Toxicity Reduction of Aflatoxin B\textsubscript{1} by Vitamin C in Fish

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ABSTRACT

Reduction of aflatoxicosis in Nile tilapia (\textit{Oreochromis niloticus}) fish was examined by adding vitamin C to aflatoxin B\textsubscript{1} contaminated diets in a feeding trial for 8 weeks. Five experimental groups were carried out, the 1\textsuperscript{st} group (control) fed basal diet without aflatoxin B\textsubscript{1} or vitamin C, the 2\textsuperscript{nd} group fed basal diet supplemented with 3 mg aflatoxin B\textsubscript{1}/kg. The other groups (3-5) fed basal diet with 3 mg aflatoxin B\textsubscript{1} plus 250, 500 and 750 mg vitamin C/kg diet, respectively. A total number of 150 fish (average body weight 10.10 ± 1.05 g) was used in 3 replicate glass aquaria (per group) of 10 fish per aquarium. Aflatoxin B\textsubscript{1} significantly (P<0.05) decreased body weight gain, relative growth rate, all tested blood parameters (total protein, albumin, globulin, AST and ALT) and economical efficiency. The mortality rate significantly (P<0.05) increased (53.33% versus 6.67% for the control) by aflatoxin B\textsubscript{1}. All levels of vitamin C significantly (P<0.05) improved all parameters which negatively affected by aflatoxin B\textsubscript{1}, the best results were obtained by 500 mg vitamin C/kg diet. The data from the present study demonstrate that adding 500 mg vitamin C/kg diet contaminated with 3 mg aflatoxin B\textsubscript{1} may provide a safe and practical method for alleviate of aflatoxin B\textsubscript{1} toxicity in fish diet and improve the economical efficiency.

Key words: Aflatoxin, Fish, Vitamin C, Performance, Blood.

INTRODUCTION

Aflatoxins are mycotoxins produced as secondary metabolites by \textit{Aspergillus flavus} and \textit{A. parasiticus} (Cheeke and Shull, 1985). In Egypt, the aflatoxins and other mycotoxins are frequently...
detected in feedstuffs (Abdelhamid, 1990 and 2009; Aziz et al., 1997 and Hassan et al., 2002), commercial fish-feeds and some of aquatic fauna (Abdelhamid et al., 1997). The ingestion of aflatoxin contaminated diets led to hazard effects on fish production and health (Jantrarotai and Lovell, 1990; Abdelhamid et al., 1997 and 2002a,c,d,e & 2007a & b; Hussein et al., 2000; Shehata et al., 2003 and Zaki et al., 2008). The problems with mycotoxins do not end at feed refusal or reduction of animal performance but many of these mycotoxins transfere into the meat or milk (Devegowda et al., 1998 and Shehata, 2002).

Immunosuppression enhanced by the consumption of aflatoxin-contaminated feed, which is very common in many tropical countries. Practically, it is not possible to destroy the contaminated feed, therefore to avoid the effect of this toxic substance increasing animal immuntty must be made (Zaky et al., 2000 and Sahoo and Mukherjee, 2003). The ability of vitamin C to stimulate the immune response and protection against bacterial infection has now been established in fish (Abdelhamid et al., 1995a,b & e and Nayk et al., 2007). Vitamin C alleviate the aflatoxin effect on rabbits (Salem et al., 2001), rats (Abd El-Mageed, 1987) and guinea pigs (Netke et al., 1997). Rearly literature were carried out on using of vitamin C in the prevention of aflatoxicosis in fish (Sahoo and Mukherjee, 2003). Therefore, the present investigation was undertaken to study the protective effects of different levels of vitamin C on aflatoxin B1 in fish feeds.

MATERIALS AND METHODS

The experimental work was carried out in the Aquaculture Research Lab., Abbassa, Abo-Hamad, and Animal Production Dept., Zagazig Univ., Egypt. Five experimental groups were carried out, the 1st group (control) fed basal diet without aflatoxin B1 or vitamin C, the 2nd group fed basal diet with 3 mg aflatoxin B1/ kg, while the other groups (3-5) fed basal diet with 3 mg aflatoxin B1 plus 250, 500 and 750 mg vitamin C / kg diet, respectively. Commercial pelleted diet product of Factory of General Authority for Fish Resources Development was used in the experiment, it consisted of fish
TOXICITY REDUCTION OF AFLATOXIN B\(_1\) BY VITAMIN C IN FISH

meal, soybean meal, meat meal, yellow corn, bone meal and a mixture of vitamins and minerals. The chemical composition of basal diet was adopted according to A.O.A.C. (1980). The basal diet contained (as dry matter basis) 80.00, 29.00, 6.50, 4.93, 39.57, 20.00% for OM, CP, CF, EE, NFE and ash, respectively.

\textit{Aspergillus flavus} MD 341, was used for production of aflatoxin \(B_1\) on liquid media (2% yeast extract and 20% sucrose). The aflatoxin concentration was determined according to the method of A.O.A.C. (1990). The media contained aflatoxin \(B_1\) alone. The media was sprayed on diet to obtain the required aflatoxin \(B_1\) level. Vitamin C (20%) product of United Co. For Chem. 7 Med. Prep., Egypt was included at 1.25, 2.5 or 3.75 g (dissolved in tap water and sprayed on contaminated diet) / kg diet to obtain 250, 500 and 750 mg vitamin C/ kg diet. After vitamin C addition the diet was stored in black plastic bags to prevent vitamin C degradation. In each treatment, a total number of 30 fish (average body weight 10.10 ± 1.05 g) was used in 3 replicate glass aquaria of 10 fish Nile tilapia (\textit{Oreochromis niloticus}) per aquarium. The dimensions of each aquarium was 150 x 150 x 50 cm, these aquariums were supplied with dechlorinated tap water up to 80% of its highest and continuous aeration was adapted by using an air pump and airstones. Fish wastes were filtered by siphon method each day and the water was completely changed every 3 days. Mean water temperature was 27.0 ± 2°C. The fish were fed 2 times a day (900 and 1600 h.) at a rate of 3% of the total body weight. The fish were weighted every 2 weeks for 8 weeks. At the end of the experiment, blood samples were taken from the cadual vien of 6 fish for each treatment (2 fish/replicate). Serum was separated and stored at -20 °C to analysis, then analyzed for total protein, albumin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by using commerical kits from Diamond Diagnostics Company, Egypt. Data of the trial were statistically analyzed using the General Linear Model Program of SAS (1996).
RESULTS AND DISCUSSION

1- Growth performance

The average body weight gain and relative growth rate were significantly (P<0.05) decreased by aflatoxin B1 addition in comparison with those of control (Table 1). These results agree with the findings of Jantrarotai and Lovell (1990), El-Said, (1997) and Shehata et al. (2003 and 2009) on Oreochromis aureus. Decreasing of growth rate by aflatoxin may be due to disturbance in metabolic processes of carbohydrate, lipid and protein metabolism and loss of appetite (Cheeke and Shull, 1985). Also, it might be due to detoxification process in the body utilizing glutathione enzymes, which partly composed of methionine and cysteine, hence this detoxification processes deplates the metabolic availability of methionine leading to poor growth and feed efficiency (Devegowda et al., 1998).

Vitamin C addition significantly (P<0.05) improved body weight gain, the best results were obtained by 500 mg/ kg diet which reached to the significant (P<0.05) level comparing with other levels and insignificant level with control. These results of vitamin C agree with those obtained by Salem et al. (2001) on rabbits. These results of vitamin C may be due to increaseing feed intake, digestibility of nutrients which had biological role in digestive enzyme biosynthesis and activation (Earp et al., 1970 and Abd El-Mageed, 1987). Also, vitamin C improve the immunity of fish by enhance the phagocytic ratio and serum lysozyme activity (Sahoo and Mukherjee, 2003). Also, the respiratory burst activity of blood neutrophils and antibody levels were significantly higher in Indian major carp and rohu (Labeo rohita Ham.) fingerlings fed 100 mg vitamin C in the form of ascorbyl polyphosphate (Nayek et al., 2007).

2- Blood parameters

Total protein and albumin concentrations were significantly (P<0.05) decreased in fish fed aflatoxin B1 contaminated diet (Table 2). These results agreed with the results obtained by Mamdouh (1996), El-Said (1997), Hussein et al. (2000) and Shehata et al. (2003) on Oreochromis niloticus. The
TOXICITY REDUCTION OF AFLATOXIN B₁ BY VITAMIN C IN FISH

Table (1): Effect of dietary aflatoxin B₁ and addition of vitamin C on fish performance (Means ± Sd).

<table>
<thead>
<tr>
<th>Items</th>
<th>Week</th>
<th>Treatments</th>
<th>Live body weight (g)</th>
<th>Body weight gain (g/2 weeks)</th>
<th>Relative growth rate (%)</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Aflatoxin B₁ + 250 mg Vitamin C/kg diet</td>
<td>Aflatoxin B₁ + 500 mg Vitamin C/kg diet</td>
<td>Aflatoxin B₁ + 750 mg Vitamin C/kg diet</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.00 ± 0.10</td>
<td>10.20 ± 0.10</td>
<td>10.20 ± 0.10</td>
<td>10.20 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td></td>
<td>11.78 ± 0.08</td>
<td>11.03 ± 0.08</td>
<td>11.33 ± 0.08</td>
<td>11.23 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>12.56 ± 0.53</td>
<td>11.85 ± 0.10</td>
<td>12.43 ± 0.18</td>
<td>12.23 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>14.10 ± 0.13</td>
<td>13.38 ± 0.33</td>
<td>13.98 ± 0.20</td>
<td>13.68 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>15.93 ± 0.10</td>
<td>15.05 ± 0.05</td>
<td>15.78 ± 0.20</td>
<td>15.38 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>10.20 ± 0.10</td>
<td>10.30 ± 0.10</td>
<td>10.23 ± 0.08</td>
<td>10.00 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>1.28 ± 0.11</td>
<td>0.13 ± 0.01</td>
<td>0.83 ± 0.03</td>
<td>1.03 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>1.28 ± 0.18</td>
<td>0.27 ± 0.03</td>
<td>0.85 ± 0.05</td>
<td>1.00 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>1.54 ± 0.11</td>
<td>0.68 ± 0.08</td>
<td>1.50 ± 0.06</td>
<td>1.45 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>1.83 ± 0.11</td>
<td>0.67 ± 0.05</td>
<td>1.70 ± 0.05</td>
<td>1.70 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>1.48 ± 0.04</td>
<td>0.44 ± 0.01</td>
<td>1.25 ± 0.01</td>
<td>1.40 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>8.53 ± 0.50</td>
<td>8.54 ± 0.17</td>
<td>5.46 ± 0.19</td>
<td>7.43 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>7.86 ± 0.40</td>
<td>1.75 ± 0.10</td>
<td>5.30 ± 0.53</td>
<td>6.74 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>8.77 ± 0.34</td>
<td>4.33 ± 0.51</td>
<td>8.89 ± 0.80</td>
<td>8.42 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>9.58 ± 0.38</td>
<td>4.09 ± 0.47</td>
<td>9.25 ± 0.29</td>
<td>9.48 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td></td>
<td>8.69 ± 0.10</td>
<td>2.76 ± 0.06</td>
<td>7.36 ± 0.20</td>
<td>8.14 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>0-8</td>
<td></td>
<td>6.67 ± 3.34</td>
<td>53.33 ± 10</td>
<td>10.00 ± 5.78</td>
<td>10.00 ± 0.0</td>
</tr>
</tbody>
</table>

a, b, c... Means in the same row bearing different letter significantly (P<0.05).

decrease in total protein and albumin may be attributed to aflatoxin interaction with protein synthesis and cellular integrity in liver (Patterson, 1976 and Srivastava, 1984), since plasma proteins are used for energy production during pollutant toxicity or increasing of protein catabolism induced by stress in order to supplementary energy (Pfeifer and

77
Table (2): Effect of dietary aflatoxin B₁ and addition of vitamin C on serum parameters of fish. (Means ± Sd).

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>Aflatoxin B₁</th>
<th>Aflatoxin B₁ + 250 mg Vitamin C/kg diet</th>
<th>Aflatoxin B₁ + 500 mg Vitamin C/kg diet</th>
<th>Aflatoxin B₁ + 750 mg Vitamin C/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>4.21 ± 0.23</td>
<td>3.001 ± 0.25</td>
<td>3.80 ± 0.16</td>
<td>4.03 ± 0.27</td>
<td>3.82 ± 0.31</td>
</tr>
<tr>
<td>Index</td>
<td>100</td>
<td>71.26</td>
<td>90.26</td>
<td>95.72</td>
<td>90.74</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.15 ± 0.23</td>
<td>2.63 ± 0.13</td>
<td>3.10 ± 0.56</td>
<td>3.16 ± 0.15</td>
<td>3.16 ± 0.23</td>
</tr>
<tr>
<td>Index</td>
<td>100</td>
<td>83.49</td>
<td>98.41</td>
<td>100.32</td>
<td>100.32</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>1.06 ± 0.18</td>
<td>0.37 ± 0.10</td>
<td>0.70 ± 0.07</td>
<td>0.87 ± 0.11</td>
<td>0.66 ± 0.13</td>
</tr>
<tr>
<td>Index</td>
<td>100</td>
<td>34.91</td>
<td>66.04</td>
<td>82.08</td>
<td>62.26</td>
</tr>
<tr>
<td>AST (u/l)</td>
<td>32.00 ± 4.00</td>
<td>21.50 ± 2.19</td>
<td>29.00 ± 3.36</td>
<td>31.50 ± 3.02</td>
<td>31.33 ± 1.33</td>
</tr>
<tr>
<td>Index</td>
<td>100</td>
<td>67.19</td>
<td>90.63</td>
<td>98.44</td>
<td>97.91</td>
</tr>
<tr>
<td>ALT (u/l)</td>
<td>30.33 ± 3.21</td>
<td>21.30 ± 3.21</td>
<td>29.00 ± 1.73</td>
<td>28.63 ± 2.89</td>
<td>29.07 ± 2.31</td>
</tr>
<tr>
<td>Index</td>
<td>100</td>
<td>70.23</td>
<td>95.61</td>
<td>94.39</td>
<td>95.85</td>
</tr>
</tbody>
</table>

a,b,c... Means in the same row bearing different letter significantly (P<0.05).

Weber, 1979). The activities of AST and ALT enzymes (Table 2) decreased significantly (P<0.05) in fish fed aflatoxin B₁ contaminated diet. These results agreed with the findings of Abd El-Wahhab (1996) and Abd El-Baki et al. (2002). Reduction of AST and ALT may be due to toxic hepatosis (Abdelhamid and Dorra, 1993).

Addition of vitamin C significantly (P<0.05) improved blood parameters measured, whereas, the values of blood parameters for vitamin C groups become nearly similar to those of control group. These results agreed with those of Sahoo and Mukherjee (2003) who reported that vitamin C improved the immunity system of fish treated by aflatoxin B₁. Also, Nayak et al., (2007) reported that vitamin C significantly (P<0.05) increased serum total protein and globulin of fish.

3- Mortality rate

The mortality rate (Table 1) was significantly (P<0.05) increased in fish fed aflatoxin B₁ contaminated diet (53.33% versus 6.67% of control). These results
agreed with those reported by El-Said, (1997) who reported that aflatoxin increased mortality in Oreochromis niloticus. Also, Shehata et al. (2003) reported that 9 mg aflatoxin B$_1$/ kg feed caused 47.62% mortality in Oreochromis niloticus in comparison with 4.76% of control. However, the effect of mycotoxin on fish depends on potency of mycotoxin, dose, species and strain of fish, state of health, stage of life, temperature of the water and presence or absence of substances that can modify the toxicity (El-Said, 1997).

The incidence of death may be due to the disturbance of organs function, since, the aflatoxicosis caused liver neoplasm, necrosis of hepatocytes and degenerative changes in pancreatic and kidney tissues of rainbow trout (Halver, 1967). Also, Lovell (1991) reported that aflatoxin caused damage of liver and other organs which led to death.

Vitamin C significantly (P<0.05) reduced the mortality rate. Since, it was 10% versus to 53.33% for aflatoxin B$_1$ alone. Decreasing the mortality rate by vitamin C agreed with the findings of Nayek et al. (2007) who reported that vitamin C reduced the mortality rate in Indian major carp. These results may be due to improve animal performance, immunity and general health (Abd El-Mageed, 1987; Nayak et al., 2007).

4- Economical efficiency

The economical efficiency (Table 3) indicated that vitamin C improved the economical efficiency which negatively affected by aflatoxin B$_1$. The best improve was occurred by 500 mg/ kg diet.

It could be concluded from the results of this work that adding vitamin C (specially 500 mg/kg diet) to a diet contaminated with 3 mg aflatoxin B$_1$/ kg diet may provide a safe and practical method to alleviation of aflatoxin B$_1$ toxicity and improve the economical efficiency of fish diet.

REFERENCES

Table 3. Effect of dietary aflatoxin B<sub>1</sub> and addition of vitamin C on economical efficiency by fish.

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt; + 250 mg Vitamin C/kg diet</th>
<th>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt; + 500 mg Vitamin C/kg diet</th>
<th>Aflatoxin B&lt;sub&gt;1&lt;/sub&gt; + 750 mg Vitamin C/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total gain (g)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>5.92</td>
<td>1.76</td>
<td>5.00</td>
<td>5.60</td>
<td>5.20</td>
</tr>
<tr>
<td>Total feed intake (g)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>21.78</td>
<td>18.77</td>
<td>21.24</td>
<td>21.82</td>
<td>21.49</td>
</tr>
<tr>
<td>Total feed cost (piastres)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5.45</td>
<td>4.69</td>
<td>5.36</td>
<td>5.56</td>
<td>5.53</td>
</tr>
<tr>
<td>Selling price (piastres)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>7.10</td>
<td>2.11</td>
<td>6.00</td>
<td>6.72</td>
<td>6.24</td>
</tr>
<tr>
<td>Net revenue (piastres)&lt;sup&gt;5&lt;/sup&gt;</td>
<td>1.65</td>
<td>-2.58</td>
<td>0.64</td>
<td>1.16</td>
<td>0.71</td>
</tr>
<tr>
<td>Relative revenue (%)&lt;sup&gt;6&lt;/sup&gt;</td>
<td>100</td>
<td>-156.36</td>
<td>38.79</td>
<td>70.30</td>
<td>43.03</td>
</tr>
</tbody>
</table>

<sup>1</sup>= final weight – initial weight.
<sup>2</sup>= final weight + initial weight/2 x .03 x 56 (8 weeks).
<sup>3</sup>= total feed intake x price (price of 1 kg diet was 250, 250, 252.5, 255 and 257.5 piastres (pt) (price 2009). One kg of vitamin C costed 2000 pt.
<sup>4</sup>= total gain x 1.2 (one kg 1200 pt).
<sup>5</sup>= selling price – feed cost
<sup>6</sup>= net revenue for treatment/ net revenue of control x 100.


TOXICITY REDUCTION OF AFLATOXIN B₁ BY VITAMIN C IN FISH


Abdelhamid A.M.; F.I. Magouz; M.F.E. Salem and M.K. Mohsen (2002b). Effect of...


TOXICITY REDUCTION OF AFLATOXIN B1 BY VITAMIN C IN FISH


Shehata S.A.; Kh.M. El-Melegy; M.S. Ebrahim and R.A.
Abou-Seif (2009). Reduction the toxicity of aflatoxin B₁ by tafla clay, honey and Nigella sativa in fish. (Under publish).


تقليل سمية الأفلاتوكسين B₁ بواسطة فيتامين C في السمك
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3- معمل بحوث الزراعة المائية، العباسية، أبو حامد، مصر.

أجريت التجربة غذائية لتقليل النسب المئوية بالأفلاتوكسين لأسماء البلطي النيلي بضافات فيتامين ج للعلاقة الملتوية بالأفلاتوكسين B₁ لمدة 8 أسابيع. تم عمل 5 مجموعات تجريبية، غذيت المجموعة الأولى (كنترول) على علبة أساسية لا تحتوي على الأفلاتوكسين، أو فيتامين ج لكل كجم علبة، وتغذت المجموعة الثانية على نفس العلبة مع 3 مجم أفلاتوكسين B₁ لكل كجم علبة. وتغذت المجموعات الأخرى (5.1) على نفس العلبة مع 3 مجم أفلاتوكسين B₁ لكل كجم علبة بالإضافة إلى 250 و 500 و 750 مجم فيتامين ج لكل كجم علبة على التوالي. استخدم 150 سمكة (متوسط وزن الجسم 10.05 ± 0.1 جم)، 3 أحواض زجاجية لكل مجموعة بكل حوض 10 سمكات.

ذات الفائدة العلامة بالأفلاتوكسين B₁ إلى انخفاض معنوي في معدل الزيادة الوزنية، معدل النمو النسبي، كل قياسات الدم التي تم تقديرها (البروتين الكلي، اليولوبتوب، ALT, AST) إنزيمات نقل الأمينات والكافافة الاقتصادية. زادت معتنقا (متوسط 5%) نسبة النفوذ بالأفلاتوكسين B₁ (حيث كانت 53.33% بالمقارنة ب 6.67% للكنترول).

الإضافات المستويات المختلفة من فيتامين ج 750 مجم فيتامين ج لكل القياسات التي تأثرت سلباً بالأفلاتوكسين B₁، وأفضل النتائج تم الحصول عليها من استخدام 500 مجم فيتامين ج لكل كجم علبة.

من نتائج هذه الدراسة يمكن استنتاج أن إضافة 500 مجم من فيتامين ج لكل كجم علبة ملوثة ب 3 مجم أفلاتوكسين B₁ طريقة آمنة وعملية لتقليل الأثر السام لعلاقه السمك الملوثة بالأفلاتوكسين B₁ وتحسين الكفاءة الاقتصادية.