JOURNAL OF THE
ARABIAN AQUACULTURE SOCIETY
Vol. 5 No 2 December 2010

Nutritional Attempts to Detoxify Aflatoxic Effects in Diets of Tilapia Fish (*Oreochromis niloticus*)

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ABSTRACT

This study was conducted to investigate the toxic effects of aflatoxin B_1 (AFB1) on mono-sex Nile tilapia *Oreochromis niloticus* fingerlings and attempting to detoxify these drastic effects by using some nutritional agents. Therefore, one percent of each of these nutritional agents, namely *Piper nigrum L* and *Coriandrum sativum* meal were added to aflatoxic (150 ppb aflatoxin B_1) diets for fingerlings. These diets were offered 7 days a week at 3% daily of actual biomass. The feeding experiment lasted 15 weeks. The aflatoxic diet led to the worst fish growth performance, survival rate, feed and protein utilization, internal organs indices, carcass composition, residues of AFB1 (ppb) in the whole body of fish, some blood parameters, protein profile and activities of the some plasma enzymes of the experimented fish. Dietary pepper meal inclusion alleviated aflatoxicosis symptoms in fish, since it improved all the above tested parameters of fish. Generally, the obtained results in the present study indicated that the pepper was the best detoxifying agent of aflatoxin, followed by coriandrum meal.

Keywords: ***Nile tilapia - Piper nigrum L – Coriandrum sativum –Aflatoxin.***

INTRODUCTION

Mycotoxins are produced by certain filamentous fungi, in foods as a result of fungal growth. They cause toxic diseases, termed mycotoxicoses, when ingested by higher vertebrates and other animals. Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes leading to deaths (Bath *et al*., 2001 and Murjani, 2003).

The AFB1 is classified as group one carcinogen by International

Agency for Research on cancer. Also, toxigenic *Aspergillus flavus* isolates produce aflatoxins B1, and B2 while toxigenic *Aspergillus parasiticus* isolates produce B1, B2, G1, and G2 (Cotty and Jaime-Garcia 2007).

The 25% of the world's crops are affected by mycotoxins, of which the most notorious are aflatoxins. They are considered the most carcinogenic, mutagenic and teratogenic poisonous by-products of the growth of *A. flavus* and *A. parasiticus* molds. Moreover they are important contaminants of certain foods and animal feeds because of their ability to produce aflatoxins. These metabolites cause damage liver and animals tested and aflatoxin subtle, reduce growth rates and losses in feed efficiency and sometimes leading to mortalities (Abdel-Wahab *et al.,* 2007). Therefore, Scientifics is efforts have been used herbs or natural plants (cinnamon, chamomile, and black pepper), which detoxify mycotoxins of aflatoxin (Abdelhamid *et al.* 1985). Moreover *ginger*, (Abdelhamid *et al.*, 2007). black seed, liquorices, garlic meal, onion meal, fenugreek seeds and cinnamon (Salem *et al.*, 2009) and some spices such as black pepper and coriandrum (Reddy and Farid, 2009) are used. Nile tilapia (*Oreochromis niloticus)* may represent a model (as a sensitive model for mycotoxicosis), since this fish is highly susceptible to nutritional deficits and is extremely vulnerable to toxic from various chemicals and poisons including aflatoxin B_1 (AFB1). (Abdelhamid *et al*. 2002c) stated that feeding 10 ppm AFB1 – contaminated feed for 10 weeks had adverse effects on the fish growth rate, PCV%, Hb content diet and Erythrocytic count. More levels of AFB1 caused necrosis and basophilia of hepatocytes, enlargement of blood sinusoids in the head kidney, accumulation of iron pigments in the intestinal mucosa epithelium and necrosis of gastric glands. Therefore, the present work aims to study the drastic effects of AFB1 on growth performance, survival, nutrient utilization, some organs indices, carcass composition, residues of AFB1, and some parameters of blood hematology, protein profile and activities of the plasma enzymes of the experimented fish *O. niloticus*. Also, this study was conducted to evaluate the ability of some nutritional agents, namely *Piper nigrum L* and *Coriandrum sativum* meal (at a level of 1%) to detoxify the drastic effects of this dangerous toxin AFB1 on Nile tilapia fish for 15 weeks.

MATERIALS AND METHODS

A group of 120 of mono-sex Nile tilapia (*O. niloticus)* fingerlings were obtained from a private fish farm at Kafr El-Sheikh, Egypt; with an average initial body weight of

10.00±0.2g. Fish were adapted in the aquaria for one month before the beginning of the feeding experiment. The fish in all treatments were distributed into the aquaria at stocking rate of 15 fish per aquarium (40x40x50cm). The experimental treatments were tested in three aquaria for each treatment.

A basal diet (30.38% crude protein, 8.79% ether extract, 4.40% crude fiber, 6.24% ash, 478.4 Kcal gross energy/100g DM) was formulated from commercial ingredients (fishmeal 10%, soybean meal 38%, yellow corn 35.5%, plant oil 4%, wheat bran 12% and vit. & min. mixture 0.5%). The basal diet was considered as a control (T1). The estimated amount of oil was gradually added (few drops) gradually, and the mixing of diet was continued for 20 minutes. After the homogenous mixture was obtained, 40ml water per 100g diet was slowly added to the mixture. These ingredients were pressed by manufacturing machine (pellets size 1mm), they were milled and toxin AFB1 was added at a content diet of 150ppb to all diets (T_2, T3, and T_4), except the control (T_1). Anti-toxin (black pepper and Coriandrum) was added at a content diet of 1%. The ingredients and supplements were obtained from a local market in Kafr El-Sheikh, Egypt. Aflatoxin B_1 was produced according to Abdelhamid *et al*., 2006). Content diet of the produced aflatoxin B_1 was calculated and incorporated into the experimental diets at a rate of 150 ppb.

The experiment lasted 15 weeks. During the experimental period the fish were fed the experimental diets at a rate of 3% of the live body weight daily, 7 days a week. The diets were offered twice daily, at 8 a.m. and 2 p.m. The amount of the feed was adjusted bi-weekly based on the actual body weight changes. Light was adjusted at14h light: 10h dark as a daily photoperiod.

At the end of the experiment, three fish from each aquarium were taken immediately to determine the residues of AFB1 in the whole fish body. Also, the remained fish were sampled from each aquarium and kept frozen for chemical analysis. The chemical analyses of the basal diet and whole fish body were carried out according to the AOAC (2000). Aflatoxin B_1 determinations in the media extract and the basal diet were determined according to (AOAC, 2000). Water quality parameters were measured weekly. Temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pH-meter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter).

Individual fish body weight was measured biweekly to adjust feed quantity and to calculate growth performance and feed utilization in the form of: Average weight gain (g/fish) AWG = Average final weight (g) – Average initial weight (g), Average daily gain (g/fish/day) ADG = AWG (g)/Experimental period (days), Specific growth rate (SGR, %/day) = [In final weight – In initial weight] x 100/Experimental period (d), Feed conversion ratio (FCR) = Feed intake (g)/Live weight gain (g), Protein efficiency ratio (PER) = Live weight gain (g)/Protein intake (g), Protein productive value (PPV %) = Retained protein (g)/Protein intake (g) x 100, and Survival rate (SR%) = End number of the alive fish/Beginning number of fish x 100.

At the end of the experiment, the liver, spleen, kidneys and gonads were removed and weighted individually. Organ indices were calculated, where: Hepato-somatic index (HSI) = Liver weight (g) x 100/Gutted fish weight (g), Spleeno-somatic index (SSI) = Spleen weight (g) x 100/fish weight (g), Kidney – somatic index (KSI) = Kidneys weight (g) x 100/fish weight (g) and Gonads-somatic index (GSI) = Gonad weight (g) x 100/fish weight (g).

Blood samples from the different groups were collected from the caudal peduncles of fish in plain centrifuge tubes. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt). Also, total erythrocytes count (RBCs) and total leucocytes count (WBCs) were measured on by an A_o Bright –Line Haemocytometer model (Neubauer imroved, Precicolor HBG, Germany). Other blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of total protein, albumin, and globulin and Alanine Aminotransferase (ALT) by using a spectrophotometer (model 5010, Germany) and commercial kits.

The obtained numerical data were statistically analyzed using SPSS (1997) of variance. and least significant difference was calculated according to Duncan *(*1955).

RESULTS AND DISCUSSION

Quality parameters of rearing water

All tested water quality criteria were suitable for rearing Nile tilapia *O. niloticus* fingerlings. Since water temperature ranged between 26 and 27^{o}C, pH values 7 – 8 and dissolved oxygen 5 – 6 mg/l. the results are

similar to that Abdelhamid *et al*. (2004) and Abdel-Hakim *et al*. (2002).

Growth performance and survival rate

Data concerning average total gain (ATG), Average daily gain (ADG), specific growth rate (SGR) and survival rate (SR) are presented in Table (1). Results show that values of ATG, ADG, SGR and SR differ significantly ($P<0.05$) among diets. But the results clearly show that the T1 (Control diet), T3 (aflatoxin-contaminated diet plus 1% black pepper meal), and T4 (aflatoxin-contaminated diet plus 1% Coriandrum meal) were slightly better than T2 ($Diet_1$ + AFB1 150ppb). On the other hand, there were no significant differences when comparing T3, and T4. In this context, similar negative effects of AFB1 on different growth performance parameters and survival rate of tilapia fish were recorded by Abdelhamid *et al* (2007). Recently, Salem *et al*. (2009) found that the effects of aflatoxins B_1 (AFB1) on the growth performance and survival rate of *O. niloticus* fish were significant by decreases.

Meanwhile, in the present study, black pepper and coriandrum meal had positive effects may be due to their chemical and physical properties and/or their positive effects on the digestive system. Pepper and coriandrum stimulates digestion and influence positively the terminal enzymes of the digestive process (Abdel-Wahab *et al*., 2007). On the other hand, Abdelhamid *et al*. (2002c) found that none of the tested medicinal herbs (thyme, sunflower, ginger, black cumin and/ or garlic) completely overcome the effects of food aflatoxicosis. However, pepper is known to inhibit the cycloxygenases, enhance the cellular immune response (Salem *et al*., 2009).

Table (1): ***Means* ± standard errors of the growth performance of the experimented tilapia fish as affected by the dietary treatments for 15 weeks.***

Treat. No.	Initial weight g	Final weight g	TWG g/fish	ADG g/fish/day	SGR %/day	Survival %	SR
T1	10.07±0.00	39.33±0.10a	29.26±0.05a	0.28±0.01a	1.30±0.02a	100.00±0.00a	
T2	10.48±0.10	30.32±0.07c	19.84±0.01c	0.19±0.02c	1.01±0.04c	86.66±0.06 c	
T3	9.81± 0.40	36.68±0.19b	26.87±0.04b	0.26±0.01b	1.26±0.05b	93.33±0.08 b	
T4	9.95±0.10	35.22±0.15b	25.27±0.05b	0.24±0.01b	1.20±0.04b	93.33±0.07 b	

Means (within the same column) with unlike superscripts are significantly different (P≤0.05).

T_1- (control diet). T_2- ($diet_1$ + AFB150ppb). T_3- ($diet_1$ +AFB1 150ppb + 1% black pepper meal (*Piper nigrum L*). T_4- ($diet_1$ +AFB1 150ppb + 1% *Coriandrum sativum*).

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Feed and protein utilization

All criteria studied and presented in Table (2) showed that T1, T3 and T4 were better (P≤0.05) treatment in comparison with the T2 group concerning FI, FCR, PER, and PPV in tilapia fish experiment. On the other side, there was no significance between T1, T3 and T4 in data of FCR, PER, and PPV. Black pepper and coriandrum stimulates digestion and influences positively the terminal enzymes of digestive process (Abdel-Wahab *et al*., 2007) and Salem *et al*., 2009).

Similar negative effects of AFB1 on feed and protein utilization parameters of tilapia fish were recorded by Abdelhamid *et al*.(2002b). This negative effect of AFB1 may be attributed to pathological alterations in the gastro-intestinal tract (Murjani, 2003). The present results agree with the findings of Nguyen *et al*. (2002) who reported a clear reduction in feed consumption in a direct relation to the dietary AFB1 level for *O. niloticus*.

Table (2): ***Feed intake and conversion as well as protein utilization in the experimented tilapia fish (x*± SE) as affected by the dietary treatments after the 15 weeks experiment.***

Treatment	Feed intake g/fish	FCR	PER	PPV %
T1	40.00±1.00 a	1.37±0.03 b	2.41±0.02 a	27.98±0.19 a
T2	35.83±0.50 c	1.80±0.01 a	1.82±0.00 b	17.46±0.30 b
T3	38.60±1.00 b	1.44±0.00 b	2.29±0.01 a	25.08±0.39 a
T4	36.56±1.00 b	1.45±0.01 b	2.28±0.01 a	26.12±0.12 a

a,b,and c means in the same column had different letters significantly (p<0.05) differ.
T_1- (control diet). T_2- ($diet_1$ + AFB150ppb). T_3- ($diet_1$ +AFB1 150ppb + 1% pepper meal (*Piper nigrum L*). T_4- ($diet_1$ +AFB1 150ppb + 1% *Coriandrum sativum*).

Also they suggested that fish fed diets containing 10 and 100 mg AFB1/kg were observed to expel feed after ingestion. These authors added that the high levels of aflatoxin-B_1 (10 and 100 mg AFB1/kg) led to decreased feed intake. On the other hand, Svobodova *et al*. (1982) proved that AFB1 at doses of 20 to 200 µg/Kg of feed did not show any effects on feed and protein utilization.

Body composition

Values of dry matter (DM), crude protein (CP), ether extract (EE) and Ash of the fish body are summarized in Table (3). The results of carcass composition of Nile tilapia showed no significance effects (P>0.05). Also clearly show that the T2 gave slightly lower CP than the other treatments.

These results agree with the finding of Abdelhamid *et* al. (2004) and Salem *et al*., (2009) who found that no differences were observed for EE and protein contents among the experimental diets.

Yet, the positive effects of pepper and coriandrum used in the present study may be due to their adsorptive characteristics as mentioned above, so prevent or reduce absorption of AFB1 and hence prevent its negative effects on carcass composition of *O. niloticus*.

Table (3): ***Proximate chemical analysis (on dry matter basis %) of the whole tilapia body as affected by the experimental diets (x^{-}* ± SE).***

Treat. No.	DM	CP	EE	ASH	GE Kcal/100g
Initial	23.80	55.60	20.00	24.00	598.56
T1	24.91±0.50	59.00±0.03	19.50±0.01	21.00±0.01	600.58
T2	23.00±0.23	57.50±0.45	21.50±0.26	21.00±0.21	611.06
T3	23.85±0.27	58.54±1.06	19.34±0.96	22.12±0.01	601.07
T4	23.04±0.11	58.50±1.08	19.40±0.36	22.10±0.809;'	601.34

(Gross energy) (Kcal/100g), based on 5.6Kcal/g protein, 9.44 Kcal/g lipid, 4.1 Kcal/g carbohydrate, according to (Jobling, 1983).

T_1- (control diet). T_2- ($diet_1$ + AFB150ppb). T_3- ($diet_1$ +AFB1 150ppb + 1% pepper meal (*Piper nigrum L*). T_4- ($diet_1$ +AFB1 150ppb + 1% *Coriandrum sativum*).

Internal organs indices

Data of the internal organs indices of the tested tilapia fish are given in Table (4). Hepato somatic index (HSI), and Kidney somatic index (KSI), increased in T2 diet. But Gonads somatic index (GSI), and Spleen somatic index (SSI) increased in T3, and T4 where asAbdelhamid *et al* (2006) reported that all tested indices reflected remarkable ($P<0.05$) and gradual increased proportional to the dietary AFB1 levels. Abdelhamid *et al*. (2006), and Reddy & Farid, (2009) reported that the aflatoxic diet (100ppb AFB1) led to significant increase ($P<0.05$) in all organs indices comparing with the control diet (zero ppb AFB1). In the present study, the effect of *Piper nigrum L* and *Coriandrum sativum* may be due to their adsorptive characteristics as mentioned before, so preventing or reducing the absorption of AFB1 and hence preventing its negative effects on organs indices of fish.

Residues of aflatoxin in the whole fish body

The data concerning aflatoxin residues in the whole fish body are shown in Table (5). The control fish were free from the aflatoxin; whereas, T_2 showed the highest level (99.00 ppb aflatoxin B_1) followed by T4 (38 ppb), and T_3 (35 ppb) respectively. So, T3 was the best treatment in reducing these residues.

Table (5): ***Residues of aflatoxin B_1 in the tilapia fish as affected by the dietary treatments extended for 15 weeks.***

Treatments	AFB1 in whole fish body (ppb)
T1 (control)	0.00
T2	99.00
T3	35.00
T4	38.00

T_1- (control diet). T_2- ($diet_1$ + AFB150ppb). T_3- ($diet_1$ +AFB1 150ppb + 1% pepper meal (*Piper nigrum L*). T_4- ($diet_1$ +AFB1 150ppb + 1% *Coriandrum sativum*).

In this respect, (Abdelhamid *et al*., 2004) reported that AFB1 residues in the *O. niloticus* flesh showed a cumulative effect related to levels of dietary AFB1 and feeding period. Also, Soliman *et al*. (1998) mentioned that the significant increase of aflatoxin residues was observed in *O. niloticus* flesh after 6 months. Abdelhamid *et al.* (2004) and Salem *et al*. (2009) found residues of AFB1 in the whole body *O. niloticus* at the end of the experiment and tended to decrease after a freezing periods.

Clinic pathological findings

a. Biochemical Parameters

The results of Table (6) show in significance differences in total protein, albumin, globulin and A/G

ratio. On the other side the liver enzymes (AST) and (ALT) values were significantly increased in T2. While there were no significant differences (P>0.05) among other treatments (T1, T3, and T4). These findings agreed with the results of Shehata *et al.* (2003) insuring that aflatoxic diets led to pathological alteration diets in the liver (Abdelhamid *et al*., 2004).

b. Hematogram

The results of hematogram revealed increases in RBCs count and HB. value and significant decrease of the leukocytic count in the following groups T1, T3, and T4 as presented in Table (7). These results are similar to the findings of Abdelhamid *et al*. (2006) and Salem *et al* (2009). On the other hand the significant increase in white blood cells count in our herein the T2 group nearest to Abdelhamid *et al* (2004).

The improvement in the hemaogram may be due to the effectsof *Piper nigrum* L and *Coriandrum sativum* to over come the necrosis and basophilia of hepatocytes, enlargement of blood sinusoids in the head kidney (congestion, shrinking of glomeruli and melanosis were observed), accumulation of iron pigments in the intestinal mucosa-epithelium, and necrosis of gastric glands done by AFB1 (Marzouk *et al.,* 1994).

CNCLUSIONS

It could be concluded from the feeding experiment that aflatoxin contamination of fish diets caused many drastic effects in all tested parameters and it is very dangerous from the view point of fish production and public health. It could be recommended for the use 1% *Piper*

Table (6): ***Protein profile and activities of plasma enzymes of experimental fish groups.***

Treatment	Total protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)	A/G ratio	ALT (U/L)	AST (U/L)
T1	**3.44±0.50**	**1.30±0.10**	**2.14±0.05**	**0.61±0.05**	**26.5±0.50b**	**26.5±0.50b**
T2	**3.00±0.25**	**1.25±0.05**	**1.75±0.01**	**0.71±0.01**	**34.5±0.02a**	**35.5±0.06a**
T3	**3.15±0.45**	**1.20±0.05**	**1.95±0.50**	**0.61±0.03**	**28.2±0.50b**	**29.5±0.07b**
T4	**3.20±0.45**	**1.15±0.15**	**2.05±0.10**	**0.56±0.02**	**28.3±0.03b**	**29.5±0.10b**

a,b, means in the same column had different letters significantly (p<0.05) differ.

T_1- (control diet). T_2- ($diet_1$ + AFB150ppb). T_3- ($diet_1$ +AFB1 150ppb + 1% pepper meal (*Piper nigrum L*). T_4- ($diet_1$ +AFB1 150ppb + 1% *Coriandrum sativum*).

Table (7): ***Hematogram of O.niloticus groups post-treatment with AFB1, with pepper and coriandrum***

Treatment	RBCs (x106/mm³)	Hb (g/100ml)	WBCs (x10³/mm³)
T1	**1.50±0.0005a**	**6.85±0.006a**	**28.85±0.005b**
T2	**1.00±0.0003b**	**5.10±0.004b**	**36.15±0.005a**
T3	**1.20±0.0004ab**	**6.00±0.003a**	**28.35±0.005b**
T4	**1.10±0.0002ab**	**6.10±0.005a**	**28.85±0.005b**

a and b, means in the same column had different letters significantly (P<0.05) differ.
T_1- (control diet). T_2- (diet$_1$ + AFB150ppb). T_3- (diet$_1$ +AFB1 150ppb + 1% pepper meal (*Piper nigrum L*). T_4- (diet$_1$ +AFB1 150ppb + 1% *Coriandrum sativum*).

nigrum L or 1% *Coriandrum sativum* to alleviate the toxic effects of AFB1 contaminated diets. Moreover, we need a lot of scientific efforts in this trend to use of the natural agents to detoxify of mycotoxins (particularly aflatoxin) in diets of fish.

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محاولات غذائية لإزالة سمية علائق اسماك البلطي الملوثة بالأفلاتوكسين.

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1 المعمل المركزي لبحوث الثروة السمكية بالعباسة- ابو حماد محافظة الشرقية,مصر-

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أجريت هذه الدراسة للكشف عن التأثيرات السامة للأفلاتوكسين ب$_1$ على أصبعيات البلطي النيلى وحيد الجنس ، وكذا لمحاولة إزالة تلك الآثار السيئة باستخدام بعض الإضافات الغذائية. لذلك تم إضافة 1 % من كل من هذه المواد وهى مادة (الفلفل الاسود و الكزبرة) لعلائق أسماك البلطي النيلى الملوثة بالأفلاتوكسين (150 جزء فى البليون أفلا توكسين ب$_1$). قدمت هذه العلائق على مدار 7 أيام فى الأسبوع بمعدل 3% من الكتلة الحيوية الحقيقية للأسماك فى الأحواض الزجاجية ، حيث مُثلت كل معامله فى مكررات(3 أحواض) ، وتم تغذية الأسماك على هذه العلائق لمدة 15 أسبوعاً. حيث أوضحت النتائج أن العلائق الملوثة بالأفلاتوكسين أدت إلى تأثيرات سيئة على كل من معدل النمو و الإعاشة للأسماك ، الاستفادة من الغذاء والبروتين ، ودلائل الأعضاء الداخلية ، والتحليل الكيماوي لجسم الأسماك ، وكذا سجلت النتائج وجود متبقيات من الأفلاتوكسين ب$_1$ فى جسم الأسماك المعاملة ، كما أثر هذا السُم تأثيرات سيئة على قياسات الدم المختلفة للأسماك. كذلك أظهرت النتائج أن العليقة المحتوية على الفلفل الاسود و الكزبرة قد خففت من تلك التأثيرات السيئة للأفلاتوكسين على الأسماك ، حيث تحسنت كل القياسات السابقة الذكر للأسماك المعاملة بالأفلاتوكسين. بصفة عامة أوضحت النتائج المتحصل عليها فى هذه الدراسة الحالية أن الفلفل الاسود ُيعد أفضل مادة مستخدمة لإزالة التأثيرات السيئة للأفلاتوكسين ب$_1$ يليه الكزبرة على التوالي.