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REDUCTION OF THE DIETARY TOXICITY OF T_{-2} TOXIN AND DIACETOXYSCIRPENOL (DAS) BY GARLIC IN FISH

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ABSTRACT

Nine experimental groups in a 3 X 3 factorial design were used to evaluate the efficiency of garlic in detoxification of T_{-2} toxin and DAS in fish diets. The experimental groups were: Control (commercial diet); 2% garlic; 4% garlic; T_{-2} toxin (4 mg /Kg diet); T_{-2} toxin plus 2% garlic; T_{-2} toxin plus 4% garlic; diacetoxyscirpenol (DAS) (10 mg/kg diet); DAS plus 2% garlic; DAS plus 4% garlic. There were 3 replicate aquariums of 10 fish Nile tilapia (*Oreochromis niloticus*) per aquarium for each experimental group. The fish were maintained on the tested diets for 3 weeks and fed at a rate of 2% of the total body weight. T_{-2} toxin and DAS had bad effect on the biological performance of fish. It caused loss ($P \leq 0.05$) in live body weight; increase in mortality rate ($P \leq 0.01$); reduction ($P \leq 0.01$) in values of hemoglobin, hematocrite, total protein and albumin and increase ($P \leq 0.05$) in activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Furthermore, the tissues of body organs (liver: kidney and spleen) suffered from these toxic effects, since liver and kidney showed sever destruction; focal coagulative necrosis or hydropic degeneration beside inactivation of hepatopancrease. Hemorrhages, congested sinusoids or ellipsoids were seen in spleen. Adding garlic to the contaminated diets reduced the toxic effect of the two toxins on growth performance; mortality rate; blood parameters and the histological structure of the tested tissues. Most of results indicated that addition of 2% garlic showed higher improvement than that of 4%.

INTRODUCTION

T_{-2} toxin and diacetoxyscirpenol (DAS) are mycotoxins produced as secondary metabolites by *F. sporotrichioides* and *F. semitectum*, respectively (*Ueno, 1987*). T_{-2} toxin and DAS belonging to trichothecenes. More than 40 naturally occurring trichothecenes have been identified, the most notable with regard to animals are T_{-2} toxin, DAS and vomitoxin (*Cheeke and Shull, 1985*). In Egypt, the fungi isolated from corn grains produced T_{-2} toxin and DAS (*El-Maghraby et al., 1995*), also, vomitoxin and DAS was naturally found in feed stuffs (Abdelhamid, 1983&1990, respectively). However, during the winter season in India; Pakistan; Egypt and South Africa, the high moisture conditions may result in producing T_{-2} toxin; zearalenone; vomitoxin; ochratoxin, etc. (*Devegowda et al, 1998*). Several diseases of farm animals and humans are attributed to T_{-2} toxin; DAS and other trichothecenes (*Fekete and Huszenicza, 1993*). Usually farmed fish have an opportunity to eat moldy fed. Where fish are reared intensively with commercial fish feed, there is a

chance that mycotoxins will contaminate fish feed (*Hintikka, 1989 and Abdelhamid et al., 1988*).

Trichothecenes cause (in most animal species) nausea; vomiting; feed refusal; inflammation; epithelial necrosis; diarrhea; abortion; hemorrhage; hematological changes; pervious disturbance and depletion of lymphoid cells in thymus; spleen and bone marrow (Immunological disorders) (*Cheeke and Shull, 1985 and Ueno, 1987*).

The adsorbents fed with mycotoxin contaminated diets reduced its bioavability and thereby reduced its effects in animals. The major advantages of the adsorbents include low cost, safety and easy addition to animal feeds. But the problem that most of adsorbents had little or without effect on adsorption of trichothecenes (*Devegowda, et al. 1998 and Shehata, 2002*). Garlic (*Allium Sativum*) has been grown widely in many countries. In Egyptian, Indian and Chinese civilization was used as flavoring agents, food and folk medicine (*Mohamed et al. 2000*). Garlic is know to have a broad-spectrum antibacterial; antifungal; antiprotozoal and nematicidal activities, as well as pesticidal action against a variety of species (*Ali et al., 2000*). Garlic contains S-allyl cysteine and S-allyl mercapto cysteine, which play a role in increasing both glutathione S-transferase and peroxidase in cells. Glutathione S-transferase is critical for detoxification and gene expression. For this reason, garlic had a beneficial effect in prevention the carcinogenicity and mutagenicity of aflatoxin (*Yamasak, et al., 1991 and El-Mofty, et al., 1994*). Also, garlic has protective effect against immunotoxicity. Most of trichothecenes had high ability in immunotoxicity and inhibition of protein synthesis.

The aim of the present work was to study the effect of crude dietary garlic on detoxification of $T\text{-}_2$ toxin and DAS in fish feed.

MATERIALS AND METHODS

The experimental work was carried out in the Aquaculture Research Lab., Abbassa, Abo-Hamad, Egypt. Nine experimental groups in a 3 x 3 factorial design were used to evaluate the efficiency of garlic in reducing the toxicity of $T\text{-}_2$ and DAS in fish diets (Table 1). Fresh minced garlic that purchased from market, Egypt, was added to a ground commercial diet, which was pelleted again. The chemical analysis of the commercial diet was adopted according to A.O.A.C. (1980) as shown in Table 2. The $T\text{-}_2$ crystalline toxin was dissolved in a 1:1 (v / v) mixture of methanol and sodium chloride (0.9 %) and sprayed on the pelleted diet to obtain 4 mg $T\text{-}_2$ toxin / kg diet. The same method was used for diacetoxyscirpenol (DAS) to obtain 10 mg DAS / kg diet as shown in Table 1. Standard of $T\text{-}_2$ toxin and DAS was purchased from Sigma Chemical Company, USA. For each of nine treatments, there were 3 replicate glass aquaria of 10 fish Nile tilapia (*oreochromis niloticus*) per aquarium for a total of 270 fish of mean live body weight 37.65$\pm$0.04 grams. The dimension of each aquarium was 150 x 50 x 50 cm, these aquariums were supplied with dechlorinated tap water and continue aeration was adapted by using an air pump and airstones. Sediment was filtered by siphon method each day and the rearing water was

completely changed every 3 days. Mean temperature degree of water was 22.0±2.0C°. The fish were fed 2 times a day (900 and 1600 h.) at a rate of 2% of the total body weight as recommended by Parrel et al. (1986).

The fish were weighted weekly for 3 weeks. At the end of the experiment, 6 fish from each treatment (2 fish / replicate) were sacrificed for collection of the blood and organs. Blood samples with or without EDTA were taken from the caudal vein using sterilized syringe. Blood samples were collected without EDTA and centrefuged at 3000 rpm for 15 minutes. Serum was separated and stored at –20 C° to analysis. The hemoglobin and hematocrite values were determined by the methods of *Frankel and Reitman (1963)* and *Strumia (1954)*, respectively. Serum was analyzed for total protein, albumin, aspartate aminotransferase (*AST*) and alanine aminotransferase (*ALT*) by using commercial kits purchased from Diamond Diagnostics Company, Egypt.

The internal organs (liver; kidney and spleen) were removed from the body and subjected to the clinical examination and kept in formalin solution (10%) for histological study. After fixation, the specimens were dehydrated; cleared; embedded in praffin wax and blocked. Sections of 6 microns thickens were cut using a rotary microtome, mounted and stained by Haematoxylin and Eosin (*Carleton et al., 1980*).

The data were statistically analyzed by the *ANOVA* as described by *Snedecor and Cochran (1967)*, as 3 x 3 factorial treatment arrangement. Means were tested for differences using Duncan's multiple range test (*Duncan, 1955*).

Table (1): The experimental design.

No.	Treatments		
	T_2 toxin	DAS	Garlic
1	0	0	0
2	0	0	2%
3	0	0	4%
4	4 mg/kg diet	0	0
5	4 mg/kg diet	0	2%
6	4 mg/kg diet	0	4%
7	0	10 mg/kg diet	0
8	0	10 mg/kg diet	2%
9	0	10 mg/kg diet	4%

Table (2): Chemical composition (%) of the commercial control diet.

Items	Proximate analysis						
	DM	OM	CP	CF	EE	NFE	Ash
As fed	92.92	74.76	29.14	6.85	3.47	35.30	18.16
On dry matter basis	100	80.46	31.36	7.37	3.73	38.00	19.54

RESULTS AND DISCUSSION

Effects of T_2 toxin; DAS; garlic and their combinations in fish supplementation on:

1. Growth performance:

Data presented in Table (3) show that, T_2 toxin and DAS had bad effects ($P \leq 0.05$) on the growth performance (live body weight, body weight

gain, relative growth rate). Since it caused loss in live body weight. These results agree with the findings of *Poston et al., (1982)* who reported that $T-_2$ toxin at levels of 1; 2.5; 5; 10 and 15 mg/kg diet in rainbow trout fingerlings caused a clear growth depressing effect which was significant for levels above 5 mg/kg. The decrease in growth may be due to the potent inhibition of protein synthesis in eucaryotic cells of trichothecenes ($T-_2$; DAS and others) treated fish (*Cheeke and Shull, 1985*). Also, it might be due to depressed efficiency of feed use as a result for expelled the feed from the mouth of fish (*Poston et al., 1982*).

Adding garlic to contaminated diets reduced the toxic effect of the two toxins. However, using 2% garlic was better than 4% for the growth performance in fish. The beneficial effect of garlic may be due to it's content of vitamins; menerals and essential amino and fatty acids (Kamanna and Chandrasekhara, 1980), also garlic has thyroid like activity that suggest to stimulate growth (El-Nawawi, 1991). The present results agreed with those obtained by *Horton et al., (1991)* and *El-Kaiaty et al, (2002)*. They found that garlic increased daily body weight gain in broiler chicks and layer hens, respectively. These results for garlic may be due to it's constituents that have protective effects against materials which induced immunotoxicity and inhibit protein synthesis such as trichothecenes (*Ueno, 1987*). Also, garlic had a beneficial effect for inhibition of carcinogenicity and immunotoxicity effects of aflatoxin (*Yamasaki et al., 1991 and El-Mofty et al., 1994*). Yet, Abdelhamid et al., (2002 a&b) did not found any positive effect of garlic on aflatoxic fish and rat, respectively. The lower growth values of 4% garlic versus 2% may be due to the depression effect of feed intake for the high level of garlic.

2 Mortality rate (%):

Results presented in Table (3) show that mortality rate was increased significantly ($P \leq 0.05$) in fish fed contaminated diets (11.11 and 32.22% for $T-_2$ toxin and DAS, respectively in comparison with 3.33% for the control). These results agreed with the findings reported by *Poston et al., (1982)* who mentioned that $T-_2$ toxin at levels of 10 and 15mg/kg diets increased significantly the mortality rate in rainbow trout fingerlings. Also, similar trends for DAS effect on mortality rate was found by *Marasas et al., (1967)* who found that mortality rate in rainbow trout fed diet contaminated by 4 mg/kg DAS was 16% in the 12 days of treatment versus 32.2% in the present study at 21 days with 10 mg/kg DAS. The incidence of death may be due to the disturbance of organs function, since the treatment of mycotoxins caused accumulation fluid in the abdominal cavity (ascites); hemorrhagic enteritis; focal hemorrhages in muscles; enlargement of gall bladder and spleen; hematopioetic necrosis and necrosis of the epithelium; gastric glands and primary lamellae of the gills (*Poston et al. 1982 and Koski, 1985*). Using 2 and 4% garlic reduced the effect of mycotoxins on mortality rate. Since, it reduced to 11.11 and 14.44% for $T-_2$ and DAS, respectively, versus 21.11% in the zero garlic group. The ability of garlic to decrease the mortality rate may be due to its content of some constituents that stimulate the immunity system.

Table (3): Effect of T_{-2} toxin; diacetoxyscripenal (DAS); garlic and their interaction on fish performance.

Items	Live body weight (g)				Weekly body weight gain (g)			Relative growth rate (RGR) (%)			Mortality rate (%)
	Initial	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	
Toxin effect:	ns	**	**	**	**	**	*	**	**	**	**
Control	37.60±0.08	38.73±0.11^{a}	39.90±0.16^{a}	41.37±0.22^{a}	1.13±0.04^{a}	1.17±0.05^{a}	1.47±0.11^{a}	3.06±0.10^{a}	2.94±0.14^{a}	3.68±0.28^{a}	3.33±1.67^{c}
T-z toxin	37.63±0.06	35.65±0.17^{b}	34.54±0.29^{b}	32.21±1.20^{b}	-1.97±0.18^{b}	-1.11±0.27^{b}	-1.27±1.11^{b}	-5.25±0.48^{b}	-3.11±0.62^{b}	-3.68±3.24^{b}	11.11±2.0^{b}
DAS	37.71±0.05	35.70±0.86^{b}	33.75±0.72^{b}	32.21±1.07^{b}	-2.01±0.88^{b}	-1.95±0.39^{b}	-1.54±0.59^{b}	-7.08±1.92^{b}	-4.62±1.08^{b}	-3.62±1.82^{b}	32.22±4.01^{a}
Garlic (G) effect:	ns	*	*	*	**	*	*	*	*	**	**
0.0	37.65±0.10	36.25±0.92^{b}	35.04±1.28^{b}	33.06±2.10^{b}	-1.39±1.01^{b}	-1.22±0.49^{b}	-1.98 ±1.10^{b}	-5.39±2.68^{c}	-1.63±1.35^{b}	-5.65±3.16^{b}	21.11±6.33^{a}
2%	37.69±0.05	37.37±0.49^{a}	37.19±0.72^{a}	37.47±1.05^{a}	-0.26±0.53^{a}	-0.24±0.45^{a}	0.39±0.30^{a}	-0.69±1.30^{a}	-0.64±1.15^{a}	-1.05±0.98^{a}	11.11±3.51^{b}
4%	37.61±0.03	36.40±0.58^{b}	35.96±1.08^{b}	35.76±1.43^{a}	-1.20±0.59^{ab}	-0.44±0.53^{a}	0.20±0.50^{a}	-3.22±1.57^{b}	-1.57±1.46^{b}	-1.09±1.72^{a}	14.44±4.44^{b}
Interaction:	ns	**	**	*	**	*	*	**	*	*	**
Control × 0.0G.	37.69±0.25	38.69±0.34^{a}	39.88±0.51^{a}	41.04±0.63^{a}	1.00±0.09^{a}	1.19±0.16^{a}	1.16±0.12^{a}	2.65±0.21^{a}	3.08±0.04^{a}	2.91±0.27^{a}	3.33±3.34^{e}
Control × 2%G.	37.56±0.11	38.81±0.11^{a}	40.00±0.07^{a}	41.69±0.18^{a}	1.25±0.01^{a}	0.19±0.03^{a}	1.69±0.25^{a}	3.33±0.01^{a}	3.07±0.10^{a}	4.23±0.64^{a}	3.33±3.34^{e}
Control × 4%G.	37.56±0.04	38.69±0.11^{a}	39.81±0.18^{a}	41.38±0.22^{a}	1.13±0.07^{a}	1.12±0.07^{a}	1.57±0.03^{a}	3.01±0.19^{a}	2.89±0.18^{a}	3.94±0.07^{a}	3.33±3.34^{e}
T_{-2} × 0.0 G.	37.44±0.03	35.97±0.26^{bc}	33.96±0.29^{c}	29.31±2.20^{c}	-1.47±0.23^{c}	-2.01±0.04^{c}	-4.65±0.24^{c}	-3.93±0.61^{c}	-5.59±0.06^{c}	-13.69±7.04^{e}	16.67±3.34^{c}
T_{-2} × 2% G.	37.81±0.04	35.88±0.01^{c}	35.56±0.37^{b}	35.40±0.29^{b}	-1.93±0.03^{c}	-0.32±0.03^{b}	-0.16±0.02^{b}	-5.10±0.06^{c}	-0.89±0.26^{b}	0.45±0.57^{b}	6.67±3.34^{d}
T_{-2} × 4% G.	37.63±0.07	35.11±0.20^{c}	34.10±0.01^{c}	33.41±0.51^{b}	-2.52±0.27^{c}	-1.01±0.20^{bc}	-0.69±0.05^{b}	-6.70±0.70^{c}	-2.88±0.66^{bc}	2.02±1.51^{b}	10.00±0.00^{c}
DAS × 0.0 G.	37.81±0.11	34.10±0.64^{d}	31.27±0.02^{d}	28.83±0.01^{c}	-3.71±0.67^{d}	-2.83±0.06^{c}	-2.44±0.01^{c}	-9.81± 1.76^{d}	-8.30±1.97^{d}	-7.8±0.01^{d}	43.33± 6.67^{a}
DAS × 2% G.	37.69±0.03	37.60±0.85^{ab}	36.02±0.26^{b}	35.31±0.62^{b}	-0.09±0.01^{b}	-1.58±0.06^{c}	-0.71±0.04^{b}	-0.24±1.08^{b}	-4.20±1.49^{bc}	-1.97±1.02^{c}	23.33±3.34^{b}
DAS × 4% G.	37.63±0.07	35.41±0.13^{c}	33.97±0.61^{c}	32.49±1.68^{b}	-2.22±0.20^{c}	-1.44±0.07^{c}	-1.48±0.17^{b}	-5.90±0.52^{c}	-4.07±2.24^{c}	-4.36±5.10^{c}	30.00±5.78^{ab}

Means in the same column bearing different letters differ significantly (P ≤ 0.05 or 0.01).
ns not significant at P ≤ 0.05.
RGR = (final live body weight – initial live body weight) / initial live body weight x 100.

3. Blood parameters:

Hemoglobin (g/dl) and hematocrite (%) values that presented in Table (4) were significantly ($P \leq 0.05$) decreased due to the effect of $T\text{-}_2$ toxin and DAS. These findings agreed with those reported by *Poston et al., (1982)*. This may be attributed to hemorrhagic diathesis associated with defective blood coagulation; disturbance in organs function (liver and spleen) and probably the inhibitory action of trichothecenes on protein synthesis (*Cheeke and Shull, 1985*).

Addition of garlic to the contaminated diets caused an increase in hemoglobin and hematocrite values, however, this increase was significant ($P \leq 0.01$) for hemoglobin only. These results agree with those obtained by *Horton et al., (1991)*, who reported that 0.1% dried garlic increased (not significantly) the hemoglobin and hematocrite values. Garlic had some constituents, which may play a role in stimulating the immunity system and function of organs related to blood cells formation such as thymus, spleen and bone marrow (*Jeong and Lee, 1998*). Furthermore, Ali et al., (2000) reported that garlic could increase the total number of leukocytes; heterophils; and basophils cells.

Some chemical constituents of blood are shown in Table 4. Total protein and albumin concentrations were decreased significantly ($P \leq 0.05$) due to the toxins effect. The decrease in serum protein and albumin may be attributed to the inhibition of protein synthesis caused by trichothecenes in eucaryotic cells. Since some of these mycotoxins inhibit peptidyl transferase and others causing breakdown of polyribosomes, thereby impairing protein synthesis (*Cheeke and Shull, 1985*). The activities of AST and ALT enzymes were increased significantly ($P \leq 0.05$) by feeding $T\text{-}_2$ and DAS contaminated diets.

Fish groups fed 2 or 4 % garlic had higher values of total protein and albumin versus those of the control group. Also, activities of AST and ALT enzymes were improved in garlic groups.

4. Clinical signs of $T\text{-}_2$ and DAS toxicity:

The fish fed $T\text{-}_2$ or DAS contaminated diets were collected at the bottom of the aquarium and lost their interest for eating. Necropsy revealed hemorrhagic enteritis, focal hemorrhages in muscles, enlargement of gall bladder and spleen. These results agreed with those reported by Poston et al. (1982).

5. Histopathological examination:

The results of histological examination reveled that, the control group were in normal state for the examined organs (liver; kidney and spleen), Fig. 1; 2 and 3. Moreover, adding garlic (2 or 4%) improved the immunity elements. Since, the hepatic sinusoids and blood vessels appeared hyperemic with activation of hepatopancreas (Fig. 4). Also, proliferations of hemopoietic elements with dilated and hyperemic blood vessels and capillaries and presence of melanomacrophage centers were observed in spleen (Fig. 5). The renal tubules of kidney and glomeruli were apparently normal. Numerous melanomacrophage centers could be seen scattered in renal tissue (Fig. 6).

Table (4): Effect of T-2 toxin; diacetoxyscirpenol (DAS); garlic and their interaction on blood parameters.

Items	Traits					
	Hemoglobin (g /dl)	Hematocrite (%)	Total protein (g /dl)	Albumin (g/dl)	AST (u/l)	ALT (u/l)
Toxin effect:	**	**	**	**	**	**
Control	10.92 ± 0.41^{a}	50.00 ± 2.89^{a}	5.11 ± 0.36^{a}	3.83 ± 0.14^{a}	25.25 ± 0.88^{b}	9.33 ± 0.43^{b}
T_{-2}	9.39 ± 0.24^{b}	44.00 ± 1.91^{b}	3.30 ± 0.14^{b}	2.70 ± 0.07^{b}	36.44 ± 2.27^{a}	10.00 ± 0.45^{ab}
DAS	9.55 ± 0.18^{b}	42.56 ± 1.46^{b}	3.42 ± 0.12^{b}	2.57 ± 0.08^{b}	36.33 ± 5.32^{a}	12.50 ± 1.23^{a}
Garlic (G) effect:	**	**	**	ns	**	**
0.0	9.13 ± 0.19^{b}	48.00 ± 1.33^{b}	3.33 ± 0.19^{b}	2.87 ± 0.19	41.53 ± 5.0^{a}	11.83 ± 1.25^{a}
2%	10.42 ± 0.23^{a}	51.67 ± 2.74^{a}	4.44 ± 0.45^{a}	3.17 ± 0.22	28.75 ± 1.0^{b}	10.42 ± 0.57^{ab}
4%	10.31 ± 0.47^{ab}	48.33 ± 1.22^{b}	4.05 ± 0.31^{ab}	3.07 ± 0.25	27.75 ± 1.93^{b}	9.58 ± 0.62^{b}
Interaction:	ns	**	ns	ns	**	**
Control × 0.0 G.	9.50 ± 0.12	46.00 ± 1.73^{c}	4.00 ± 0.29	3.50 ± 0.29	23.25 ± 1.59^{e}	8.50 ± 0.06^{c}
Control × 2%G.	11.25 ± 0.14	61.00 ± 1.73^{a}	6.08 ± 0.59	4.00 ± 0.12	27.75 ± 1.01^{de}	9.50 ± 0.58^{c}
Control × 4%G.	12.00 ± 0.58	43.00 ± 1.16^{cd}	5.24 ± 0.14	4.00 ± 0.23	24.75 ± 0.72^{de}	10.00 ± 1.16^{c}
T_{-2} × 0.0 G.	9.00 ± 0.58	41.00 ± 0.58^{de}	3.00 ± 0.12	2.60 ± 0.12	44.33 ± 2.46^{b}	10.50 ± 0.29^{bc}
T_{-2} × 2% G.	10.00 ± 0.29	51.00 ± 2.31^{b}	3.50 ± 0.29	2.80 ± 0.12	30.00 ± 1.16^{cd}	9.25 ± 0.43^{c}
T_{-2} × 4% G.	9.17 ± 0.09	40.00 ± 1.16^{de}	3.40 ± 0.23	2.70 ± 0.10	35.00 ± 1.16^{c}	10.25 ± 1.30^{bc}
DAS × 0.0 G.	8.90 ± 0.06	37.67 ± 0.67^{e}	3.00 ± 0.06	2.50 ± 0.12	57.00 ± 1.16^{a}	16.50 ± 1.16^{a}
DAS × 2% G.	10.0 ± 0.12	43.00 ± 0.58^{cd}	3.75 ± 0.14	2.70 ± 0.17	28.50 ± 2.89^{d}	12.50 ± 0.29^{b}
DAS × 4% G.	9.75 ± 0.14	47.00 ± 1.73^{bc}	3.50 ± 0.12	2.50 ± 0.17	23.50 ± 1.73^{e}	8.50 ± 0.87^{c}

Means in the same column bearing different letters differ significantly ($P \leq 0.01$).
ns not significant at $P \leq 0.05$

The toxic effect of mycotoxins ($T-_2$ or DAS) on liver was shown to be sever and manifested by degeneration or focal coagulative necrosis of hepatic tissue (Fig. 7); inactivation of hepatopancreas (Fig. 8); extravasted erythrocyte and congested sinusoids and pancreatic blood vessels. The hepatopancrease showed loss of zymogenic granules or destruction. Concerning kidney, it showed edema and hemorrhages in the interstitial tissue (Fig. 9). Also, the renal tubular epithelium suffered from hyaline or hydropic degeneration (Fig. 10); pyknotic nuclei with cytoplasmolysis and coagulative necrosis were also seen. Fibrosis edema around archinephric duct was common (Fig. 11). Furthermore, Results showed sever lymphoid necrosis and depletion of hemopoietic cells (Fig. 12), beside hemorrhages and congested ellipsoids in spleen (Fig. 13).

Garlic addition by 2 or 4% improved the histopathological lesions. Since, the hepatic cells showed mild degenerative changes or appeared normal. Interstitial and portal lymphocytic aggregations beside activation of hepatopancrease were evident (Fig. 14). Mild dilatation of hepatic sinusoids was observed. Similarly, kidney tissues showed mild hydropic or vacuolar degeneration in the epithelial lining of renal tubules and lymphocytic infiltration in the glomeruli and interstitial tissue with proliferation of hemopioetic elements (Fig. 15). Proliferation and activation of the lymphoid and hemopioetic tissue with dilated blood vessels (Fig. 16), and presence of melanomacrophage cells were also seen in spleen tissues (Fig. 17).

It is clear that the improvement as a result of garlic addition to $T-_2$ toxin was better than that with DAS. Since, the group of DAS plus 2% garlic showed activation and proliferation of hemopoeitic elements, mild congestion of blood vessels and capillaries and scattered melanomacrophages centers. Also, hyaline degenerated tubular epithelium could be seen in some renal tubules.

The present findings agreed with those reported by *Karppanen and Westerling (1986)*, who mentioned that rainbow trout fish that treated with trichothecenes toxins (Deoxynivalenol, $T-_2$ or DAS) in their feed suffered from hemorrhages and edema in different organs of the body and accumulation of zymogen granules in the acinar cells of the pancreas, rupturtion of these cells, and escape of zymogen in the surrounding tissues. Moreover, the pancreatic tissue appeared almost totally destroyed. Also, *Vanyi et al. (1989)* stated that the pathological examination of rabbit organs showed several lesions due to $T-_2$ toxin effect, such as centrolubular hepatic degeneration and necrosis of reticulo-endothelial system cells in the liver, tubulonephrosis, and necrosis in the lymphiod tissues. Furthermore, they suggested that the degenerative changes found in the paranchymal cells of the liver and kidneys may be connected with the systematic effect of $T-_2$ toxin. The ability of garlic to decrease the lesions of toxins may be due to its beneficial effect in inhibit the necrotic changes in body organs (*Soni et al., 1993*). In conclusion, in the light of the present knowledge it could suggest adding garlic to fish diet at 2% level to reduce the lesions of mycotoxins ($T-_2$ or DAS) that may be presence in its feed.

Fig. (1): Liver of control group. H&E × 300.

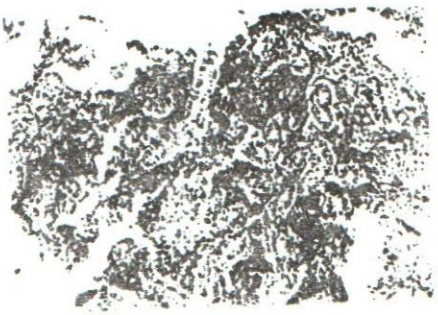

Fig. (2): Kidney of control group. H&E × 300.

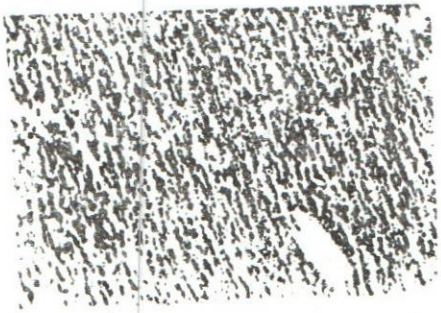

Fig. (3): Spleen of control group. H&E × 300.

Fig. (4): Liver (2% garlic), activation of hepatopancreas. H&E × 300.

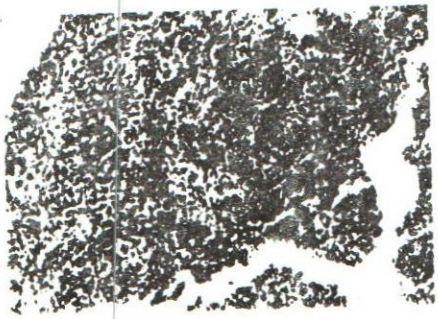

Fig. (5): Spleen (2% garlic), proliferation of hemopioetic elements with presence of melanomacrophage centers. H&E × 300.

Fig. (6): Kidney (2% garlic), numerous melanomacrophage centers internal tissue. H&E × 300.

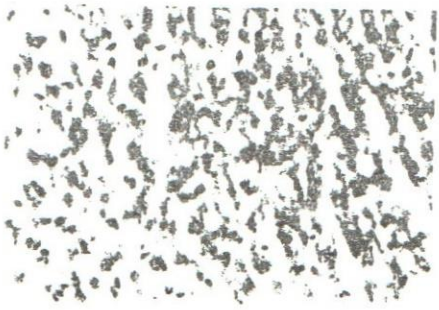

Fig. (7): Liver (T_2), Degeneration or necrosis of the hepatic tissue, H&E × 300.

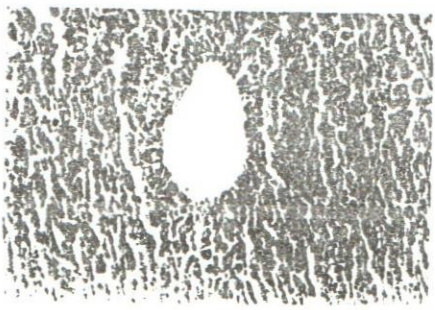

Fig. (8): Liver (DAS), inactivation of hepatopancreas, H&E × 300.

Fig. (9): Kidney (T_2), hemorrhage and edema in renal tissue, H&E × 300.

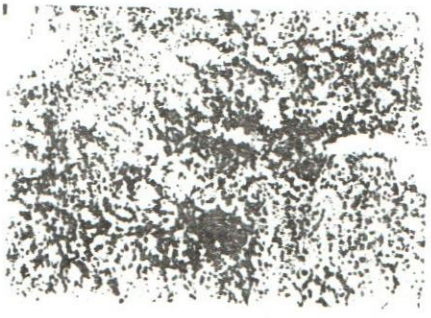

Fig. (10): Kidney (DAS), hyaline or haydropic degeneration of the renal tubular epithelium, H&E × 300.

Fig. (11): Kidney (DAS), edema and fibrosis around archinophric duct, H&E ×300.

Fig. (12): Spleen (T_2), depletion and necrosis of hemopoietic cells, H&E × 300.

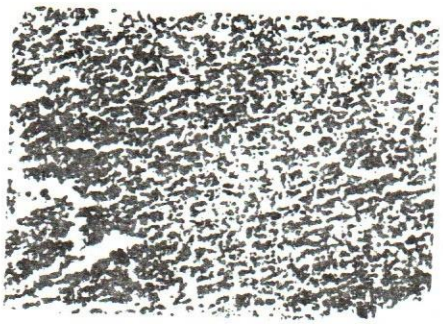

Fig. (13): Spleen (DAS), hemorrhages and congestion of ellipsoids, H&E× 300.

Fig. (14): Liver (4% garlic + DAS), portal lymphocytic aggregation and activation of hepatopancreas, H&E × 300.

Fig. (15): Kidney (4% garlic + T_2), proliferation of hemopoietic elements and lymphocytic infiltration in glomeruli and interstitial tissue, H&E × 300.

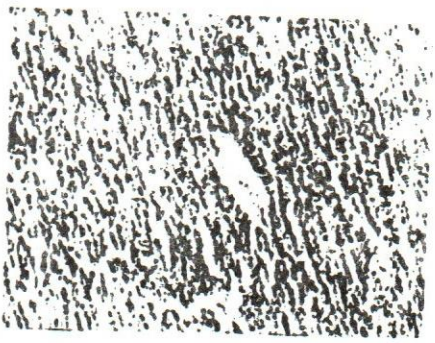

Fig. (16): Spleen (4% garlic + T_2), activation of hemopoietic and lymphoid tissue, H&E × 300.

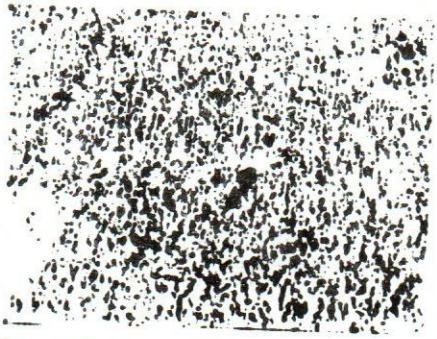

Fig. (17): Spleen (4% garlic + DAS), melanomacrophage centers scattered in spleen tissue, H&E × 300.

REFERENCES

Abdelhamid, A.M.(1983). Mykotoxin – Nachweis in Lebens – und Futternitteln des subtropischen Klimas. Z. Tierphysiol., Tierernährg. U. Futtermitelkde. 50: 4–5.

Abdelhamid, A.M.(1990). Effect of feeding rabbits on naturally molded and mycotoxin – contaminated diet. Arch. Anim. Nutr., Berlin, 40: 55-65.

Abdelhamid, A.M.; F.F. Khalil and M.A. Ragab (1998). Problem of mycotoxins in fish production. Egypt. J. Nutr. & Feeds, 1 (1) 63–71.

Abdelhamid, A.M.; F.F. Khalil; M.I. El-Barbary; V.H. Zaki and H.S.Husien (2002a). Feeding Nile tilapia on Biogen® to detoxify aflatoxic diets. Proc. 1st Ann. Sc. Conf. Anim. & Fish Prod., Mansoura 24 & 25 Sep., pp: 207–230.

Abdelhamid, A.M.; A.E. Salam; G.A. Abd Allah and S.H. El-Samra (2002b). Effect of feeding male rats on aflatoxic diets without or with medicinal herbs (thyme, safflower, ginger, black cumin and/or garlic). Proc. 2nd Conf. Foodborne Contamination and Egyptians Health, 23 – 24 April, El-Mansoura, pp: 99–121.

Ali, M.A.; O.S. Barakat; M.F. Nofal; E.M. Abo-Etta and A.A. Salem (2000): Effect of garlic and black cumin seeds on the immune system and some intestinal microorganisms of Maamourah laying hens. Proc. of the 10th Microbiology Conference, 11-14 Nov. (2000) Cairo, Egypt, pp. 208-218.

Carleton, R.A.; B. Drury and E.A. Wallington (1980): Histological Technique for Normal and Pathological Tissue and Identification of Parasites. Fifth Edition, Oxford Univ. Press, New York, Toronto.

Cheeke, P.R. and L.K. Shull (1985): Natural Toxicants in Feeds and Poisonous Plants, pp. 393 - 476. AVI Publishing Company, Westport, C .T.

Devegowda, G.; M.V.L.N. Raju; N. Afzali and H.V.L.N. Swamy (1998): Mycotoxins picture worldwide: Novel solutions for their counteraction. In. T.P. Lyons and K.A. Jacques (Eds.) Biotechnology in the Feed Industry, pp. 241-255. Proc. of Alltech's 14th Annual Symposium, Nottingham, U. K.

Duncan, D.B. (1955): Multiple range and multiple F. test. Biometric, 11: 1-42.

El-Kaiaty, A.M.; A.Z.M. Soliman and M.S.H. Hassan (2002): The physiologyical and immunological effects of some natural feed additives in layer hen diets. Egypt. Poultry Sci., 22 (1): 175-203.

El-Nawawi, G.H. (1991): Some of non-conventional ingredients in broiler ration. M. Sc. Thesis, Fac. of Agric. Ain Shams Univ.

El- Maghraby, O.M.; I.A. El-Kady and S. Soliman (1995): Mycoflora and fusarium toxins of three types of corn grains in Egypt with special references to production of trichothecene toxins. Microbiology Res., 150 (3): 225-232.

El-Mofty, M.M.; S.A. Sakr; Y.H. Essaw and H.S. Abdel-Gawad (1994): Preventive action of garlic on aflatoxin B1 induced carcinogenesis in the toad bufo regularis. Nutr. Cancer, 21 (1): 95-100.

Fekete, S. and G. Huszenicza (1993): Effects of T_2 toxin on ovarian activity and some metabolic variables of rabbits : Laboratory Animal Sci., 43 (6): 646-649.

Frankel, S. and S. Reitman (1963): Grandwohl's Clinical Laboratory Methods and Diagnosis. Ibid. Vol. 2, "Haematology", Acad. Press London.

Hintikka, E.L. (1989): Trichothecene poisonings on fish. In: Fusarium Mycotoxins, Taxonomy and Pathogenicity. Elsevier Science, Amstrdam - Oxford - New York - Tokyo, pp. 131-138.

Horton, G.M.J.; M.J. Finnell and B.M. Prasad (1991): Effect of dietary garlic (*Allium sativum*) on performance, carcass composition and blood chemistry changes in broiler chickens, Can. J. Anim. Sci., 71: 939-942.

Jeong, H.G. and Y.W. Lee (1998): Protective effects of diallyl sulfide on N-nitrosodimithylamine - induced immunosuppression in mice. Cancer Letters, 11, 134 (1): 73-79.

Kamanna, V.S. and N. Chandrasekhara (1980): Fatty acid composition of garlic (*Allium sativum Linnaeus*) lipids. Nutr. Abstr. Rev., 51: 2238 (Abstr.).

Karppanen, E. and B. Westerling (1986): Poisonings by fusarium toxins and cases investigated by the national veterinary institute (in Finnish). Suomen Eläinlääkärilehti (Finnish Veterinary Journal) 92: 515–523.

Koski, P. (1985): Studies on the pathology caused by trichothecenes (fusarium mycotoxins) in farmed rainbow trout (*Salmo gairdneri*). MSc. Thesis, Stirling Univ., Scotland.

Marasas, W.F.O.; E.P. Samalley; P.E. Degurse; J.R. Bamburg and R.E. Nichols (1967): Acute toxicity to rainbow trout (*Salmo gairdneri*) of a metabolite produced by the Fungus fusarium tricinictum. Nature, 214: 817-818.

Mohamed, F.R.; S.M.S. Siam and A.K. Alm El-Din (2000): The influence of garlic and onion on productive performance and some physiologyical traits in laying hens. Egypt. Poultry Sci., 20 (1): 123-144.

Parrel, P.; I. Ali and J. Lazard (1986): Le dêveloppement de l'aquaculture an Niger: un exemple de elevage de Tilapia en zone saheliene, Bois et Forêts des Tropiques, 212,71.

Poston, H.A.; J.L. Coffin and G.F. Combs (1982): Biological effect of dietary T-2 toxin on rainbow trout, *Salmo gairdneri*. Aquatic Toxicology, 2: 79-88.

Shehata, S.A. (2002): Detoxification of mycotoxin contaminated animal feedstuffs. Ph. D. Thesis, Zagazig Univ., Fac.of Agric., Egypt.

Snedecor, G.W. and W.G. Cochran (1982): Statistical Methods. 7th Ed. Iowa State Univ. Press, Ames, Iowa.

Soni, K.B.; A. Rajan and R. Kuttan (1993): Inhibition of aflatoxin induced liver damage in ducklings by food additives. Mycotoxin Research, 9: 22–26.

Strumia, M.M. (1954): Macromethod for hematocrite determination. Amer. J. Clin. Path., 24: 1016-1018.

Ueno, Y. (1987): Trichothecenes in food, In : P. Kroch (Ed) Mycotoxins in Food. pp. 123-147. Academic Press, Harcourt Brace Jovanovich, London.

Vanyi, A.; R. Glavits; S. Fekete and J. Jamas (1989): The pathological effects, metabolism and excretion of T-$_2$ toxin in rabbits. J. Appl. Rabbit Res., 12: 194–200.

Yamasaki, T.; R.W. Teel and B.H. Lau (1991): Effect of allixin, a phytoalexin produced by garlic, on mutagenesis, DNA-binding and metabolism of aflatoxin. Cancer Letters, 59 (2): 89-94.

تقليل سمية T$_2$ توكسين والداى أسيتوكسى اسكربينول بواسطة الثوم فى علائق السمك.

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أجريت تجربة عاملية ٣ × ٣ لدراسة كفاءة الثوم فى تقليل سمية T$_2$ والداى اسيتوكسى اسكربينول للبلطى النيلى، حيث استخدمت تسع معاملات وهى : كونترول (عليقة تجارية)، ٢% ثوم (طازج مهروس أضيف إلى العليقة التجارية التى سبق طحنها ثم أعيد تصبيعها مرة أخرى)، ٤% ثوم ، T$_2$ توكسين (٤ ملجم/كجم عليقة)، T$_2$ توكسين بنفس التركيز السابق + ٢% ثوم، T$_2$ توكسين + ٤% ثوم، داى استيوكسى إسكربينول (١٠ ملجم/كجم عليقة)، داى اسيتوكسى اسكربينول + ٢% ثوم، داى استيوكسى اسكربينول + ٤% ثوم.

استخدم فى كل معاملة ٣٠ سمكة (متوسط الوزن عند البداية ٣٣,٦٥ جم ± ٠,٠٤) وزعت على ثلاث مكررات بكل مكررة ١٠ سمكات. تم وضع العليقة للسمك بمعدل ٢% من وزن الجسم.

وجد أن T$_2$ توكسين، الداى أسيتوكسى اسكربينول لهما تأثيرات سيئة على أداء السمك حيث أحدثت : انخفاض معنوى على مستوى ١% فى وزن الجسم وزيادة معنوية فى معدل النفوق وانخفاض معنوى على مستوى ١% أيضا فى قيم الهيموجلوبين، الهيماتوكريت، البروتين الكلى والالبيومين. كذلك أحدثت زيادة معنوية (على مستوى ١%) فى نشاط انزيم الأسبرتيت أمينو ترانز فيريز (AST) وإنزيم الألانين أمينو ترانزفيريز (ALT). الفحص الهستولوجى أظهر وجود تأثيرات ضارة لهذه السموم على أعضاء الجسم المختلفة (الكبد، الكلية، الطحال) حيث أحدثت تدمير حاد فى خلايا الكبد والكلية وتجمع لفجوات ميتة أو تحلل مائى وكذلك تدهور فى نشاط الكبد البنكرياسى. كذلك لوحظ نزيف وتجمعات دموية على الطحال.

إضافة الثوم للعلائق الملوثة بالسموم السابقة خفف التأثير السام لهما على معدل النمو، معدل النفوق، تركيب الدم والتركيب الهستولوجى للأعضاء التى تم دراستها. معظم النتائج أشارت إلى أن إضافة ٢% ثوم حققت نتائج أفضل من ٤%.