REDUCTION OF THE DIETARY TOXICITY OF T-2 TOXIN AND DIACETOXYSCIRPENOL (DAS) BY GARLIC IN FISH
Shehata, S.A.1; Askar A.A.2 and M.S. Mohamed3
1Department of Animal Production, Fac. of Agric., Zagazig Univ., Zagazig, Egypt.
2Department of Poultry Production, Fac. of Agric., Zagazig Univ., Zagazig, Egypt.
3Aquaculture Research Lab., Abbassa, Abo-Hamad, Egypt.

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 < 0.05) in live body weight; increase in mortality rate (P < 0.01); reduction (P < 0.01) in a values of hemoglobin, hematocrite, total protein and albumin and increase (P < 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 severe destruction; focal coagulative necrosis or hydropic degeneration beside inactivation of hepatopancreas. 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 Huszenicz, 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

The adsorbents fed with mycotoxin contaminated diets reduced its
bioavailability 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 known 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 mercaptocysteine, 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
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-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-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-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-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-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
(oereochromis niloticus) per aquarium for a total of 270 fish of mean live body
weight 37.65±0.04 grams. The dimension of each aquarium was 150 x 50 x
50 cm, these aquaria 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 ± 2.0°C. 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 hematocrit 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 thickness 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 × 3 factorial treatment arrangement. Means were tested for differences using Duncan's multiple range test (Duncan, 1955).

Table (1): The experimental design.

<table>
<thead>
<tr>
<th>No.</th>
<th>T-2 toxin</th>
<th>DAS</th>
<th>Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>4%</td>
</tr>
<tr>
<td>4</td>
<td>4 mg/kg diet</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>4 mg/kg diet</td>
<td>0</td>
<td>2%</td>
</tr>
<tr>
<td>6</td>
<td>4 mg/kg diet</td>
<td>0</td>
<td>4%</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>10 mg/kg diet</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>10 mg/kg diet</td>
<td>2%</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>10 mg/kg diet</td>
<td>4%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Items</th>
<th>Proximate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM</td>
</tr>
<tr>
<td>As fed</td>
<td>92.92</td>
</tr>
<tr>
<td>On dry matter basis</td>
<td>100</td>
</tr>
</tbody>
</table>

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 ≤ 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; minerals 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-Molty 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 ≤ 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; hematopoietic 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.
|       | GS 4% C |       |       | 2% C |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-------|---------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|       |         |       |       |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|       |         |       |       |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|       |         |       |       |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|       |         |       |       |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|       |         |       |       |      |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |

**Notes:**
- Means in the same column bearing different letters differ significantly (p < 0.05).
- ns (not significant at p > 0.05).


**LWG** = Weekly body weight gain.

**RGR** = Relative growth rate (RGR).

**Mortality** = Percentage of mortality.

**Table 3:** Effect of T. toxin; dieltoxicyscinin (DAS); garlic and their interaction on fish performance.

<table>
<thead>
<tr>
<th>Item</th>
<th>Initial</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>5th week</th>
<th>6th week</th>
<th>7th week</th>
<th>8th week</th>
<th>9th week</th>
<th>10th week</th>
<th>11th week</th>
<th>12th week</th>
<th>13th week</th>
<th>14th week</th>
<th>15th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGR (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LWG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Shehata, S.A. et al.

3. Blood parameters:

Hemoglobin (g/dl) and hematocrite (%) values that presented in Table (4) were significantly (P ≤ 0.05) decreased due to the effect of T-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 ≤ 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 ≤ 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 ≤ 0.05) by feeding T-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-2 and DAS toxicity:

The fish fed T-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 revealed 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>
<thead>
<tr>
<th>Items</th>
<th>Hemoglobin (g/dl)</th>
<th>Hematocrite (%)</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>AST (u/l)</th>
<th>ALT (u/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Toxin effect:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.92 ± 0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.00 ± 2.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.11 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.83 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.25 ± 0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.33 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sup&gt;−&lt;/sup&gt;</td>
<td>9.39 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.00 ± 1.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.30 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.70 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.44 ± 2.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.00 ± 0.45&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DAS</td>
<td>9.55 ± 0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.56 ± 1.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.42 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.57 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.33 ± 5.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.50 ± 1.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Garlic (G) effect:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>9.13 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.00 ± 1.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.87 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.53 ± 5.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.83 ± 1.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2%</td>
<td>10.42 ± 0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.67 ± 2.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.44 ± 0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.75 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.42 ± 0.57&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4%</td>
<td>10.31 ± 0.47&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>48.33 ± 1.22&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.05 ± 0.31&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.07 ± 0.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>27.75 ± 1.93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.58 ± 0.62&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Interaction:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control × 0.0 G.</td>
<td>9.50 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.00 ± 1.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.50 ± 0.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.25 ± 1.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.50 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control × 2% G.</td>
<td>11.25 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61.00 ± 1.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.08 ± 0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.75 ± 1.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.50 ± 0.58&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control × 4% G.</td>
<td>12.00 ± 0.58</td>
<td>43.00 ± 1.16&lt;sup&gt;ca&lt;/sup&gt;</td>
<td>5.24 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.00 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.75 ± 0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.00 ± 1.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sup&gt;−&lt;/sup&gt; × 0.0 G.</td>
<td>9.00 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.00 ± 0.58&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.00 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.60 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44.33 ± 2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.50 ± 0.29&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sup&gt;−&lt;/sup&gt; × 2% G.</td>
<td>10.00 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.00 ± 2.31&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.50 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.80 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.00 ± 1.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.25 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sup&gt;−&lt;/sup&gt; × 4% G.</td>
<td>9.17 ± 0.09&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>40.00 ± 1.16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.40 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.70 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.00 ± 1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.25 ± 1.30&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>DAS × 0.0 G.</td>
<td>8.90 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.67 ± 0.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.00 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.50 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.00 ± 1.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.50 ± 1.16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>DAS × 2% G.</td>
<td>10.0 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.00 ± 0.58&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>3.75 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.70 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.50 ± 2.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.50 ± 0.29&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>DAS × 4% G.</td>
<td>9.75 ± 0.14&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>47.00 ± 1.73&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.50 ± 0.12&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.50 ± 0.17&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>23.50 ± 1.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.50 ± 0.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in the same column bearing different letters differ significantly (P < 0.01). ns not significant at P < 0.05.
The toxic effect of mycotoxins (T-2 or DAS) on liver was shown to be severe and manifested by degeneration or focal coagulative necrosis of hepatic tissue (Fig. 7); inactivation of hepatopancreas (Fig. 8); extravasated erythrocyte and congested sinusoids and pancreatic blood vessels. The hepatopancreas 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 severe 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 hepatopancreas 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 hemopoietic elements (Fig. 15). Proliferation and activation of the lymphoid and hemopoietic 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 hemopoietic 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 Karpanen and Westerling (1986), who mentioned that rainbow trout fish that treated with trichotheccenes 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, rupture 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 centrilobular hepatic degeneration and necrosis of reticulo-endothelial system cells in the liver, tubulonephrosis, and necrosis in the lymphoid tissues. Furthermore, they suggested that the degenerative changes found in the parenchymal 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 hemopoietic 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 hydropic degeneration of the renal tubular epithelium. H&E × 300.

Fig. (11): Kidney (DAS), edema and fibrosis around archinophreric 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


تقليل سمية T2 توكسـٍسن والـدائم أسِتيوكسـٍسن أسـكربينول 

بواصِطة الثوم في عالِقلا السمك.

* مصري عبد الحافظ شحاته. ** على عبد الرزاق عسكر. *** محمد صلاح محمد.

قسم الإنتاج الحيوي - كلية الزراعة - جامعة الزقاقر - مصر.

قسم الدواجن - كلية الزراعة - جامعة الزقاقر - مصر.

* * * 

عمل بحوث الزراعات المائية - العباسية - أبو حماد - مصر.

أجرت تجربة عاملية ل دراسة كفاءة الثوم في تقليل سمية T2 و الدائم أسِتيوكسـٍسن أسـكربينول للبلطي النبيل، حيث استخدمت ستمعاملات، وهي: كونترل (علبة تجارية) 20% توم (طـازج مهروس أضيف إلى العلبة التجارية التي سبق طحنها ثم أعيد تصنيعها مرة أخرى)، 2% توم T2 توكسـٍسن (4 ملجم/كم علبة)، T2 توكسـٍسن بنفس التركيز السابق + 2% توم، T2 توكسـٍسن + 4% توم، داى أسِتيوكسـٍسن أسـكربينول (10 ملجم/كم علبة)، داى أسِتيوكسـٍسن أسـكربينول + 2% توم، داى أسِتيوكسـٍسن أسـكربينول + 4% توم

استخدم في كل معاملة 30 سمكة (متوسط الوزن عند البداية 13 ± 5 جم) وزعت على ثلاث مكررات بكل مكررة 10 سمكات. تم وضع العلبة للسمك بسعة 20% من وزن السمك.

وجَد أن T2 توكسـٍسن، النائي أسِتيوكسـٍسن أسـكربينول لهما تأثيرات سلبة على أداء السمك حيث أحدثت انتفاخ عضوي على مستوى 1% في وزن السمك وزادت مفعوله في معدل النفوذ وانخفاض مفعوله على مستوى 1% أيضا في ميغاسماك، أمين كلف، البروتين الكلي والبروتينات. كذلك أحدثت زيادة معنوية (على مستوى 0.01) في نشاط الأنزيمات، أمين ترارا فريرز (AST) وانزيم الأنزيمات، أمين ترارا فريرز (ALT). يظهر النتائج المخبرية أن وجود تأثيرات ضارة لهذه السموم على أعضاء الجسم المختلفة (أحد، الكلية، الطحال) حيث أحدث تدمير حاد في حلايا الكبد والكلية وجميع الانتهاءات، ميتة أو تحمل سمك.

وذلك يكون قد تؤدي تنشيط كليتريباستي، كذلك لوحظ نزف وتحمج دموية على الطحال.

إضافة الثوم لمعالجة الملوثة بالسموم السائبة خلف النباتات السمية لها على معدل النمو، معدل النفوذ. تركيب السموم والتركيب البيولوجي للأعضاء التي تم دراستها. معظم النتائج أشارت إلى أن إضافة 2% توم حققت نتائج أفضل من 4%.