AN ATTEMPT FOR REDUCTION OF AFLATOXICOSIS B_1 IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) THROUGH MEDICINAL PLANT.

M.F.I. Salem

Central Laboratory for Aquaculture Research-Abbassa, Sharkia, Sakha Aquaculture Research Unit. Egypt.

(Received 10/2/2012, Accepted 30/3/2012)

SUMMARY

This study was conducted to investigate the toxic effects of aflatoxin B1 (AFB1) on Nile tilapia (*Oreochromis niloticus*) fingerlings and to attempt detoxify these drastic effects by using some medicinal seeds and leaves. Therefore, 0.5% each of these medicinal plants namely; Dried Basil Seeds (D.B.S), Requette Seed Meals (R.S.M), Green tea (G.T.), and Fennel Seeds meal (F.S.M) was added to an aflatoxin (150 ppb aflatoxin B1) diet for fingerlings. These diets were offered 6 days a week at 3% daily of actual biomass in glass aquaria in duplicate (2aquaria / treatment) in an indoor feeding experiment lasted 15 weeks. The aflatoxic diet has adversely affected the growth performance, survival rate, feed and protein utilization and carcass composition in fish and residues of AFB1 (ppb) in the whole body of fish and the tested parameters of blood hematology and biochemistry of the experimented fish. Dietary Dried Basil Seeds, Requette Seed Meals, Green tea, and Fennel Seeds meal inclusion alleviated aflatoxicosis symptoms by fish, since it improved all the above tested parameters of aflatoxicated fish. Generally, the obtained results in the present study indicated that the (D.B.S., R.S.M., G.T., and F.S.M.) could be used as detoxifying agents for aflatoxins.

Keywords: *Nile tilapia - Dried Basil Seeds – Requette Seed Meals - Green tea - Fennel Seeds Meal - aflatoxin B1.*

INTRODUCTION

Aflatoxins are potent toxic, carcinogenic, mutagenic, immunosuppressive agents, produced as secondary metabolites by mainly fungus *Aspergillus* (*A.*) *flavus* and *A. parasiticus* on variety of food products. Among 18 different types of aflatoxins identified, major members are aflatoxin B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is normally predominant in amount in cultures as well as in food products (Horvath, 1998, Cotty and Jaime-Garcia, 2007, and Salem *et. al.*, 2010). They cause a toxic disease, termed as aflatoxicosis, when ingested through contaminated foodstuff by higher vertebrates and other animals. Aflatoxin losses to livestock and poultry producers include subtle effects on the health status as immune suppression, reduced growth rates and losses in feed efficiency sometimes leading to mortalities (FAO 2002, and Abdel-Wahab *et al* 2007). Toxic fungi can also invade various feed - and foodstuffs and hence affect agricultural animals (Abdelhamid and Saleh, 1996) and humans (Abdelhamid *et al.*, 1999). Moreover, these toxigenic fungi occur also in and / or on moist houses, libraries, air conditioners, feed mills, dust, air, insects,etc... (Abdelhamid, 2008). Aflatoxins in fish diets has become a serious problem and it leads severe health and economical impacts in developing countries. The use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antimycotoxins and other therapeutic drugs. Medicinal plants are important elements of traditional medicine in virtually all cultures. It has been used in developing countries as well as using extended to developed countries (Lewis and Ausubel, 2006). Therefore, some scientific efforts have been conducted to use the herbs, some spices or natural plants (green tea, cinnamon, chamomile,ginger, and black pepper) which, detoxify mycotoxins as aflatoxin (Abdelhamid *et al.* 2002 and Ibrahim, 2004 and Kunio Suzuki *et al.* 2006). Coriandrum meal, black seed, liquorices, garlic meal, onion meal, fenugreek seeds and cinnamon were used also (Salem, *et al.* 2010) besides some spices such as black pepper, coriandrum, basil seeds, and roquette seed meals (El-Dakar, 2004 ; El-Dakar *et. al.* 2005 and Reddy and Farid, 2009). Dried Basil Seeds and leaves (D.B.S) play an important role in the orientation of food in fish DBS may be acting on growth, feed and protein conversion and nutrient retention efficiencies by its constitutions of the volatile oil rich in ocimene, methyl chavecol and linalool which were the predominant effective compounds in the volatile oil (El-

Dakar *et al.*2005). Requette Seed Meals (R.S.M) consisted of digestive and stimulant effect through their aromatic substance or essential oils, oil contains more than 100 components, and it is a rich source of polyunsaturated fatty acids (palmitic, oleic, linoleic, and linolenic) and nutrient components thiamin, riboflavin, pyridoxine, niacin, folacin, and some heavy metals, (Khalifea, 1995).Tea flush and Green tea (G.T.), the unfermented tea exerted has the strongest antimicrobial and antimycotoxin activity followed by the partially fermented tea products such as Longjing, Tieh-Kuan-Ying, Paochung, and Oolong teas. Green tea may protect against blood cell cancer, drinking five or more cups of green tea per day may reduce the risk of blood- and lymph-based cancers by about 50 per cent, says a new study from Japan. Antioxidants from black tea may aid diabetics. Polysaccharides from black tea may blunt the spike in sugar levels after a meal more than similar compounds from green and oolong tea, and offer potential to manage diabetes (Cheng-Chun Chou *et al.*, 1999). Fennel Seeds meal (F.S.M) (*Foeniculum vulgare L.*) is a potential source of natural antioxidant due to increasing digestive enzymes activities (Saleh, 2004).

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 insult from various chemicals and poisons including aflatoxin B1 (AFB1). Many authors including Abdelhamid,*et al.* (2006) stated that feeding at least 10 ppm AFB1 – contaminated feed for 10 weeks had adverse effects on the fish growth rate, PCV%, Hb concentration and Erythrocytic count . More added 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 aimed to study the drastic effects of AFB1 on the growth performance, survivability, nutrient utilization, some organs indices, carcass composition, residues of AFB1, and some parameters of blood hematology, protein profile and activities of the plasma enzymes, muscular and abdominal areas of the experimented fish *O. niloticus*. Also, this study was conducted to evaluate the ability of some nutritional agents namely, green tea, Dried Basil Seeds Meal , Requette Seed Meals, green tea and Fennel Seeds Meal (at a level of 0.5%) to detoxify the drastic effects of this dangerous toxin AFB1 on Nile tilapia fish for 15 weeks.

MATERIALS AND METHODS

This study was conducted to evaluate the ability of some medicinal and aromatic plants namely Dried Basil Seeds (T3), Requette Seed Meals (T4), Green Tea (T5), and Fennel Seeds Meal (T6), at a level of 0.5% to detoxify the drastic effects of this dangerous toxic AFB1 on the Nile tilapia fish for 15 weeks.

1- Water quality parameters:

Water quality parameters were measured weekly including temperature via a thermometer, pH using Jenway Ltd., Model 350-pH meters and dissolved oxygen using Jenway Ltd., Model 970-dissolved oxygen meters.

2- Experimental fish

A group of 180 Nile tilapia (*O. niloticus*) fingerlings with an average initial body weight of 7 g were used in this study. Fish were obtained from a fish farm at Tolombat 7 and transported to the wet lab, Faculty of Agriculture, Kafr El-Sheikh. They were maintained in the aquaria for one month before the beginning of the experiment for acclimatization purpose. Fish were stocked at a rate of 15 fish in each aquarium. Two aquaria per treatment were used. Each aquarium, which measured (60x35x40 cm) containing 70 L of water was supplied with well-aerated and dechlorinated tap water, four air stones were used for aerating the aquaria water. The fish were fed during the acclimatization period on artificial basal diet at a rate of 3% of the body weight, 2 times daily. At the beginning of the experiment period, fish were distributed into the aquaria at a stocking rate of 15 fish per aquarium. The experimental treatments were tested in two aquaria for each.

3- Experimental diets

A basal diet (25% crude protein, 17.80 ether extract, 8.50 crude fiber, 6.40 ash, 42.3 NFE, 453.14 Kcal/100g DM gross energy and 55.17 mg CP/Kcal GE, P/E ratio) was formulated from the commercial ingredients (fish meal 10%, soybean meal 32%, yellow corn 30%, sunflower oil 4%, wheat bran 14 % 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 operation was continued for 20 minutes. After homogenous mixture was obtained, forty ml water per hundred g diet was slowly added to the mixture

according to Shimeino *et al.*, (1993). These ingredients were pressed by manufacturing machine (pellets size 1mm), they were milled and toxin AFB1 was added at a concentration of 150 ppb to all diets (T2, T3, T4, T5, T6), except the control (T1). Anti-toxin (D.B.S), (R.B.M), (G.T.M), and (F.S.M) were added at a concentration of 0.5%. The ingredients and supplements were bought from the local market, aflatoxin B1 was produced through pellets fermentation using *Aspergillus parasiticus* NRRL 2999 according to the method described by Abdelhamid and Mahmoud (1996). Concentration of the produced AFB1 was calculated and incorporated into the experimental diets at a rate of 150 ppb.

4- Experimental procedure:

The experiment continued for 15 weeks. During the experimental period, the fish were fed the experimental diets at a rate of 3% of the live body weight daily. The diet was introduced twice daily, at 8 a.m. and 2 p.m. The amount of feed was adjusted weekly based on the actual body weight changes. Samples of water were taken daily and another sample was taken before and after adding the diets weekly from each aquarium to determine water quality parameters. Light was controlled by a timer to provide a 14h light: 10h dark as a daily photoperiod.

At the end of the experiment, six fish from each aquarium was 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 analysis of the basal diet and whole fish body were carried out according to A.O.A.C. (1984). AFB1 determinations in the media extract and the basal diet were determined as described by Abdelhamid (1990).

5-Growth parameters:

Average total gain (ATG), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER %), protein productive value (PPV %) and survival rate (SR) was calculated according to the following equations:

a- TWG (g/fish) = [Average final weight (g) – Average initial weight (g). **b-** ADG (g/fish/day) = [TWG (g)/experimental period]. **c-** SGR (%/day) = [Ln final body weight–Ln initial body weight] x 100/experimental period]. **d-** FCR= Feed Intake, dry weight (g)/Live weight gain, (As reported by De Selva and Anderson, 1995). **e-** PER (%) = Live weight gain (g)/ protein intake (g), (as reported by De Selva and Anderson, 1995).

f- PPV (%) = 100[final fish body protein (g) – initial fish body protein (g)/crude protein intake (g). **g-** SR = 100[total No. of fish at the end of the experimental /total No. of fish at the start of the experimental].

6 - Total tissue residues:

Total tissue residues of AFB1 were estimated by TLC (Thin Layer Chromatography) method described by Abdelhaomid, (1981). And hepatosomatic indices H.S.I = Liver weight x 100/Gutted fish weight (Jangaard *et al*; 1967).

7-Blood parameters determination:

At the end of the experiment, fish in each aquarium were weighed and 3 fish were taken randomly for blood sampling. Anti coagulated blood samples were performed immediately for counting red and white blood cells. Then, the blood samples were centrifuged at 3500 rpm for 15min to obtain blood plasma for determination of total protein, albumin, globulin (by differences), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using commercial kits and spectrophotometer (model 5010, Germany).

Hematological parameters:

Red blood cells count (RBCs× 10^6/mm) and white blood cells count (WBCs ×10^3/mm) were counted on an A0 Bright –Line Haemocytometer model (Neubauer improved, Precicolor HBG, Germany).

Biochemical parameters:

Total blood plasma protein was determined by using a commercial kit (Spain React Company, Spain) according to the method recommended by Gornall *et al.* (1949). Plasma albumin was determined by using a commercial kit (Spain React Company, Spain) according to the method recommended by Weichsebum (1976), however, plasma globulin was calculated by subtracting albumin from total plasma protein concentration according to Doumas and Biggs (1976). AST and ALT were determined by using a commercial kit (Randox Company, Germany) according to the method described by Varley (1976).

8- Muscular and abdominal areas:

The fish were examined also for infiltration / muscular areas using Echo Scan (H5/s) Ultrasonic Diagnostic Instrument, Budapest Remeny Co. according to (Salem, 2008).

9- Statistical analysis:

The obtained numerical data were statistically analyzed using SPSS (1997) for one-way analysis of variance. When F- test was significant, least significant difference was calculated according to Duncan (1955).

RESULTS AND DISCUSSION

1-Water quality parameters of rearing fish:

All tested water quality criteria were suitable for rearing Nile tilapia *O. niloticus* fingerlings as cited by Abdel-Hakim *et al.* (2002). Since water temperature ranged between 25 and 26°C, pH values between 7 and 8 and dissolved oxygen were between 5 and 7 mg/l. In the same trend, Abdelhamid *et al.* (2004), tested water quality (temperature °C, pH value, and dissolved oxygen mg/l) criteria and reported similar values for rearing Nile tilapia fish *O. niloticus*.

2-Growth performance and survival rate:

Data presented in Table (1) showed that aflatoxin B1 (AFB1) had negative effects ($P<0.05$) on the growth performance (live body weight, body weight gain, specific growth rate). And showed that there were no significant ($P\geq0.05$) differences among the initial body weights in all treatment. While average total weight gains (TWG), average daily gain (ADG) and specific growth rate (SGR) of the experimental fish were the best for the fish fed T_1 (control). However, T_3 (aflatoxin-contaminated diet plus 0.5% Basil Seeds) and T_4 (aflatoxin-contaminated diet plus 0.5% Requette Seed), were better than T2 (aflatoxin B1 150 ppb alone). In general, the fish fed diet 2 (contained AFB1 alone) recorded the lowest values for AWG, ADG, SGR and SR compared to the other diets. On the other hand, there were no significant differences among the fish fed on diets T3, T4, T5, and T6. These results agree with the finding of Abdelhamid. (2008) and Salem *et. al.* (2009) who reported that AFB1 toxin at levels 100ppb, and 150ppb in fish diet fingerlings without adding any spices caused a clear growth depressing effect which was significant. This depression effect might be due to depressed efficiency of feed used as a result for expelled the feed from the mouth of fish (Nguyen *et al.*, 2002). Adding some medicinal plants and some spices to contaminated diet such as (D.B.S), (R.B.M), (G.T.M), and (F.S.M) reduced the toxic effect of the AFB1 and stimulated growth performance in fish. The beneficial effect of (D.B.S), (R.B.M), (G.T.M) and (F.S.M) may be due to its content of volatile oil and its high levels of ocimene, methyl chavecol and linalool which considered the predominant effective compounds in the volatile oil (El-Dakar *et al.*2005), aromatic substance or essential oils (Khalifea, 1995), polysaccharides (Cheng-Chun Chou *et al.*, 1999), digestive enzymes activities (Saleh, 2004), and beneficial effect for inhibition of carcinogenicity and immunotoxicity effects of AFB1 (Nguyen *et al.* 2002). The present results agreed with those obtained by El-Dakar *et al.* (2004), Asmaa Abdelmonem et al., (2002), (Cheng-Chun Chou *et al.*, (1999) and Shalaby (2004).

Servival rate results presented in Table (1) showed that mortality rate was increased significantly ($P<0.5$) in fish fed contaminated diets (150ppb AFB1 without any addition) in comparison with other treatments. These results agreed with the findings reported by Salem *et. al.* (2009) who mentioned that AFB1 at levels of 100ppb and 150ppb significantly increased the mortality rate in tilapia. The ability of (D.B.S), (R.B.M), (G.T.M) and (F.S.M) to decrease the mortality rate may be due to its content of some constituent that stimulate the immunity system.

3-Efficiency of Feed and Protein Utilization:

Data in Table (2) show the feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), and hepatosomatic indices (HSI). No differences ($p<0.05$) were found in feed intake, feed conversion ratio, protein productive value, and hepatosomatic indices among T1, T3, T4, T5, and T6, but differences ($p<0.05$) between fish fed diet T2 and all the experimental diets were found for all the tested parameters. The best results were found when fish fed diet T3 (that containing 0.5%D.B.S), diet T4 (containing 0.5 %R.S.M)., diet T5 (containing 0.5 %G.T.M), diet T6 (containing 0.5 % F.S.M, and the control diet. Diet T2 showed the lowest FI, FCR, PER and PPV. The increasing feed and nutrient utilization as affected by adding 0.5%D.B.S, 0.5%R.S.M, 0.5%G.T.M, and 0.5% F.S.M may confirm the results obtained by Abdlmonem *et al,* (2002); Shalaby, *et al.* (2004); and

Eldakar *et al.*(2004 and 2005). The seniority of dietary D.B.S followed by R.S.M., G.T.M., and F.S.M., in improving these parameters may be due to its contents of volatile oil and some effective components (Eldakar *et al.* 2005). Hepato somatic index (HSI) was increased in T2 (AFB1), but not significantly (P>0.05) affected by the dietary treatments, T1, T3, T4, T5, and T6 (Table, 2).

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

Initial weight	Final weight	TWG g/fish	ADG g/fish/day	SGR %/day	SR %
10.07±0.01	40.83±0.00a	30.76±0.006a	0.29±0.005a	1.33±0.05a	100.00±0.00a
10.48±0.03	25.22±0.07c	14.74±0.003c	0.14±0.001c	0.84±0.04c	50.00±0.05 c
9.80±0.004	36.65±0.09b	26.85±0.009b	0.26±0.005ab	1.26±0.05ab	93.33±0.03 b
10.09±0.06	35.89±0.08b	25.80±0.008b	0.25±0.005b	1.21±0.04b	95.66±0.66ab
9.59±0.005	32.92±0.04b	23.33±0.006b	0.22±0.005b	1.18±0.01b	93.33±0.00 b
10.31±0.03	33.90±0.01b	23.59±0.005b	0.23±0.005b	1.13±0.01b	95.66±0.03ab

**Means (within the same column) with unlike superscripts are significantly different (P≤0.05).*
T_1- (Control diet), T_2- ($Diet_1$ + $AFB_1$150ppb), T_3- ($Diet_1$ +AFB_1 150ppb + 0.5% D.B.S), T_4- ($Diet_1$ +AFB_1 150ppb + 0.5% R.S.M, T_5- ($Diet_1$ + AFB_1 150 ppb + 0.5% G.T.M), and T_6-($Diet_1$ +AFB_1 150ppb + 0.5% F.S.M).

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

Treat. No.	FI (g/fish)	FCR	PER	PPV %	HSI %
T1	60.44±1.73a	1.96±0.04b	2.04±0.12a	36.39±0.05a	2.10±0.02b
T2	55.80±0.17b	3.78±0.03a	1.06±0.01c	14.33±0.07b	3.40±0.04a
T3	58.30±0.94a	2.17±0.07b	1.84±0.07b	34.55±0.05a	2.20±0.05b
T4	57.90±0.92a	2.24±0.07b	1.78±0.10b	31.09±0.02a	2.39±0.01b
T5	56.64±0.36a	2.43±0.01b	1.65±0.04b	28.24±0.03a	2.32±0.10b
T6	59.70±1.06a	2.53±0.05b	1.58±0.03b	29.49±0.04a	2.25±0.06b

**Means (within the same column) with unlike superscripts are significantly different (P≤0.05).*
T_1- (Control diet), T_2- ($Diet_1$ + $AFB_1$150ppb), T_3- ($Diet_1$ +AFB_1 150ppb + 0.5% D.B.S), T_4- ($Diet_1$ +AFB_1 150ppb + 0.5% R.S.M, T_5- ($Diet_1$ + AFB_1 150 ppb + 0.5% G.T.M), and T_6-($Diet_1$ +AFB_1 150ppb + 0.5% F.S.M).

4-Biochemical analysis of the diets and whole fish body:

a- Chemical composition of the experimental diets:

The chemical analysis revealed that no differences were observed among all diets in DM, CP, and ash, while there were some differences observed among different diets for EE, and CF, these differences may be due to the ingredients themselves. The CP content was between 25 to 25.50% on DM basis, the calculated energy was similar in the tested diets, where the GE values ranged between 453.14 and 463.66 Kcal/100g, as mentioned in the materials and methods. Such level was within the range suggested by NRC (1993) in the practical diets for tilapia. However, it was nearly similar to that used by Abdel-Maksoud *et al.* (1998).

b- Chemical composition of the whole fish body

The proximate chemical analysis of the whole body of the tested tilapia fish is given in Table 3. Dry matter (DM) content was not significantly different for all treatments and there were high significant (P<0.5) differences among the dietary treatments in CP, EE and ash contents. The highest CP was observed in the fish groups of T1, T_3, T_4, T5, and T_6 and the lowest values were found in groups T_2. The highest EE was observed in the fish group of T2.

Similar results were recorded by Abdelhamid *et al.* (2002) concerning fish carcass analyses. The same adverse effects of AFB_1 on carcass composition of *O. niloticus* were recorded too by Khalil (1997) and Salem (2002).

The present results agreed with the findings of Salem (2002) who found that the control group of fish had the highest (P < 0.05) DM and CP values and the lowest (P < 0.05) EE percentage. The latest

author added that percentages of DM and CP decreased as the levels of the aflatoxin B_1 increased, while the value of EE increased with increasing the levels of AFB_1. Also, Abdelhamid *et al.* (2006) and Mehrim *et al* (2006) concluded that aflatoxin B_1 significantly reduced DM and CP contents of the *O. niloticus* fish carcass, but it significantly increased EE and ash contents of the fish.

In accordance with the present findings, Abdelhamid *et al.* (2002) reported that the aflatoxic diets significantly ($P < 0.01$) reduced the fish flesh crude protein content but increased its fat and ash contents proportional to the dietary levels of the aflatoxin. Yet, the positive effects of T6, T7, and T8 used in the present study may be due to increased immunity that reduces the effect of toxin and hence hided its negative effects (to some extent) 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 ($\bar{x}$* ± SE).

	DM%	CP	EE	ASH	GE Kcal/100g**
Initial	24.80	55.00	18.50	19.00	560.54
T1	23.81±0.50	60.50±0.50a	20.00±0.01b	19.50±0.01ab	605.30±0.00
	23.26±0.03	57.00±0.04b	23.40±0.26a	19.60±0.21ab	607.55±0.00
	23.45±0.07	60.00±0.06a	21.14±0.96b	18.86±0.01b	612.88±0.00
	24.10±0.01	59.50±0.08a	21.10±0.36b	19.40±0.80ab	611.92±0.00
	23.25±0.09	59.00±0.01a	21.20±0.45b	19.80±0.20ab	609.15±0.00
	23.37±0.01	59.40±0.07a	20.40±0.31c	20.20±0.13a	608.03±0.00

**Means superscripted (in the same column) with different letters significantly (P≤0.05) differ.*
*** Gross energy was calculated by multiplication the factor 4.1, 5.6 and 9.44 kcal GE/g DM carbohydrate, protein and fat, respectively (Jobling, 1983).*

5- Residues of aflatoxin in the whole fish body:

The data concerning aflatoxin residues in the whole fish body are shown in Table 4. The control fish were free from the AFB1; whereas, T_2 reflected the highest level being 96.50 ppb aflatoxin B_1, followed by T_5, T_6, T_4 and T_3, respectively. So, T_3 (D.B.S.) and T4 (R.B.S) were the best treatments in reducing the level of the residues, followed by T_6 (F.S.M.).

In this respect, El-Banna *et al.* (1992) reported that AFB_1 residues in the *O. niloticus* flesh showed a commutative effect related to the level of dietary AFB_1 and feeding period. Also, Hussain *et al.* (1993) suggested that aflatoxin B_1, G_1 and G_2 were detected in muscles of the treated groups of walleye fish at a level up to 20 ppb. In the same trend, Soliman *et al.* (1998) mentioned that the significant increase of aflatoxin residue was observed in *O. niloticus* flesh after 6 months. Abdelhamid *et al.* (2006) and Mehrim *et al* (2006) found residues of AFB_1 in the whole body of the aflatoxicated *O. niloticus* fish at the end of the experiment and tended to decrease after freezing periods.

Table (4): Residues of aflatoxin B_1 in the tilapia fish as affected by the dietary treatments extended for 15 weeks (wet weight basis).

Treatment	AFB_1 in Fish (µg/ kg)
T1	0.00
T2	96.50
T3	20.52
T4	25.33
T5	27.40
T6	26.50

T_1- (Control diet), T_2- ($Diet_1$ + $AFB_1$150ppb), T_3- ($Diet_1$ +AFB_1 150ppb + 0.5% D.B.S), T_4- ($Diet_1$ +AFB_1 150ppb + 0.5% R.S.M, T_5- ($Diet_1$ + AFB_1 150 ppb + 0.5% G.T.M), and T_6-($Diet_1$ +AFB_1 150ppb + 0.5% F.S.M).

6- Blood analysis:

Data of some hematological parameters are illustrated in Table 5 and indicated that there were significant ($P \leq 0.05$) differences among the tested dietary treatments, in all criteria, except albumin. Yet,

T_1 (control diet) had higher (P≤0.05) RBCs followed by the treatments T3, T4, T5, and the secand treatment T6. T_2 diet had higher (P≤0.05) white blood cells (WBCs) count followed by the T_4 and T_6. There were no significant (P≥0.05) differences among the dietary treatments T1, T3, T4, T5, and T6 for the concentrations of globulin, and total protein, this may be due to their chemicals and physicals properties and its positive effects on the immune system. Since, D.B.S, R.S.M, G.T.M and F.S.M stimulate liver enzymes (Khalifea, 1995; Saleh, 2004, and Cheng-Chun Chou *et al.*, 1999). Therefore, T2 lowered total protein, albumin and globulin.

The present results concerning, AST and ALT activity had widely differences among the different treatments (Table 5) indicating a damage of the liver and probably also the kidney. Moreover, Abdelhamid *et al.* (2006) found that the activity of AST and ALT enzymes increased significantly (P≤ 0.05) in the fish fed aflatoxin-B1 contaminated diet. These finding agreed with evidence for acute aflatoxin B1 nephrotoxicity wich was provided by distended gall bladder indicating disrupted osmoregulation (i.e. water retention) as reported by Mehrim et al. (2006).

Recently In the same trend, Abdelhamid et al. 2006 and Mehrim et al., 2006 found that AFB1 caused not significant decrease in concentration of red blood cells count and significantly increase in white blood cells count and transaminases activity of aflatoxicated *O. niloticus* fish. As well as, the positive effects of some nutritional additives used in the present study, namely D.B.S, R.S.M, G.T.M, and F.S.M may be due to the increased of immunity and hence hide its negative effects on blood parameters of *O. niloticus* fish.

Table (5): Means and SE of some hematological, plasma biochemical (Kidney function), and some plasma biochemical (Liver function) parameters at the end of the 15 weeks experimental feeding of the tilapia fish.

Treat. No.	RBCs (10^6/mm)	WBCs (10^3/mm)	Total protein (g/dl)	Albumin g/dl	Globulin g/dl	AST U/L	ALT U/L
T1	1.600±0.00a	500.00±1.00c	4.00±0.50a	1.50±0.80	2.50±0.40a	34.00±4.00b	45.00±3.00b
T2	0.750±0.00b	690.00±0.00a	2.50±1.00b	1.20±0.50	1.30±0.30b	55.50±6.00a	62.00±5.00a
T3	1.250±1.00a	600.00±5.00b	3.60±1.00a	1.10±0.00	2.50±0.00a	36.00±0.00b	43.00±3.00b
T4	1.200±0.05a	650.00±2.00ab	3.50±0.20a	1.20±0.20	2.30±1.00a	41.00±1.00b	42.00±2.00b
T5	1.150±0.05a	600.00±1.00b	3.60±0.10a	1.30±0.40	2.30±0.10a	34.00±4.00b	35.00±5.00c
T6	1.100±0.05ab	610.00±1.00ab	3.60±1.00a	1.20±0.30	2.40±0.30a	32.00±2.00c	37.00±0.00c

** Means superscripted (in the same column) with different letters significantly (P≤0.05) differ.*

T_1- (Control diet). T_2- ($Diet_1$ + $AFB_1$150ppb). T_3- ($Diet_1$ +AFB_1 150ppb + 0.5% D.B.S). T_4- ($Diet_1$ +AFB_1 150ppb + 0.5% R.S.M, T_5- ($Diet_1$ + AFB_1 150 ppb + 0.5% G.T.M), and T_6-($Diet_1$ +AFB_1 150ppb + 0.5% F.S.M).

7- Musculature and abdominal areas:

This test showed the variations among the tested fish groups, where Figs. 1, 2, 3, 4, 5, and 6 presented the groups 1, 3, 4, 5, and 6 (control, 0.5% D.B.S, 0.5%R.S.M, 0.5%G.T.M, and 0.5%F.S.M) with larger musculature (white area) than in group 2(AFB1 without any addition) Fig. 2. The increase in abdominal cavity (black area) is proportional to the level of 150ppb AFB1 without addition of medicinal seed or leave. These results agreed with the findings reported by Salem, *et. al.*, (2009) who mentioned that AFB1 at levels of 100ppb and 150ppb increased significantly the abdominal cavity (black area) and decreased the musculature (white area) in tilapia.

CONCLUSIONS

From the foregoing results it could be concluded that aflatoxin contamination of fish diets caused many drastic effects in all tested parameters. It is very dangerous from the view point of fish production and public health. It could be recommended for the beneficial using of 0.5% D.B.S (or R.S.M, G.T.M, and F.S.M) to alleviate the toxic effects of AFB_1 contaminated diets. Also, from the above results, it is a must to conduct a lot of scientific efforts in this trend to use the medical or aromatic plants and other natural agents to detoxify the toxic effects of mycotoxic (particularly aflatoxic) diets of fish and other animals.

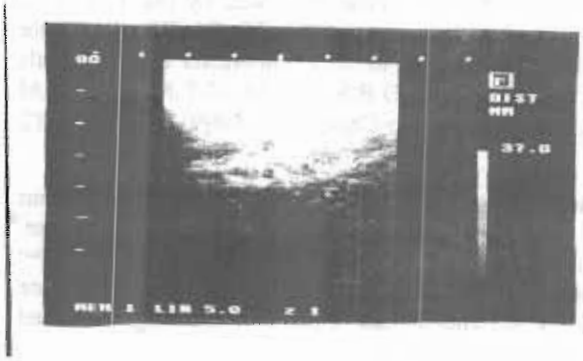
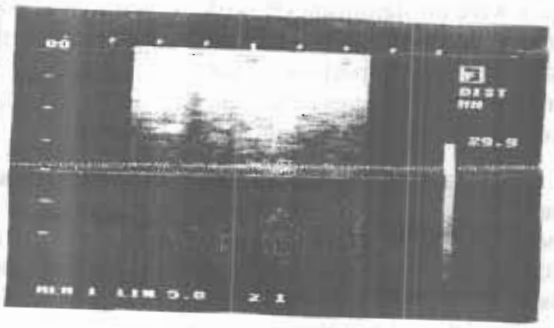


Fig.(1) Diet 1 (Control) Musculature area about 37mm



Fig(2) Diet2 (AFB1 without any added) Musculature area about 29.9mm.

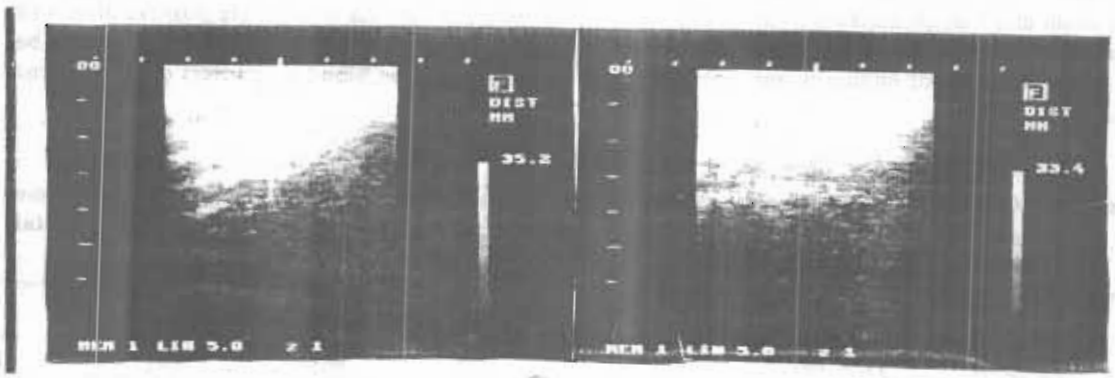


Fig.(3) Diet3 (AFB1+0.5 % D.S.B.) Musculature area about 35.2mm

Fig.(4) Diet4 ((AFB1+0.5 % R.S.M.) Musculature area about 33.4mm

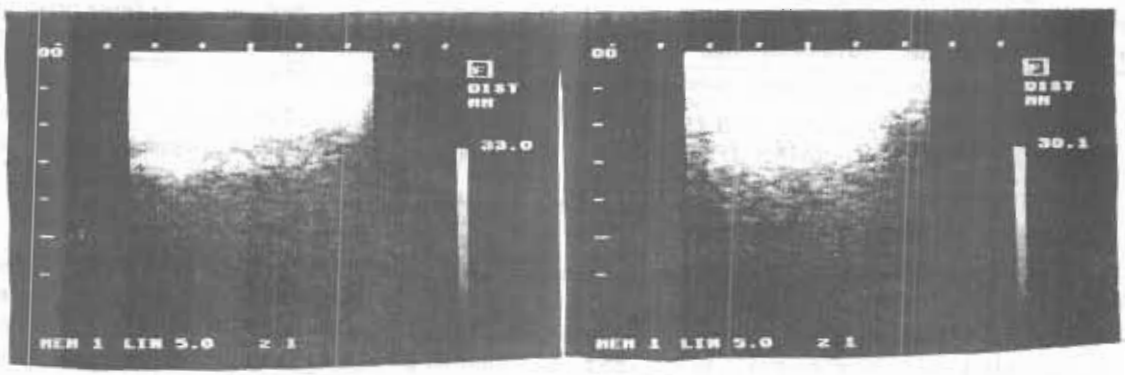


Fig.(5) Diet5(AFB1+0.5 % G.T.) Musculature area about 33mm

.Fig.(6) Diet6(AFB1+0.5 % F.S.M.) Musculature area about 30.1mm

REFERENCES

Abd El- Hakim, N. F.; M.N. Bakeer and M.A. Soltan. (2002). Water Environment for Fish Culture. Deposition No.: 4774, ISBN: 977-298-228-5.

Abdelhamid, A.M. (1981). Mykotoxine in Osterreichischen Futtermitteln. Dissertation-Bodenkulture Univ.-Wien.

Abdelhamid, A.M. (1990). Occurrence of some mycotoxins (aflatoxin, ochratoxin A, citrinin, zearalenone, and vomitoxin) in various Egyptian feeds. Arch. Anim. Nutr., 40: 647-664.

Abdelhamid, A. M. (2008). Thirty years (1978-2008) of mycotoxins research at faculty of agriculture, Almansoura University, Egypt. Engormix.com, Mycotoxins Technical Articles, 11p.

Abdelhamid, A.M. and K.I. Mahmoud. (1996). Elimination or adsorption of aflatoxin from poultry feedstuffs. Proc. Conf. Food borne Contamination & Egyptian's Health, Mansoura Univ., 26-27 Nov., pp. 61-69

Abdelhamid, A.M. and M.R. Saleh. (1996). Are aflatoxin and ochratoxin endemic mycotoxins in Egypt? Proc. Conf. Food Borne Contamination & Egyptian's Health, Mansoura Univ., Nov. 26-27, pp: 51-59.

Abdelhamid, A.M.; A.M. Ahmed and H.M. El-Meleigy (2002). Detoxification of aflatoxin-contaminated ration by some physical and chemical mean, J. Agric.Sci. Mansoura Univ., 27: 8213-8224.

Abdelhamid, A.M.; A.M. El-Mansoury; A.I. Osman and S.M. El-Azab (1999). Mycotoxins as causative for human food poisoning under Egyptian conditions. J. Agric. Sci., Mansoura Univ., 24: 2751 – 2757.

Abdelhamid, A. M.; B. R. Nemetallah; M. A. Abd Allah and T. E. Mousa (2006). Hemolytic activity in blood plasma of Oreochromis niloticus under different types of stress. The 3rd Int. Conf. for Develop. And the Env. In the Arab World, March 21-23, Assuit Univ., pp: 153-169.

Abdelhamid, A.M.; E.A. Sadik and S.A. Fayzalla (1985). Preserving power of some additives against fungal invasion and mycotoxin production in stored-crushed-corn containing different level of moisture. Acta Phytopathologica Academia Scientiarum Hungaricea, 20 (3-4): 309-320.

Abdelhamid, A.M.; M.F. Salem and A.E. Tolan (2004). Evaluation of sesame meal as a dietary protein source for Nile tilapia (*Oreochromis niloticus*) fingerlings .J.Agric. Sci.Mansoura Univ., 29:6887-6897.

Abdelmonem, Asmaa.; M.M. Shymaa and E. Ashraf (2002). Response of Red tilapia to different levels of some medicinal plants by-products: Requette seed meal, Proc., 1st. Conf. Aqua, El-Arish. 13-15 Dec., pp 247-259.

Abdel-Wahab, A. M.; M. M. Hassouna; A. M. Abdel-Maksoud and A. M. Abu-Seef (2007). Cinnamon as a feed supplement in Nile tilapia, *Oreochromis niloticus*, rations that reared in earthen ponds. Egyptian J. Nutrition and feeds, 10 (2): 331-890.

A.O.A.C. (1984). Official Methods of Analysis, 15th Ed. Association of Official Analysis of Chemists, Washington D.C.

Abdel-Maksoud, A.M.; G.E. Allam; S.M. Abou-Fatouh and R.M Abou Zied (1998). Evaluation of *Nigella* seeds, as a feed additive in diets of Nile tilapia. Fayoum. J. Agric. Res.&Dev. 12: 170-181.

Bathnagar, D. and S. Garcia (2001). *Aspergillus*. In: Labbe´, R.G., Garcı´a, S. (Eds.), Guide to Foodborne Pathogens. John Wiley and Sons, New York, pp. 35– 49.

Cheng-Chun Chou, Lon-Leu Lin, King-Thom Chung (1999). Antimicrobial activity of tea as affected by the degree of fermentation and manufacturing season. International Journal of Food Microbiology, 48, (2):125-130.

Cotty, P.J. and R. Jaime-Garcia (2007). Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int.J. Food Microbial; 119 (1-2):109-115.

De Silva, S.S. and T.A. Anderson (1995). Fish nutrition in aquaculture Ed., Champ man and Hall, 2-6 Bouday Raw, London SEI8 FIN, UK.

Doumas, B.T. and H.G. Biggs (1976). Determination of serum albumin in standard method of clinical chemistry.Vol.7 Edited by G.R. Cooper, New York, Academic Press.

Duncan, D.B. (1955). Multiple ranges and multiple F-tests. Biometrics, 11:1-42.

El-Banna, R.; M.M. Teleb; H.M. Hadi and F.M. Fakhry (1992). Performance and tissue residue of tilapias fed dietary aflatoxin. J. Veterinary Medical Giza, 40: 17-23.

El-Dakar, A.Y. (2004). Growth response of hybrid tilapia, *Oreochromis niloticus* x Oreochromis aureas, fingerlings to diets supplemented with different levels of caraway seeds. J. Agric. Sci. Mansoura Univ., 29(11): 6094-6094.

El-Dakar, A.Y.; A.Y. Shymaa; M. Shalaby; S.M. Abead and M.M. Khalafala (2005). Use of as feeding stimulation in diets of marine shrimp penaeus japonicus. Egyption J. Nutrition and feec ,8(1) Special Issue: 1065-1076.

FAO, (Food and Agriculture Organization of the United Nation) (2002). Evaluation of Certain Mycotoxins in Food: Fifty-Sixth Report of the Joint FAO/WHO Expert Committee on Food Additives, World Health organization, Geneva.

Gornall, A. G.; G. J. Bardawill and M. M. Parid (1949). Method of protein in serum blood .J.Biol.Chem., 177: 751.

Hussain, M. I.; T. Wilson and R. C. Summerfel (1993). Effect of aflatoxin-contaminated feed on morbidity and residues in walleye fish. Veterinary and Human Toxicology, 35: 396-398.

Ibrahim, D.H.E. (2004). Biochemical studies on fungi toxins of feedstuffs in Dakahli and Damietta governorates. M.Sc. Thesis. Fac. of Agric. Mansoura Univ.

Jangaarrd P.M.; R.G. Ackman; and A.J. Spois (1967).Seasonal studies of the fatty acids composition of liver, flesh, roe and milt lipid . J.Fish Res. Biol. of Canada, 24:613-627.

Jobling, S. (1983). A short review and critique of methodology used in fish nutrition studies .J.Fish Bio. 23:685-703.

Khalifea, M.M. (1995). Nigella seed oil meal as a protein supplement in broiler diets. MSc.Thesis, Faculty of Agriculture, Univ. of Alexandria.

Khalil, F.F. (1997). Effect of aflatoxin B_1 (AFB_1) contaminated diets with and without Urpason® on performance of Nile tilapia fry. Egyptian J. Nutrition and Feeds, 1: 351-368.

Kunio Suzuki, N.M. and D.K. Sakai (2006). Efficacy of green tea extract on removal of the ectoparasitic flagellate Ichthyobodo necator from chum salmon, *Oncorhynchus keta*, and masu salmon, *O.masou*), Aquaculture, 259:17-27.

Lewis, K. and F.M. Ausubel (2006). Prospects for plant-derived antibacterials Nature Biotechnology, 24:1504-1507SAS Institute. SAS/SAT User's guide-version 6.Cary, 1989.

Mehrim, A. I.; A. M. Abdelhamid; A. A. Abo Shosha; M. F. I. Salem and M. EL-Sharawy (2006). Nutritious attompts to detoxify aflatoxin diets of tilapia fish: clinical, biochemical and histological parameters, Journal of the Arabian Aquaculture Society, (1) (2): 69-89.

Nguyer, A. T.; J. M. Grizzle; R. T. Lovell; B. B. Manning and E. G. Rottinghaus (2002). Growth and hepatic lesions of Nile tilapia *Oreochromis niloticus* fed diets containing aflatoxin B_1. Aquaculture, 212: 311-319.

NRC (1993). Nutrient Requirements of Fish. National Academy Press. Washinghton.DC, P.114.

Reddy, S.V.; and W.B. Farid (2009). Properties of aflatoxin and it production fungi J. Sci. Food Agric., 70: 430 – 438.

Saleh, H.M. (2004). Effect of supplementing fenugreek seeds as glactagogue on performance of lactating ewes. Egyptian J. Nutrition and Feeds, 7(2):155-165.\

Salem, M.F.I. (2002). Effect of dietary graded levels of aflatoxin B_1 on growth performance and chromosomal behavior of Nile tilapia *Oreochromis niloticus*. Ph.D. Thesis, Kafr El-Sheikh, Tanta Univ.

Salem, M.F.I. (2008). Replacement of fish meal by silkworm, *Bombyx mori* Pupa meal, in Nile tilapia, *Oreochromis niloticus* diets. Egyptian J. Nutrition and Feeds, 11(3): 611-624.

Salem, M.F.I.; E.M. Abd El-Raouf; N.M. Eweedah and. B. S. Mohamed (2010). Influence of some medicinal plants as anti-mycotoxins in Nile tilapia (*O. niloticus*) diets. Proc. Global Fisheries&Aquaculture Research Conf., 24-26 October, pp 227-242.

Shalaby, S.M. (2004). Response of Nile tilapia, fingerlings to diets supplemented with different levels of fenugreek seed.J.Agric.Mansoura Univ., 29: 2231-2242.

Shimeino, S.; T. Masumoto and T. Hujita (1993). Alternative protein sources for fish meal diets of young yellowtail. Nippon Suisan Gakkaishi, 59:pp137-143.

Soliman, K. M.; A.M. Ayesh; M. A. Essa and K. Naguib (1998). Fix-A-tox in aquaculture: 1-Effect of aflatoxin decontamination by selective chemisorbent materials on the *Oreochromis niloticus* with considering fish processing efficiency. J. Egypt. Ger. Soc. Zoo., 25 (A) Comparative Physiologies: 1 – 19.

SPSS (1997). Statistical package for the social sciences, Revisions 6, spss Inc, Chicago, USA.

Varley, V. (1976). Practical Clinical Biochemistry. 4th Edition, New Delhi, India. Etal.

Weichsebum,T. E. (1976). Method for determination of albumin in serum blood.Amer.J.Clin.Pathol., 16-40.

محاولة تخفيف سمية الافلاتوكسين ب1 على اسماك البلطى النيلي بواسطة بعض النباتات الطبية.

محمود فؤاد اسماعيل سالم

المعمل المركزى لبحوث الثروة السمكية- بالعباسة ابو حماد- شرقية – وحدة بحوث الثروة السمكية بسخا – مصر.

أجريت هذه الدراسة للكشف عن التأثيرات السامة للأفلاتوكسين ب1 على اصبعيات البلطى النيلى ، وأيضا لمحاولة إزالة الآثار السيئة لهذه السموم عن طريق إضافة بعض النباتات والبذور الطبية، لذلك تم إضافة 0.5% من كل من هذه المواد وهى (بذور نبات الريحان، بذور الجرجير، الشاى الاخضر، و مسحوق نبات الشمر) لعلائق أسماك البلطى النيلى الملوثة بالأفلاتوكسين ب1 (150 جزء فى البليون افلاتوكسين ب1). وقدمت هذه العلائق للأسماك على مدار 6 أيام فى الاسبوع بمعدل 3% من الكتلة الحيوية الحقيقية للأسماك فى الأحواض الزجاجية، حيث مثلت كل معاملة فى مكررتين، وتم تغذية الأسماك على هذة العلائق لمدة 15 إسبوعا. ومن أهم النتائج التى تم التوصل اليها هو أن العلائق الملوثة بالأفلاتوكسين أدت الى تأثيرات سيئة على كل من معدلات النمو والإعاشة للأسماك، وأيضا تأثيرات سيئة على معدلات الإستفادة من الغذاء والبروتين ، والتحليل الكيماوى لجسم الأسماك، وكذا سجلت النتائج وجود متبقيات من الأفلاتوكسين ب1 فى جسم الأسماك المعاملة بنسبة عالية بالمقارنة بباقى المعاملات، كما أثر هذا السم تأثيرات سيئة على قياسات الدم المختلفة للأسماك، وايضا أظهرت النتائج إنخفاض مساحة العضلات التى تغذت على علائق ملوثة بالافلاتوكسين ب1 وزيادة فى مساحة التجويف البطنى نتيجة لزيادة نسبة الارتشاحات. وكذلك اظهرت النتائج أن العليقة المحتوية على بذور الريحان وبذور الجرجير ومسحوق الشاى الاخضر والشمر أعطت أقل تأثيرات سيئة للافلاتوكسين على الاسماك المعاملة، حيث تحسنت كل القياسات السابقة الذكر. وبصفة عامة أوضحت النتائج المتحصل عليها فى هذة الدراسة أن بذور الريحان وبذور الجرجير ومسحوق الشاى الاخضر والشمر لها تأثير تثبيطى على الافلاتوكسين ب1 والتى قد تصيب أعلاف الأسماك وتسبب أضرارا بالغة على صحة الأسماك مما قد يؤدى إلى حدوث خسارة إقتصادية فى مزارع الأسماك.