticus*, and fingerlings with a mean body weight of 9-11 g for *Cyprinus carpio* were used. Ochratoxin A caused loss in live body weight gains in *Oreochromis niloticus* and *Cyprinus carpio*. Addition of the adsorbents reduced the bad effects of Ochratoxin A on growth performance (Live body weight, body weight gain and specific growth rate).

Concurred the electrophoretic investigation, plasma protein of fish showed the presence of 22-24 and 18 main fractions in control group of the various sera of *Oreochromis niloticus* and *Cyprinus carpio*, respectively. The genotoxic effects of ochratoxin A led to a loss of two bands at mobility 0.01 & 0.135, while, new fraction appeared at mobility 0.96 in *Oreochromis niloticus*. Also, new fractions appeared at mobility 0.059, 0.089, 0.774 & 0.904 in *Cyprinus carpio*.

Addition of 0.5% adsorbents (Tri star, mycoband and Mould Stop Super) on contaminated diet with 7 mg ochratoxin A /kg led to appearance of new bands at mobility 0.01, 0.135 & 0.539 in *O. niloticus*, and at mobility 0.281 & 0.824 in *Cyprinus carpio* as control group. It is concluded that the adsorbents were able to resist the toxicity of Ochratoxin A and to improve the growth parameters and plasma protein in fish.

INTRODUCTION

Mycotoxins are among the most common contaminants in animal feeds, causing great losses in both the livestock industry and aquaculture. Ochratoxin-A is one of these mycotoxins, produced as toxic metabolite from *Aspergillus ochraceus*. Ochratoxin A was encountered as a naturally occurring contaminant of foodstuffs (maize) in many

countries. The LD₅₀ of Ochratoxin for the rainbow trout by intraperitoneal injection was 4.70 mg/Kg (Jantrarotai and Lovell, 1990).

Storage of feeding stuffs for a long period of time specially under local bad environmental conditions of high temperature and humidity exposes these feeds to deterioration, contamination, and losses of their nutritive value, and so, affects the consumer. The most common problems occurred during storage is the elaboration of mycotoxins. Mycotoxins are groups of fungal metabolites that may cause pathological and undesirable physiological responses in man and animals (Tetushia, 1990).

Mycotoxins are toxic fungal metabolites which are structurally diverse, common contaminants of the ingredients of animal feed and human food. To date, mycotoxins with carcinogenic potency in experimental animal models include aflatoxins, sterigmatocystin, ochratoxin, fumonisins, zearalenone, and some *Penicillium* toxins. Most of these carcinogenic mycotoxins are genotoxic agents with the exception of fumonisins, which is currently believed to act by disrupting the signal transduction pathways of the target cells (Wang and Groopman, 1999).

Ochratoxicosis is one of the animal hazards which affect our livestock with remarkable influence on the health and production. The target organs of ochratoxin A for any animal species (including fish) seem to be the kidney, gastrointestinal tract, liver, lymphoid organs, reproductive system as well as skeletal system, so, ochratoxin reduces the rate of growth (Frisvad and Samson, 1991).

Fish growth is highly flexible and is one of the complex activities where it represents the net outcome of a series of environmental and physiological factors that begin with food intake, digestion, absorption, assimilation and other metabolic activities. All these processes may affect the final fish production (Bugaev *et al.*, 1994).

The toxicokinetic profile of ochratoxin A was studied after oral or intravenous administration of 50 ng/g b.w. to fish, quail, mouse, rat and monkey. The elimination half-life varied from 0.68 h after oral administration to fish, up to 840 h after intravenous administration to monkey. The distribution volume ranged from 57 ml/kg in fish to 1500 ml/kg in quail. The plasma clearance was most rapid in quail and fish, 72 and 58 ml/kg.h, respectively, while, it was only 0.17 ml/kg.h in monkey. The bioavailability was as low as 1.6% in fish, but as high as 97% in mouse. The binding abilities of ochratoxin A to plasma proteins were found to be less than 0.2% in all species investigated (including man) except fish. A similar investigation on the toxicokinetics and binding properties of ochratoxin B was also performed. Ochratoxin B

was more readily eliminated and had a lower affinity for plasma proteins, which partly may explain its lower toxicity (Hagelberg *et al.*, 1989).

Under experimental condition, Ochratoxin-A has revealed a pronounced acute toxic effect in a number of mammals, birds and fish species, with peroral LD₅₀ in range 3.4-62.4 mg/kg body weight (World Health Organization, 1979).

This study is focused mainly on testing the ability of 0.5% adsorbents (Tri star, Mycoband and Mould Stop Super) to resist the toxicity of Ochratoxin A (7mg O.A/ Kg diet), and also, to measure the growth parameters and plasma protein fractionation in exposed to *Oreochromis niloticus* and *Cyprinus carpio*.

MATERIALS AND METHODS

1- Fish

Five groups of *Oreochromis niloticus* each one containing three replicates and each replicate containing ten fish (29.1 - 31.0 g), and five groups of *Cyprinus carpio* fingerlings each one containing three replicates and each replicate containing twenty fish (2.27-3.45 g) were used. Healthy fish were collected from the fish ponds of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia. Fishes were acclimatized in laboratory conditions for two weeks prior the experiment in aerated holding tanks with feeding rate of 3% b.w, then, to 30-glass aquarium (75x50x50 cm) of 150-liter capacity. The aquaria were supplied with dechlorinated tap water and constant aeration. The dissolved oxygen was adjusted at 5.7 mg/l, the temperature at 26 ± 2 °C and the pH at 7.2 ± 0.2.

2- Fish diet

Locally prepared fish diet containing fish meal, soybean meal, yellow corn formulation ingredients, bone meal, meat meal, mixture of vitamins, and salts was formulated in a pelleted form using glutinative substance from Atmida poultry company, Dakahlia Governorate, Egypt. The chemical composition of the experimental diet was analyzed on dry matter as in Table 1.

Table 1. Chemical analysis of the experimental diet on dry matter basis.

Items	%
Moisture	10.34
Crude protein	25.51
Ether extract	6.08
Ash	13.13
Crude fiber	5.59
Carbohydrates	39.35
Gross energy (KCal/kg)	4537.7

3- Adsorbents

Adsorbent 1 was modified Tri star (Organic acid and silicate salts). Each Kg contained 300g formic acid, 150g probionic acid, 300g glutofid, 150 g precipitate of silica, 100g calcium carbonate (German Co. for Vet. Medicine and Feed Additives).

Adsorbent 2 was modified mycoband (natural mineral compound with a high adsorption and binding capacity), product of Optivite International Ltd, Main Street. Laneham, Retford, Notts, United Kingdom.

Adsorbent 3 modified Mould Stop Super (used to control the molds and adsorption of its mycotoxin). Each Kg contained 200g calcium probionate, 100g Kaolin, 100g aluminum silicate, 10g copper sulphite, product of Smart Vet.

4- Preparation of high concentration of Ochratoxin-A

The spore of *Aspergillus ochraceus* was obtained from the Central Lab. of Residues in Agric. Products, Agric. Pesticides Research Center, Dokki, Egypt. Cultures were maintained at 4 °C on Czapek agar and subcultures were used for inoculations. Triple-baffled shake flasks (125ml) containing 50 ml of medium were inoculated with 1 ml of a spore suspension (50 spores / ml) and incubated on rotatory shaker at 250 rpm (2.54 cm circular orbit) for 9 days at 25-29 °C. Ochratoxin A was analyzed from culture filtrates as described by Bacon *et al.* (1973). Ochratoxin A was purified by preparative thin layer chromatography. The highly concentration of Ochratoxin A was diluted to minimum peroral LD₅₀ by adding extract Ochratoxin A to fish diet (7 mg Ochratoxin-A /Kg of diet) according to World Health Organization (1979).

5-The experimental design

Different fish groups were subjected for experimentation in the following manner:

1st group from *Oreochromis niloticus* and *Cyprinus carpio* fed on commercial diet and kept as control.

i- 2nd group fed on contaminated diet (7 mg ochratoxin A /kg diet).

ii- 3rd group fed on contaminated diet with 7 mg ochratoxin A /kg diet plus 0.5% of adsorbent 1.

iv- 4th group was fed on contaminated diet with 7 mg ochratoxin / kg diet plus 0.5 % of adsorbent 2.

iii- 5th group was fed on contaminated diet with 7 mg ochratoxin-A /kg diet plus 0.5% of adsorbent 3.

Fish were weighed to the nearest 0.1 g every 15 day- intervals to readjust the calculated food and estimate the growth rate. The blood samples were taken from the caudal vein of fish at the end of experiment (60 days) to obtain the serum for studying the Polyacrylamide gel Electrophoresis analysis of plasma protein. The growth performance of all tested fish groups was estimated throughout the 2 months experimental period.

6- Polyacrylamide gel electrophoresis analysis of plasma protein

Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Studier (1973). The 5 ul plasma samples were dissolved in the same volume of 2x Laemmli buffer. Mercaptoethanol was added to each tube (10%v/v, for each sample). All samples were then boiled in a water bath for 10 minutes at 100 °C and loaded on the gel after adding one drop of bromophenol blue (0.025%). The next step was to pour 4.2 liters of the run buffer into a running tank to be precooled by flowing tap water through cooling tubes. The buffer tank was filled, so that gels were completely covered. 800 ml of run buffer were saved for the upper tank. Gels were run at 175V for minutes and then were raised to 200 V for two hours. Staining gels were placed in 100ml of Coomassie Brilliant Blue R 250 staining solution, removing the staining solution from tray, 200ml-destining solution was added. Gel was agitated for one hour. After removing the solution, a new 200 ml destining solution was added to the gel, agitation was repeated for three times until gel background was clear. The gel was photographed and diagrammatically illustrated.

7- Statistical analysis

The obtained data were statistically analyzed of the mean value (X), standard deviation (S.D.), test of significance (t-Test) and analysis of variance (F- test) according to Snedecor and Cochran (1976).

RESULTS AND DISCUSSION

Reduction of ochratoxicosis in *Oreochromis niloticus* and *Cyprinus carpio* was examined by adding 3 commercial adsorbents from Egyptian market to Ochratoxin-A contaminated diet in feeding trial for 8 weeks. Data presented in Table 2 showed that Ochratoxin-A caused loss in live weight gain (0.0 & 0.45 g) when compared with control (9 & 1.97g) in *Oreochromis niloticus* and *Cyprinus carpio*, respectively. The bad effects of Ochratoxin A on growth performance (Live body weight, body weight gain and specific growth rate) agreed with the findings of Shehata *et al.* (2003) who reported that Aflatoxin B1 (9 mg/kg diet) significantly reduced (6.09; 11.25; 17.34 and 22.87%) in live body weight of *Oreochromis niloticus* treated at 2, 4, 6 and 8 weeks, respectively. Adding the adsorbents (Mycoband and mold stop super) significantly reduced the toxic effect of aflatoxin on loss of body weight (the improvement ranged from 14.53 to 95.57%). This might be due to detoxification process in the body utilization of glutathione enzyme. Glutathione is partly composed of methionine and cysteine, hence, this detoxification process depletes the metabolic availability of methionine leading to poor growth and feed efficiency (Devegowda *et al.*, 1998).

The problems with mycotoxins do not end in feed refusal or reduction or reduction of animal performance, but many of these mycotoxins are transferred into the meat or milk (Devegowda *et al.*, 1998). Addition of the adsorbents reduced the toxic effect of ochratoxin A. The average body weight gain for *Oreochromis niloticus* and *Cyprinus carpio* were 0.0 & 0.45 g / 8 weeks without adsorbents. The average improvement of Tri star, Mycoband and Mould Stop Super in body weight gain were 1.15; 0.50 & 3.07 and 0.6, 0.56 & 1.35 g / 8 weeks for *Oreochromis niloticus* and *Cyprinus carpio*, respectively (Table 2). Reduction of Ochratoxin-A effect by adsorbents may be due to its effect on stimulating the specific immune system. These results agreed with Savage *et al.* (1996) who reported that reduction of aflatoxin effect by adsorbents may be due to its effect on stimulating the specific immune system. Also Park *et al.* (1996) reported that adsorbents reduced the liver cholesterol and liver fat levels which were increased by aflatoxin. Diminished effect of the adsorbents on body weight gain agreed with the findings of Shehata *et al.* (2003) who reported that adding the adsorbents (Mycoband and mold stop super) significantly reduced the toxic effect of aflatoxin B1 (9 mg/kg diet) on the body weight of *Oreochromis niloticus* treated at 2, 4, 6 and 8 weeks (the improvement ranged from 14.53 to 95.57%).

The electrophoretic pattern of plasma protein of *Oreochromis niloticus* and *Cyprinus carpio* treated with contaminated diet (7 mg ochratoxin A /Kg of diet) in feeding trial for 8 weeks using 3 commercial adsorbents from Egyptian market was investigated . According to the percentage of frequency and appearance of the fraction, the results presented in Tables 3&4 and illustrated in Fig. 1 showed the presence of 22-24 and 18 main fractions in control group of the various sera of *Oreochromis niloticus* and *Cyprinus carpio*, respectively. The fractions were described in terms of their mobility, the faster anode migration is designated first followed by the less mobility fraction and so on. This is in accordance with the observation recorded by Elghobashy (2004) who reported that the maximum numbers of bands were 23, 25, 21 and 20 for Maryout, Manzala, Abbassa and Aswan populations, respectively for the densitometrically analysis for muscle protein patterns of five random individuals from each population of *Oreochromis niloticus*. Kamel (1999) reported that the number of bands ranged from 11-14 for the three parental strains Abbassa, Zawia and Maryout. The three strains shared five common bands at mobility of 0.01, 0.05, 0.16, 0.22 and 0.33. These bands could be used as marker bands for *O. niloticus*.

The genotoxic effects of ochratoxin A led to a loss of two bands at mobility 0.01 & 0.135, and new fraction appeared at mobility 0.96 in *Oreochromis niloticus* (Table 3). Also, new fractions appeared at mobility 0.059, 0.089, 0.774 & 0.904 in *Cyprinus carpio* are shown in Table 4 and illustrated in Fig. 1. The reduction in plasma protein bands sometimes occurred due to the increase of protein breakdown as a result of toxicants to compensate for the increase in energy demands. This is in accordance with the observation recorded by Krogh (1983) who reported that ochratoxin is a fungal metabolite (Mycotoxin) with pronounced nephrotoxic potency in all species of animals studied. It has shown teratogenic properties in 3 rodent species, and a renal carcinogen in the mouse. Protein synthesis and RNA synthetase is competitively inhibited by the toxin; renal phosphoenol pyruvate carboxykinase appears to be specifically inhibited by the compound. On the other hand, Rizkalla *et al.* (1997) studied the genotoxic effects of different doses of organophosphorous compound (Hinosan) and carbamate (Sevin) for 30 days on the electrophoretograms of sarcoplasmic proteins of common carp (*Cyprinus carpio*) with regard to the number, mobility and density of fractions.. Hinosan exposed specimen's yielded bands than control. On the other hand, in sevin treated specimens, new bands appeared. Also, some bands disappeared and others appeared.

Many different methods (physical, chemical and biological techniques) were carried out for detoxification of mycotoxins, and the most applied method for protecting animals against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to bind the mycotoxins efficiently in the gastrointestinal tract (Abdelhamid *et al.*, 2003). In this study, addition of 0.5% adsorbents (Tri star, mycoband and Mould Stop Super) on contaminated diet with 7 mg ochratoxin A /kg increased or improved the plasma proteins of *O. niloticus* and *Cyprinus carpio*. Adsorbents led to clear new bands at mobility 0.01, 0.135 & 0.539 in *O. niloticus* and at mobility 0.281 & 0.824 in *Cyprinus carpio* of control groups. These new bands might be a result of the genotoxic effects of ochratoxin A, and some unexpressed genes might have come to be expressed to face such effect. This result also agreed with Rashed *et al.* (1992) who reported that the detected change in the muscle protein of *Oreochromis* species after exposure to organophosphorous insecticides might be a result of pesticide stress, and some unexpressed genes might have come to be expressed to contradict such effect. Also Savage *et al.* (1996) reported that reduction of aflatoxin effect by adsorbents may be due to its effect on stimulating the specific immune system.

In the present study, it is concluded that, 0.5% adsorbents (Tri star, Mycoband and Mould Stop Super) was able to resist the toxicity of Ochratoxin A (7mg O.A/ Kg diet) by improving the growth parameters and reducing the bad effects of Ochratoxin-A on growth performance (Live body weight, body weight gain and specific growth rate). Also, adsorbents improved plasma proteins with new bands. These new bands might be a result of the genotoxic effects of ochratoxin A and some unexpressed genes might have come to be expressed to face such effects.

Table 2. Growth performanc of *Oreochromis niloticus* and *Cyprinus carpio* fed Ochratoxin A and antitoxin (Tri star, mycoband and mold stop bactersrop).

Fish	Treatment	Intial weight (g)		Final weight (g)		Weight gain (g)		SGR		A.D.W.G gm		F-value	P >
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
<i>O. niloticus</i>	Conrol	31.00	1.63c	40.00	0.29a	9.00	1.55a	0.00569	0.0011a	0.19991	0.0345a	63.58	0.0000***
	tox + O.A	38.70	0.60a	38.70	1.99a	0.00	1.73c	0.00028	0.0007b	0.01116	0.0282b	15.79	0.0000***
	tox + salaonity	33.80	1.08b	34.94	1.32b	1.14	1.26c	0.00073	0.0008b	0.02538	0.028ab	48.08	0.0000***
	tox + mycoband	39.20	0.54a	39.70	1.27a	0.50	1.27c	0.00209	0.0024b	0.06813	0.075ab	6	0.0024**
	tox + mold stop bactersrop	30.40	1.129c	33.47	2.56b	3.07	3.36b	-0.00002	0.0010b	-0.00004	0.0385b	3.511	0.0251*
<i>C. carpio</i>	Conrol	3.45	0.40a	5.42	0.88a	1.97	0.73a	0.0099	0.0032a	0.0437	0.0163a	1.08	0.395ns
	tox + O.A	2.27	0.37a	2.72	0.65c	0.45	0.92b	0.0038	0.0075ab	0.0099	0.0204a	11.801	0.0000***
	tox + salaonity	3.71	0.43a	4.31	0.52b	0.60	0.95b	0.0033	0.0053ab	0.0134	0.0210a	3.18	0.036*
	tox + mycoband	3.55	0.67a	4.11	0.10b	0.56	0.66b	0.0036	0.0045b	0.0125	0.0146a	1.75	0.178ns
	tox + mold stop bactersrop	3.36	0.18a	4.71	0.78ab	1.35	0.88ab	0.0073	0.0043ab	0.0301	0.01961a	0.96	0.4514ns

Means with the same letter in the same column were not significantly different (P < 0.05)

Table 3. Effect of Ochratoxin A on plasma protein fractionation of Nile tilapia (*Oreochromis niloticus*).

Lane Number	Relative Front	Mol. Wt. KDa	Average optical density								
			Control		Ochratoxin A		Tri star		Mycoband		Mould
1	0.01	122.37	6.104				32.927	14.777	59.416	22.465	39.912
2	0.059	118.169	28.694	19.999	26.836	14.972	23.302	32.573	31.959	63.815	20.645
3	0.089	115.674	20.426	30.426	6.32	29.881	10.488	34.199	9.211	42.908	7.45
4	0.135	111.992	7.522	22.903			8.27	33.01	7.445	42.071	6.166
5	0.174	108.921	17.133	34.219	15.425	36.004	16.868	25.811	13.743	29.439	17.993
6	0.194	107.314	16.15	32.53	11.007	39.174	26.96	18.487	17.32	21.475	28.6
7	0.206	106.4	24.542	22.002	7.57	38.964				22.131	
8	0.24	103.778	11.557	12.214	5.441	31.713	19.098	13.053	19.435	9.971	36.268
9	0.281	100.862	37.324	4.3	20.386	24.374		13.017		11.87	
10	0.31	98.507	67.057	9.105	66.093	20.173	58.987	13.464	59.95	14.84	33.701
11	0.363	95.016	55.925	15.959	37.368	17.727				6.94	
12	0.401	87.235	32.554	7.737	10.343	16.511	28.374	12.412	27.298	4.464	56.599
13	0.433	77.473	20	5.204	19.617	9.828	15.2	5.526	11.816	6.495	42.393
14	0.487	63.884	15.093			3.216	19.099	5.385	16.122	3.825	16.654
15	0.507	58.829	23.294	6.604	20.72	3.774	14.558	10.832	17.702	11.968	14.878
16	0.539	52.372	15.838	58.804		3.362	6.861	56.263	4.807	68.501	10.347
17	0.611	40.31	22.117	57.133	9.308	48.822	6.173	76.715	20.095	103.649	8.372
18	0.66	33.216	33.815	37.315	21.871	72.133	20.845	68.898	10.215	94.775	17.203
19	0.7	27.699	18.776	14.652	15.77	58.814	12.643	48.876	6.673	50.086	4.248
20	0.774	19.895	2.22	6.261	7.593	48.124	4.246	27.595	18.913	52.442	7.502
21	0.8	17.623								16.585	
22	0.843	14.678	23.457	18.246	33.959	26.654	25.122	19.058	50.051	7.265	38.643
23	0.88	12.263	71.352	27.694	53.396	22.085				21.907	
24	0.904	11.007	96.256	2.885	59.597	26.125	85.753	23.894	86.439	35.551	76.452
25	0.93	9.754	30.25	24.449	34.215	32.234	57.456	37.71	48.713	2.53	20.93
26	0.96	8.52			21.177	18.397	17.845	22.412	22.82	36.93	20.034

Ochratoxin A = Control + Ochratoxin A (7 mg/ kg diet)

Tri star = Control + Ochratoxin A (7 mg/ kg diet)+ Tri star

Mycoband = Control + Ochratoxin A (7 mg/ kg diet)+ mycoband

Mould = Control + Ochratoxin A (7 mg/ kg diet)+ mold stop super

Table 4. Effect of ochratoxin A on plasma protein fractionation of common carp (*Cyprinus carpio*).

Lane Number	Relative Front	Mol. Wt. KDa	Average optical density								
			Control		Ochratoxin A		Tri star		Mycoband		Mould
1	0.01	122.37	25.473	17.252	11.361	21.296	26.845	24.277	26.467	17.093	17.66
2	0.059	118.169			10.378	44.305	9.03	58.21		54.377	
3	0.089	115.674			15.452	41.825	7.1	49.396	10.852	32.627	14.872
4	0.135	111.992				29.081	13.574	43.929		38.768	
5	0.156	110.178	3.828	28.582	29.008	17.33	28.045	48.7	22.26	22.763	29.047
6	0.188	107.765	8.133	37.231	27.639	17.838	36.812	39.901	39.405	14.686	23.527
7	0.24	103.778	6.892	37.827	33.862	3.491	21.584	24.674		6.659	32.491
8	0.281	100.862	19.297	31.221		9.645	16.959	16.346	43.666	9.701	40.027
9	0.31	98.507	13.607	25.093	31.084	9.404		10.16			
10	0.337	96.931				12.353		8.469		12.85	
11	0.377	94.256	30.667	18.332	34.158	12.246	22.226	17.068	41.718	7.6	37.534
12	0.401	87.235	19.637	17.096		7.19		9.87		9.11	
13	0.433	77.473	37.952	8.853	37.289	4.866	37.014	8.548	44.881	11.515	37.132
14	0.487	63.884	34.968	4.257	29.703	3.774	34.766	10.165	48.665	7.254	30.295
15	0.507	58.829				9.191		6.799			
16	0.539	52.372	22.03	3.043	25.145	72.629	31.172	65.225	38.252	12.502	17.583
17	0.611	40.31	8.773	45.359	17.395	105.791	29.023	110.607	25.969	64.573	13.932
18	0.66	33.216	6.605	59.078	13.628	95.987	16.716	97.486	9.67	109.451	9.504
19	0.7	27.699	9.362	47.804	6.51	55.647	11.69	69.522	11.508	94.377	
20	0.719	25.58				56.845	8.151	53.507	12.401	61.616	
21	0.774	19.895			65.269	20.147	5.879	17.944	10.405	53.157	
22	0.824	15.779	19.961	29.003		14.385	7.844	9.736	9.864	11.129	9.274
23	0.88	12.263	71.405	13.685	50.268	7.507	81.699	5.151	81.729	8.274	9.056
24	0.904	11.007			29.469	22.041					
25	0.93	9.754	57.581	22.338	27.725	33.598	36.554	30.119	46.06	24.036	
26	0.96	8.52	25.897	13.486		2.341		32.671	31.151	26.344	
27	0.995	7.299				33.159	15.954	30.994	18.189	33.308	64.232

Ochratoxin A = Control + Ochratoxin A (7 mg/ kg diet)
 Tri star = Control + Ochratoxin A (7 mg/ kg diet)+ Tri star
 mycoband = Control + Ochratoxin A (7 mg/ kg diet)+ mycoband
 Mould = Control + Ochratoxin A (7 mg/ kg diet)+ mold stop super

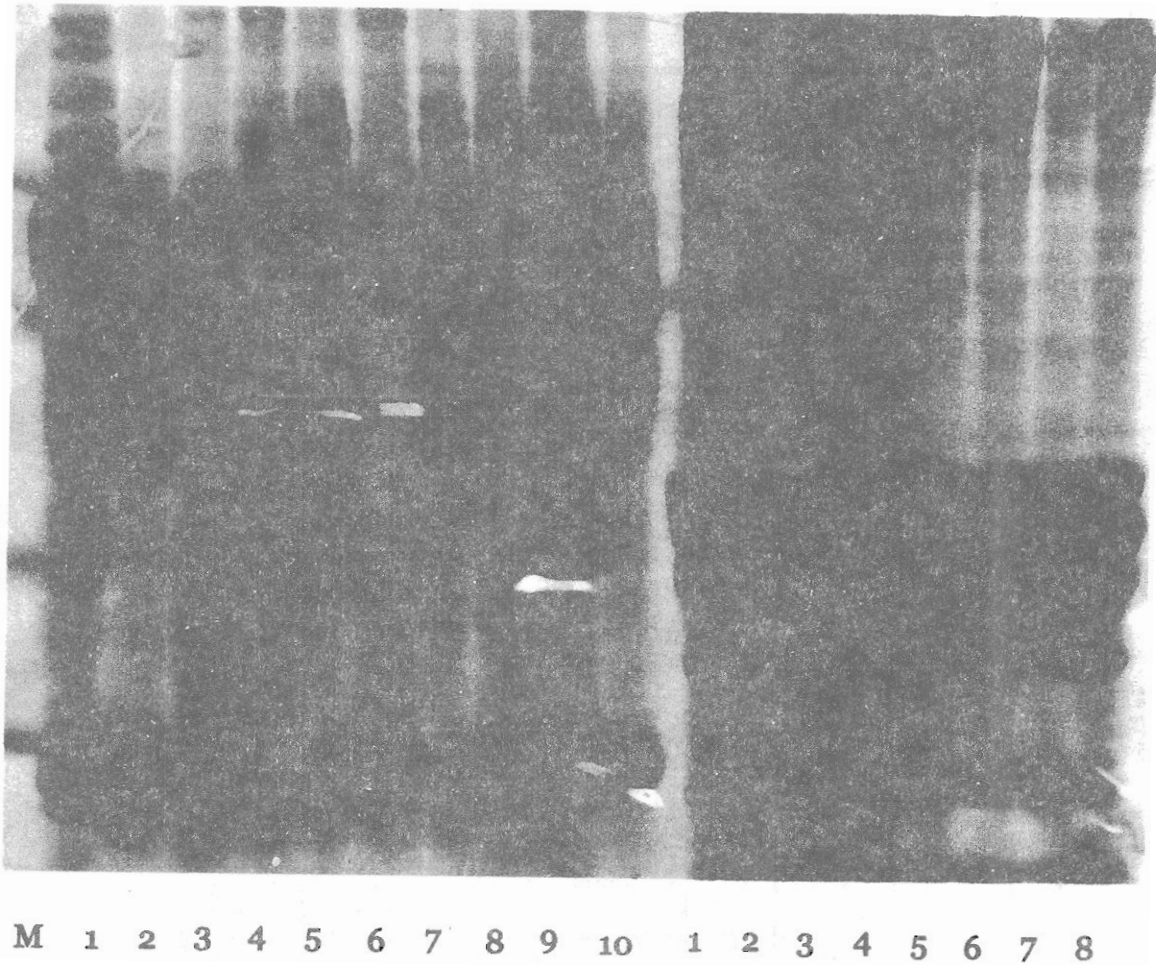


Fig.1. Variation in electrophoretic patterns for plasma protein of *Oreochromis niloticus* and *cyprinus carpio*.

M -Marker

1-Control of *Oreochromis niloticus*

2- Control of *Cyprinus carpio*

3- *Oreochromis niloticus* fed on contaminated diet.

4- *Oreochromis niloticus* fed on contaminated diet plus tri star.

5- *Cyprinus carpio* fed on contaminated diet.

6- *Oreochromis niloticus* fed on contaminated diet plus mycoband.

7- *Cyprinus carpio* fed on contaminated diet plus tri star.

8- *Cyprinus carpio* fed on contaminated diet plus mycoband.

9- *O. niloticus* fed on contaminated diet plus Mould stop super.

10- *Cyprinus carpio* fed on contaminated diet plus Mould stop super.

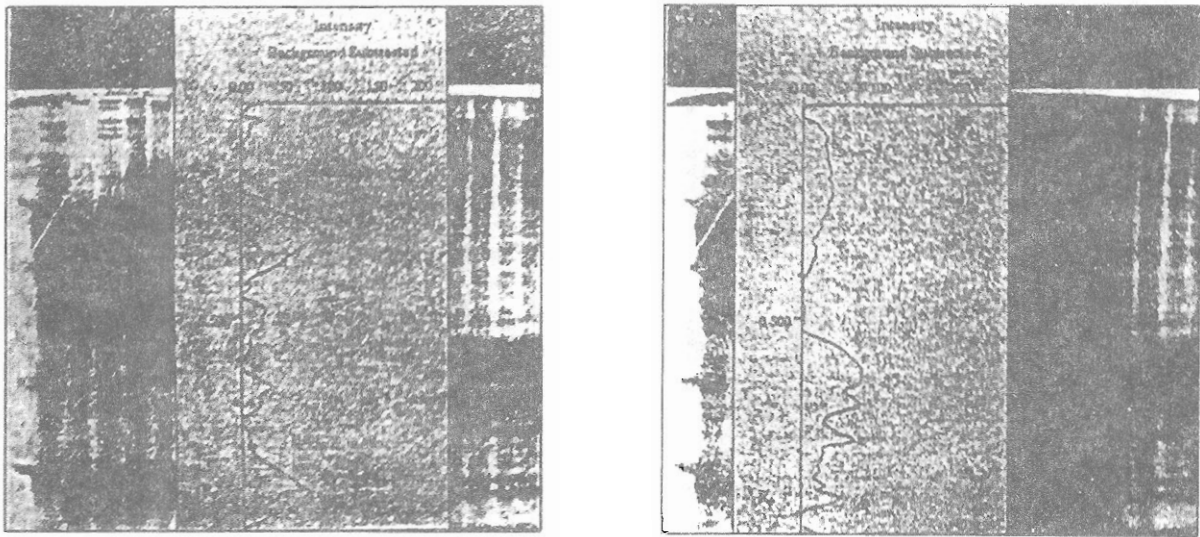


Fig. 2. SDS- PAGE Patterns of control *Oreochromis niloticus*

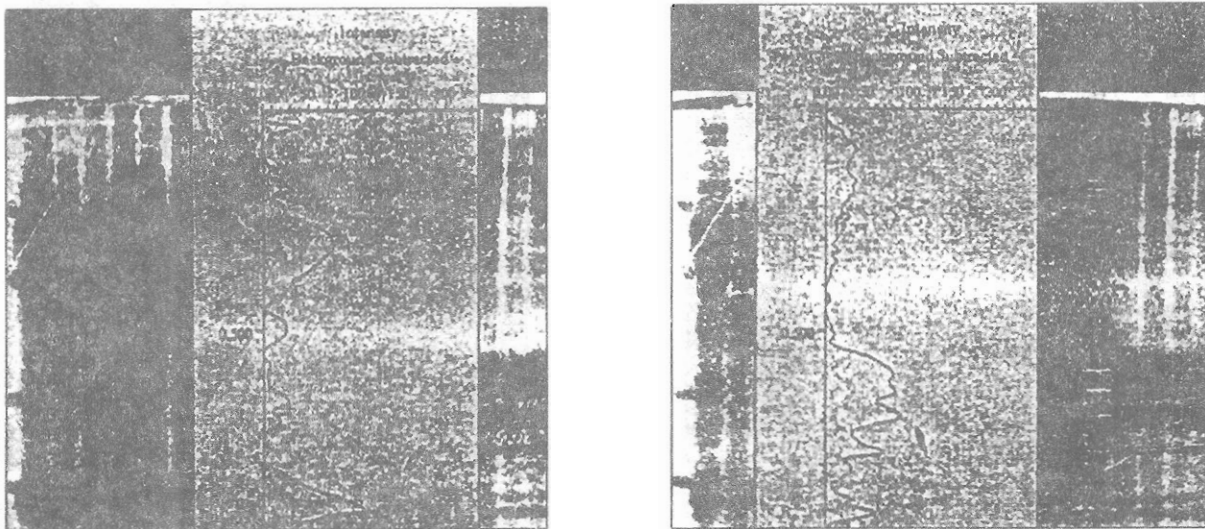
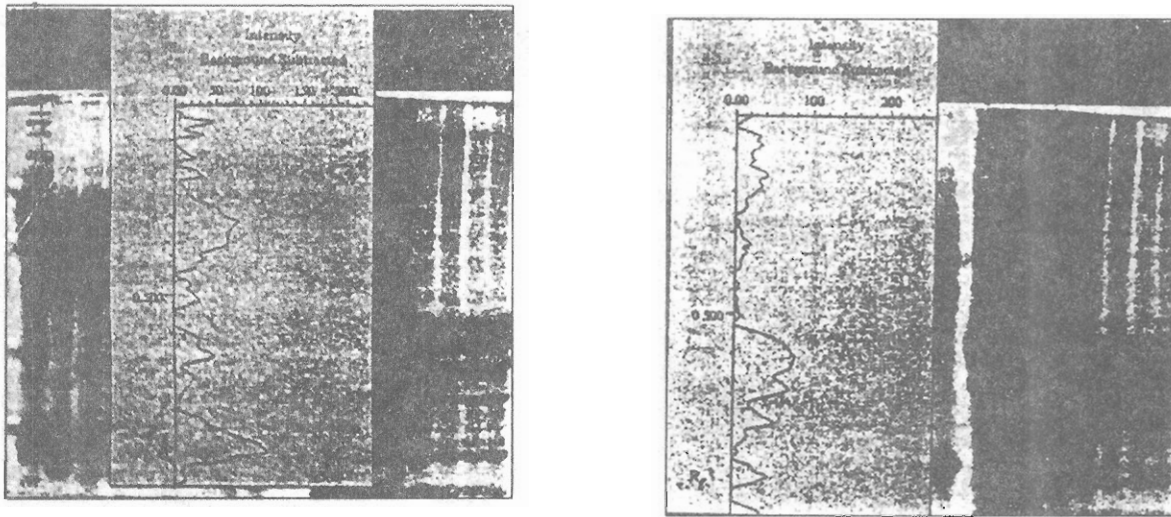
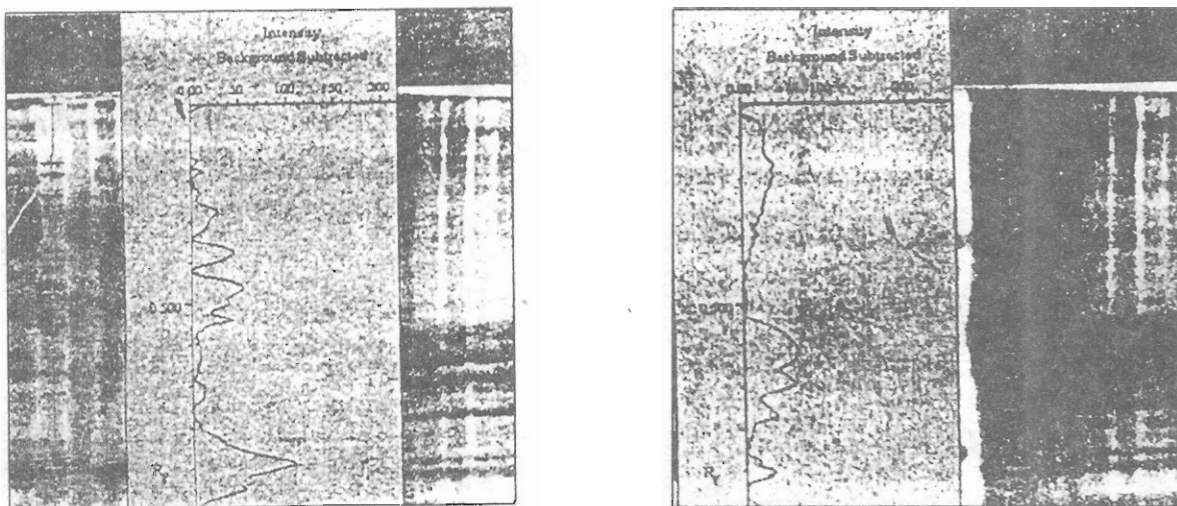


Fig. 3. SDS- PAGE patterns of control *Cyprinus carpio*

Fig.4. SDS-PAGE Patterns of *Oreochromis niloticus* fed on contaminated dietFig. 5. SDS-PAGE patterns of *Oreochromis niloticus* fed on contaminated diet plus tri star

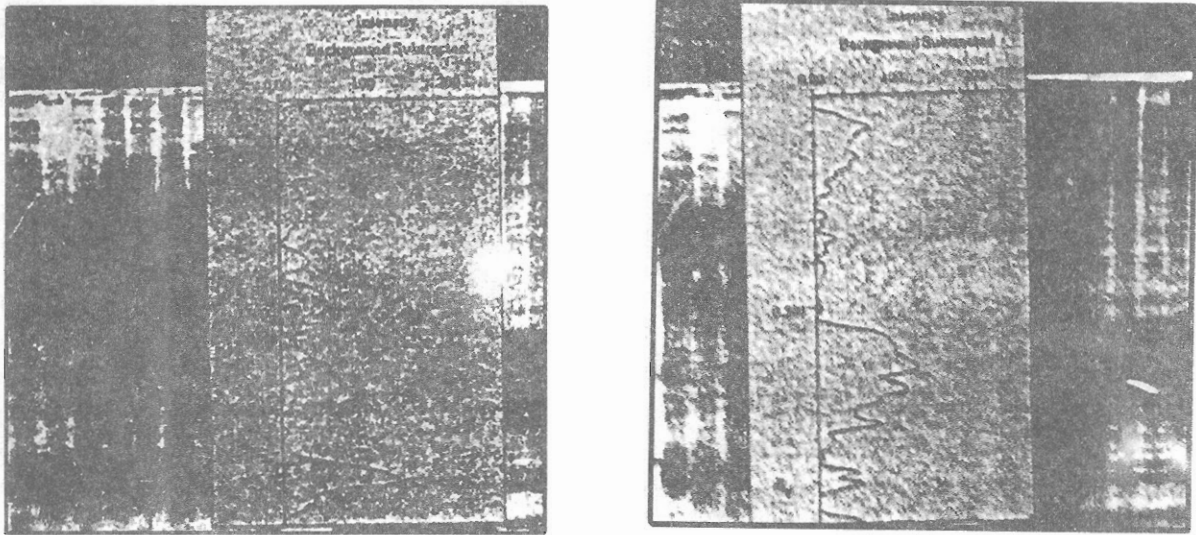


Fig. 6. SDS-PAGE patterns of *Oreochromis niloticus* fed on contaminated diet.

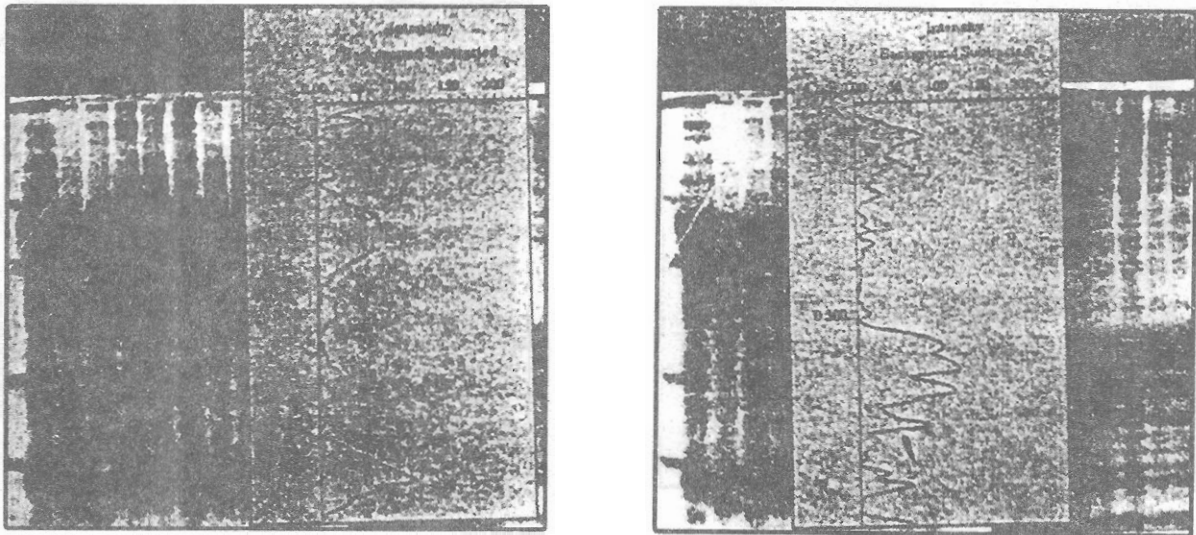


Fig.7. SDS-PAGE patterns of *Oreochromis niloticus* fed on contaminated diet plus mycoband.

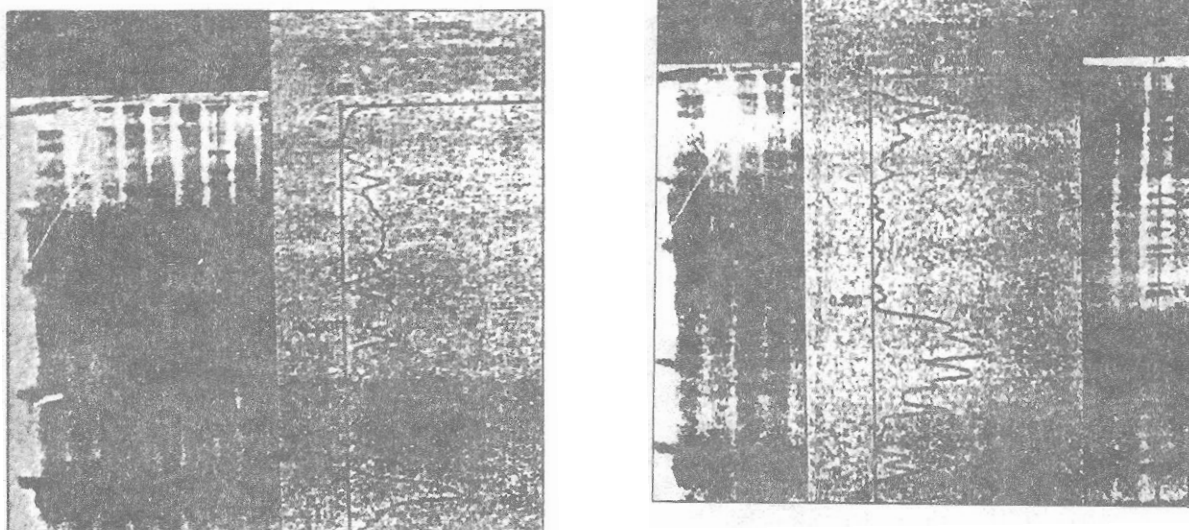


Fig.8. SDS-PAGE patterns of *Cyprinus carpio* fed on contaminated diet plus tri star.

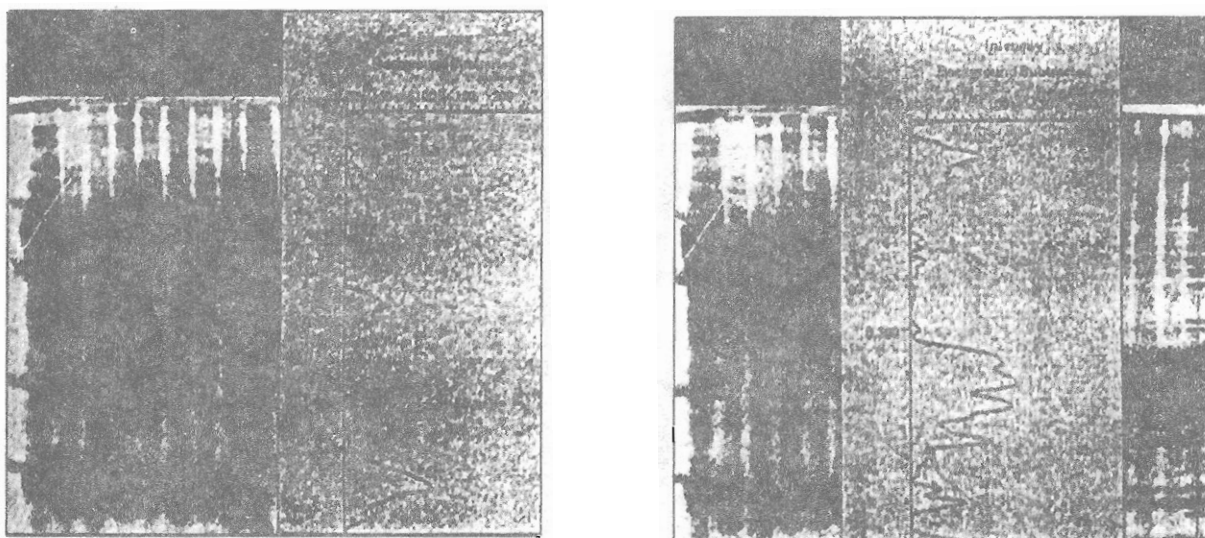


Fig.9. SDS-PAGE patterns of *Cyprinus carpio* fed on contaminated diet plus mycoband

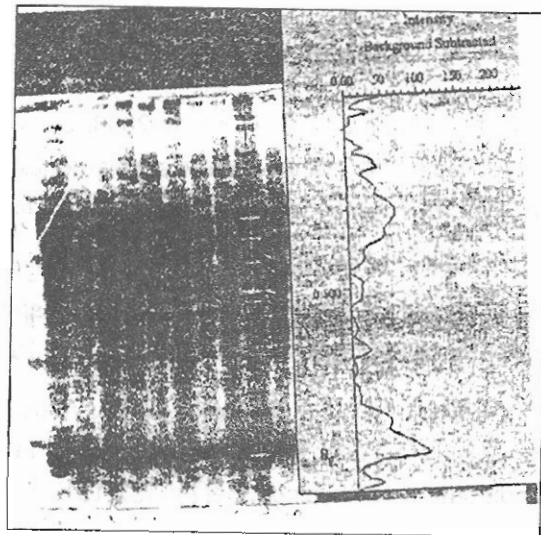


Fig.10. SDS-PAGE patterns of *Cyprinus carpio* fed on contaminated diet plus Mould Stop Super.

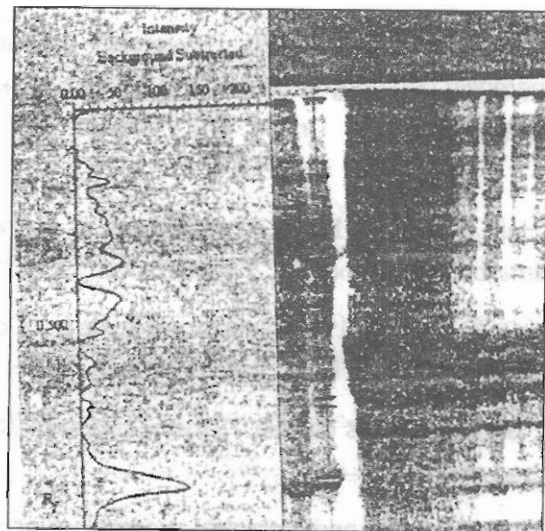


Fig.11. SDS-PAGE patterns of *Cyprinus carpio* fed on contaminated diet plus Mould Stop Super.

REFERENCES

1. Abdelhamid, A. M., A. I. Mehrim and F. F. Khalil. 2003. Detoxification of aflatoxin-contamination diet of Tilapia fish using dietary supplementation with egg shell, petafin, clay or silica. Proc 1st Egypt. –Syrian Con., 8-11 Dec., Minia.
2. Bacon, C. W., J. G. Sweeny, J. D. Robbins and D. Burdick. 1973. Production of penicillic acid and ochratoxin A on poultry feed by *Aspergillus Ochraceus*: Temperature and moisture requirements. Appl. Microbiol., 26: 155-160.
3. Bugaev, V. F., L. Bazarkina and V. A. Dubynin. 1994. Annual variation in scale growth in groups of sockeye salmon; *Onchorhynchus nerka*, in relation to feeding and temperature conditions. J. Ichthyology, 34(1): 117-131.
4. Devegowda, G., M. V. LN., Raju, N. Afzali and H. V. L. N. Swamy. 1998. Mycotoxins picture worldwide: Novel solutions for their counteraction in Lyons, T.P. and Jacques, K.A. (Ed3.) Biotechnology in the feed industry PP. 241-255. Proc.. of Alltech's 14th . Annual Symoisum Nottingham, U.K..
5. Elghobashy, H. M. A. 2004. Phylogenetic relationship and growth performance of different populations of Nile *Tilapia (Oreochromis niloticus)* under polyculture system. J. Egypt. Acad. Soc. Environ. Develop., (C-Molecular Biology). 5 (1): 11-30.
6. Frisvad, J. C. and R. A. Samson. 1991. Mycotoxin produced in species of *Penicillium* and *Aspergillus* occurring in cereals. In: J. Chelkowski (Ed) Cereal Grain Mycotoxin, Fungi and Quality in Drying and Storage. Pp. 441-476 Elsevier, Amesterdam.
7. Hagelberg, S., K. Hult, R. Fuchs. 1989. Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. J Appl Toxicol., 9(2):91-6.
8. Jantrarotai, W. and R. T. Lovell. 1990. Subchronic toxicity of dietary aflatoxin B1 to channel catfish. J. Aquatic Anim. Hlth., 2: 248-254.
9. Kamel, E. A. 1999. Genetic studies on Nile *Tilapia (Oreochromis niloticus)* in Egypt. Ph. D., Ain Shams University.
10. Krogh, P. 1983. Microbial nature and biological property of ochratoxins. Proc. Int. Symp., Mycotoxins, pp. 81-86.
11. Park, T.W., C. I. Kim and V. G. Stanley. 1996. Effect of dietary aflatoxin and Bio-Mos on cholesterol and basic nutrient content of broiler chicken meat. Annual Meeting of the Institute of food Technology, Orleans. 22-26 June.

12. Rashed, M. A., S. A. Ibrahim, A. A. El-Seoudy, F. M. Abdel-Tawab and E.E. Ahmed. 1992. Effect of pollutant with organophosphorous insecticide Tamaron on muscle protein electrophoresis to some Tilapia species. Egypt. J. App. Sci., 7(11): 498-510.
13. Rizkalla, E. H., A. A. El-Gamal, A. A. Mahmoud, A. S. Shawky and A. A. Ramadan amadan. 1997. Effect of some Pesticides on the sarcoplasmic protein fractionation of common carp (*Cyprinus carpio*). Egypt. J. Agric. Res., 75(1): 225-245.
14. Savage, T. F., P. F Cotter and E. I. Zakrzewska. 1996. The effect of feeding mannanoligosaccharide on immunoglobulins, plasma IgG and bile IgA of Wrolstad MW male turkeys. Poult. Sci., 75 (1): 129.
15. Shehata, S.A., M. S. Mohamed and G. A. Mohamed. 2003. Reducing the toxicity of aflatoxin B1 by different adsorbents in fish. J. Agric. Sci., Mansoura Univ., 28(10): 7157-7167.
16. Snedecor, G.W. and W. G. Cochran. 1976. Statistical Methods 6th. Edition, Iowa State Univ. Press, Ames, Iowa, 161-166.
17. Studier, F.W. 1973. Analysis of bacteriophage Tz early RNAs and proteins on slab gels. J. Mol. Biol., (79): 237.
18. Tetushia, G. 1990. Mycotoxins currency situation. Food Reviews International, 6(2): 265-290.
19. Wang, JS. and JD. Groopman. 1999. DNA damage by mucotoxins. Mutat Res., 8 424 (1-2): 167-181.
20. World Health Organization. 1979. Environmental Health Criteria 11, Mycotoxins, Geneva, 86-98

تأثير الأكراتوكسين وبعض المواد ضد السممية على النمو وبروتين الدم لأسماك البطى النيلى والمبروك العادى

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المعمل المركزى لبحوث الثروة السمكية بالعباسة - مركز البحوث الزراعية - وزارة الزراعة
- الدقى - جيزة - مصر

أجرى هذا البحث لدراسة التأثير الوقائى لمضادات السممية (ترائى أستار والميكوباند وسوبر أسطوب) بمعدل ٠,٥ % من العليقة ضد التسمم الناتج من مادة الأكراتوكسين المستخلصة من فطر (*Aspergillus*) بمعدل ٧ ملغ جرام / كيلو جرام غليقة على النمو وكذلك التحليل الكهربى لبروتين بلازما سمكة البطى النيلى والمبروك العادى .

أخذت مجموعتان من أسماك البطى النيلى والمبروك العادى فى حالة صحية جيدة من المزرعة الإنتاجية بالمعمل المركزى لبحوث الثروة السمكية بالعباسة ، تضم المجموعة الأولى خمس مجموعات من البطى النيلى متوسط وزن ٥٠ - ٦٠ جرام والمجموعة الثانية من أصبعيات المبروك العادى تضم خمس مجموعات متوسط الوزن ٩ - ١١ جرام . وأوضحت النتائج نقص معدلات النمو فى البطى والمبروك المغذى على الأكراتوكسين وبإضافة مضادات السممية إنخفض تأثير الأكراتوكسين وازادت معدلات النمو وهذا لزيادة المناعة عند الأسماك . وأوضحت نتائج التحليل الكهربى أن عدد الأوزان الجزيئية لبروتين البطى النيلى ٢٢ - ٢٤ والمبروك العادى فى المجموعة الضابطة . والتأثير السمى الوراثى للأكراتوكسين واضح فى إختفاء وظهور أوزان جزيئية جديدة فى البطى النيلى والمبروك وهذا يرجع لظهور بعض أوزان جزيئية للبلازما بروتين جديدة المقاومة للسممية . وبإضافة بعض المواد ضد السممية للعليقة المضافة إليها الأكراتوكسين ظهرت بعض أوزان جزيئية مره أخرى مثل المجموعة الضابطة فى البطى النيلى والمبروك العادى ويوضح التأثير المقاوم إلى بعض المواد ضد السممية وتحسين معدلات النمو والتحليل الكهربى بإضافة بعض المواد ضد السممية لعلائق الأسماك .