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Toxicity Reduction of Aflatoxin B_1 by Vitamin C in Fish

Shehata, S.A.[1]; El-Melegy, Kh.M.[2]* and Ebrahim M.S[3].

[1] Department of Animal Production, Fac. of Agric., Zagazig University, Zagazig, Egypt.

[2] Regional Center for Food and Feed, Agric. Res. Center, Ministry of Agric., Giza, Egypt.

[3]Aquaculture Research Lab. Abbassa, Abo-Hamad, Egypt.

***Corresponding Author**

ABSTRACT

Reduction of aflatoxicosis in Nile tilapia (*Oreochromis niloticus*) fish was examined by adding vitamin C to aflatoxin B_1 contaminated diets in a feeding trial for 8 weeks. Five experimental groups were carried out, the 1st group (control) fed basal diet without aflatoxin B_1 or vitamin C, the 2nd group fed basal diet supplemented with 3 mg aflatoxin B_1/ kg. The other groups (3-5) fed basal diet with 3 mg aflatoxin B_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_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_1. All levels of vitamin C significantly ($P<0.05$) improved all parameters which negatively affected by aflatoxin B_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_1 may provide a safe and practical method for alleviate of aflatoxin B_1 toxicity in fish diet and improve the economical efficiency.

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

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INTRODUCTION

Aflatoxins are mycotoxins produced as secondary metabolites by *Aspergillus flavus* and *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), commerical 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 immunty 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 under taken to study the protective effects of different levels of vitamin C on aflatoxin B_1 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 B_1 or vitamin C, the 2nd group fed basal diet with 3 mg aflatoxin B_1/ kg, while the other groups (3-5) fed basal diet with 3 mg aflatoxin B_1 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

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.

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 (*Oreochromis niloticus*) per aquarium. The dimensions of each equarium was 150 x 150 x 50 cm, these aquariums were supplied with dechlorinated tap water up to 80% of its highest and continueous 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, aspertate 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 these 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 aureaus.* 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 immunty 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 B_1 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

Table (1): ***Effect of dietary aflatoxin B_1 and addition of vitamin C on fish performance (Means ± Sd).***

Items	Week	Treatments				
		Control	Aflatoxin B_1	Aflatoxin B_1 + 250 mg Vitamin C/kg diet	Aflatoxin B_1 + 500 mg Vitamin C/kg diet	Aflatoxin B_1 + 750 mg Vitamin C/kg diet
Live body weight (g)	Initial	10.00 ± 0.10	10.30 ± 0.10	10.20 ± 0.10	10.20 ± 0.10	10.20 ± 0.10
	2	11.28^{a} ± 0.08	10.43^{c} ± 0.08	11.03^{b} ± 0.08	11.33^{a} ± 0.08	11.23^{a} ± 0.08
	4	12.56^{a} ± 0.53	10.70^{d} ± 0.10	11.88^{c} ± 0.18	12.43^{ab} ± 0.18	12.23^{b} ± 0.18
	6	14.10^{a} ± 0.13	11.38^{c} ± 0.13	13.38^{b} ± 0.33	13.98^{a} ± 0.20	13.68^{b} ± 0.23
	8	15.93^{a} ± 0.10	12.05^{c} ± 0.05	15.08^{b} ± 0.20	15.78^{a} ± 0.20	15.38^{b} ± 0.20
Body weight gain (g/2 weeks)	2	1.28^{a} ± 0.11	0.13^{d} ± 0.01	0.83^{c} ± 0.03	1.13^{ab} ± 0.03	1.03^{bc} ± 0.03
	4	1.28^{a} ± 0.18	0.27^{d} ± 0.03	0.85^{c} ± 0.05	1.10^{b} ± 0.05	1.00^{bc} ± 0.05
	6	1.54^{a} ± 0.11	0.68^{b} ± 0.08	1.50^{a} ± 0.06	1.55^{a} ± 0.06	1.45^{a} ± 0.06
	8	1.83^{a} ± 0.11	0.67^{b} ± 0.05	1.70^{a} ± 0.05	1.80^{a} ± 0.05	1.70^{a} ± 0.05
	Average	1.48^{a} ± 0.04	0.44^{c} ± 0.01	1.25^{b} ± 0.01	1.40^{a} ± 0.01	1.30^{ab} ± 0.01
Relative growth rate (%)	2	8.53^{a} ± 0.50	0.85^{d} ± 0.17	5.46^{c} ± 0.19	7.43^{b} ± 0.77	6.78^{b} ± 0.50
	4	7.86^{a} ± 0.40	1.75^{d} ± 0.10	5.30^{c} ± 0.53	6.74^{b} ± 0.41	6.16^{b} ± 0.28
	6	8.77^{a} ± 0.34	4.33^{c} ± 0.51	8.89^{b} ± 0.80	8.89^{b} ± 0.56	8.42^{b} ± 0.27
	8	9.58^{a} ± 0.38	4.09^{c} ± 0.47	9.25^{ab} ± 0.29	9.48^{a} ± 0.05	9.10^{b} ± 0.35
	Average	8.69^{a} ± 0.10	2.76^{d} ± 0.06	7.36^{c} ± 0.20	8.14^{b} ± 0.42	7.62^{c} ± 0.18
Mortality rate (%)	0-8	6.67^{c} ± 3.34	53.33^{a} ± 10	10.00^{b} ± 5.78	10.00^{b} ± 0.0	10^{b} ± 5.78

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 pollulant toxicity or increasing of protein catabolism induced by stress in order to supplementary energy (Pfeifer and

Table (2): ***Effect of dietary aflatoxin B_1 and addition of vitamin C on serum parameters of fish. (Means ± Sd).***

Items	Treatments				
	Control	Aflatoxin B_1	Aflatoxin B_1 + 250 mg Vitamin C/kg diet	Aflatoxin B_1 + 500 mg Vitamin C/kg diet	Aflatoxin B_1 + 750 mg Vitamin C/kg diet
Total protein (g/dl)	$4.21^{a} \pm 0.23$	$3.001^{b} \pm 0.25$	$3.80^{a} \pm 0.16$	$4.03^{a} \pm 0.27$	$3.82^{a} \pm 0.31$
Index	100	71.26	90.26	95.72	90.74
Albumin (g/dl)	$3.15^{a} \pm 0.23$	$2.63^{b} \pm 0.13$	$3.10^{a} \pm 0.56$	$3.16^{a} \pm 0.15$	$3.16^{a} \pm 0.23$
Index	100	83.49	98.41	100.32	100.32
Globulin (g/dl)	$1.06^{a} \pm 0.18$	$0.37^{c} \pm 0.10$	$0.70^{b} \pm 0.07$	$0.87^{b} \pm 0.11$	$0.66^{b} \pm 0.13$
Index	100	34.91	66.04	82.08	62.26
AST (u/l)	$32.00^{a} \pm 4.00$	$21.50^{b} \pm 2.19$	$29.00^{a} \pm 3.36$	$31.50^{a} \pm 3.02$	$31.33^{a} \pm 1.33$
Index	100	67.19	90.63	98.44	97.91
ALT (u/l)	$30.33^{a} \pm 3.21$	$21.30^{b} \pm 3.21$	$29.00^{a} \pm 1.73$	$28.63^{a} \pm 2.89$	$29.07^{a} \pm 2.31$
Index	100	70.23	95.61	94.39	95.85

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 B_1 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 modifiy 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.

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Table 3. Effect of dietary aflatoxin B_1 and addition of vitamin C on economical efficiency by fish.

Items	Treatments				
	Control	Aflatoxin B_1	Aflatoxin B_1 + 250 mg Vitamin C/kg diet	Aflatoxin B_1 + 500 mg Vitamin C/kg diet	Aflatoxin B_1 + 750 mg Vitamin C/kg diet
Total gain (g)[1]	5.92	1.76	5.00	5.60	5.20
Total feed intake (g)[2]	21.78	18.77	21.24	21.82	21.49
Total feed cost (piastres)[3]	5.45	4.69	5.36	5.56	5.53
Selling price (piastres)[4]	7.10	2.11	6.00	6.72	6.24
Net revenue (piastres)[5]	1.65	-2.58	0.64	1.16	0.71
Relative revenue (%)[6]	100	-156.36	38.79	70.30	43.03

1= final weight – initial weight.

2= final weight + inital weight/2 x .03x 56 (8 weeks).

3= 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.

4= total gain x 1.2 (one kg 1200 pt) .

5= selling price – feed cost

6= net revenue for treatment/ net revenue of control x 100.

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تقليل سمية الأفلاتوكسين B_1 بواسطة فيتامين C في السمك

صبري عبدالحافظ شحاتة[1]، خالد مصطفى المليجى[2]، محمد صلاح إبراهيم.[3]

1- قسم الإنتاج الحيواني-كلية الزراعة- جامعة الزقازيق- مصر

2- المركز الاقليمى للأغذية والأعلاف- مركز البحوث الزراعية-وزارة الزراعة- الدقى- مصر.

3- معمل بحوث الزراعات المائية- العباسة – أبو حماد – مصر.

أجريت التجربة تغذية لتقليل التسمم الأفلاتوكسينى لأسماك البلطى النيلى بإضافة فيتامين ج للعليقة الملوثة بالأفلاتوكسين B_1 لمدة 8 أسابيع . تم عمل 5 مجموعات تجريبية, غذيت المجموعة الأولى (كنترول) على عليقة أساسية لا تحتوى على الأفلاتوكسين B_1 أو فيتامين ج , وتغذت المجموعة الثانية على نفس العليقة مع 3 مجم أفلاتوكسين B_1 لكل كجم عليقة. وتغذت المجموعات الأخرى (3-5) على نفس العليقة مع 3 مجم أفلاتوكسين B_1 لكل كجم عليقة بالإضافة إلى 250 , 500 و 750 ملجم فيتامين ج لكل كجم عليقة على التوالى. استخدم 150 سمكة (متوسط وزن الجسم 10.05 ± 0.1 جم)، 3 أحواض زجاجية لكل مجموعة بكل حوض 10 سمكات.

أدت العليقة الملوثة بالأفلاتوكسين B_1 إلى انخفاض معنوي فى معدل الزيادة الوزنية, معدل النمو النسبى ، كل قياسات الدم التى تم تقديرها (البروتين الكلى , الألبيومين , الجلوبيولين , إنزيمات نقل الأمين ALT ,AST) والكفاءة الاقتصادية. زادت معنويا (مستوى 5%) نسبه النفوق بالأفلاتوكسين B_1 (حيث كانت 53.33% بالمقارنة ب 6.67 % للكنترول).

إضافة المستويات المختلفة من فيتامين ج حسنت معنويا كل القياسات التى تأثرت سلبيا بالأفلاتوكسين B_1، وأفضل النتائج تم الحصول عليها من استخدام 500 ملجم فيتامين ج لكل كجم عليقة.

من نتائج هذه الدراسة يمكن استنتاج أن إضافة 500 ملجم من فيتامين ج لكل كجم عليقة ملوثة ب 3 ملجم أفلاتوكسين B_1 طريقة آمنة وعملية لتقليل الأثر السام لعلائق السمك الملوثة بالأفلاتوكسين B_1 وتحسين الكفاءة الاقتصادية.