DETOXIFICATION OF AFLATOXIN CONTAMINATED RATION BY CHEMICAL, BIOLOGICAL AND SPICES METHODS IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) DIETS

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ABSTRACT

This study was done to investigate the toxic effects of aflatoxin B1 (AFB1) on Nile tilapia *(Oreochromis niloticus)* fingerlings and detoxifying these drastic effects by using some antimycotoxins. Therefore, 0.5 and 1g of each Black pepper, Filofeed plus and Cap T_2 were added to an aflatoxin diet (150 ppb) for fingerlings. These diets were offered 6 days a week at 3% daily of actual biomass in glass aquaria (three aquaria / treatment) in the wet Lab. The experiment lasted for 14 weeks (98 days). The Aflatoxic diet had adversely affected growth performance, survival rate, feed and protein utilization, muscular and abdominal areas and carcass composition of fish as well as residues of AFB1 (ppb) was observed in the viscera and muscles of fish. The addition of antitoxins alleviated aflatoxicosis of fish, moreover, it improved all the above tested parameters. Generally, the results obtained of this study recommended that adding 0.5 or 1g of Black pepper, Filofeed plus and Cap T_2 as fish feed additives could be used as detoxifying agents for aflatoxin.

Key words: Tilapia– aflatoxin B1- antimycotoxins- Black pepper - Filofeed plus - Cap T2- growth performance

INTRODUCTION

Tilapia is the third most important cultured fish group in the world, after carps and salmonids **(FAO, 2002).** Tilapia production has increased greatly in the past two decades and world production of farmed tilapia exceeded two million metric tons in 2004.Tilapia are currently raised in different types of production systems ranging from pond, tank, cage, flowing water and intensive water reuse culture systems. **(El Sayed *et al*., 2005**).

The intensification of aquaculture and globalization of the seafood trade have led to remarkable developments in the aquaculture industry. The global aquaculture industry currently accounts for over 45% of all seafood consumed. That figure has been projected to increase to 75% over the next 20 years **(FTU, 2007).** In Egypt, fish

production from aquaculture represented about 60% of total fish production sources **(GAFRD, 2007).**

The mycotoxigenic fungi involved with the human food chain belong mainly to three genera: *Aspergillus*, *Fusarium* and *Pencillium*. While *Fusarium species* are destructive plant pathogens producing mycotoxin before, or immediately post harvesting, *Penicillium* and *Aspergillus species* are more commonly found as contaminants of commodities and foods during drying and subsequent storage (**Abdelhamid, 1989 and Sweeney and Dobson, 1998).** Also**, Farr *et al.* (1989)** added that *Aspergillus flavus* and *Aspergillus Parasiticus* are important contaminants of certain foods and animal feeds because of their ability to produce aflatoxin.

Aflatoxins are considered the most carcinogenic, mutagenic and teratogenic poisonous by – product of the growth of the molds *Aspergillus flavus* and *Aspergillus parasiticus,* are the most studied and widely known mycotoxins**.** There are four major groups of aflatoxins: B1, B2, G1 and G2. Aflatoxins M1**,** a metabolite of aflatoxins B1 in mammals, may be found in the milk of animals eating feeds contaminated by Aflatoxins B1**(Conner, 1993 and FAO, 2002).** Aflatoxins B1, B2, G1 and G2 are classified as Group1 human carcinogens whereas M1 is classified as Group 2 probable human carcinogen **(Ioannou – Kakouri *et al.,* 1999).**

Aflatoxins are a major contaminant in aqua feeds and considered as a causative agent for fish mortality, morbidity and low productivity besides its residues in fish carcass leading to economic losses, human toxicity and affects public health specially in Egypt **(El – Fiky and Zaki, 1994; Abdelhamid and Saleh, 1996; and Abdelhamid *et al.,* 1998).** Practically, it is not possible to destroy the contaminated feed, therefore to prevent aflatoxicosis in fish, increasing animal immunity **(Zaky *et al.,* 2000 and Sahoo and Mukherjee, 2003)** and detoxification chemically **(CAST, 2003)** or biologically **(Nayek *et al.,* 2007)** were used.

Therefore, the present work aimed to study the drastic effect of AFB1 on the growth performance, survivability, nutrient utilization, some organs indices, carcass composition, residues of AFB1, and muscular and abdominal areas of the experimental fish Nile tilapia *(O. niloticus)* fingerlings. Also, this study was conducted to evaluate the ability of some nutritional agents, namely Black pepper (as spices additives), Filofeed plus (as chemicals additives) and Cap T2 (as biological additives) at levels of 0.5 and 1g from each to detoxify the drastic effects of this dangerous toxin AFB1 on Nile tilapia fish.

MATERIALS AND METHODS

The present study was carried out at the fish lab, Sakha Aquaculture Research Unit, Central Laboratory for Aquaculture Research-Abbassa, during summer season 2014. Feeding experiment was conducted for 14 weeks.

1- Experimental fish:

The experimental fish, *(Oreochromis niloticus*), were collected from a private farm in EL-Riyadh, Kafr El-Sheikh governorate. The experiment started in August 2014 and lasted up to November 2014. The fingerlings were placed in a fiberglass tank and randomly distributed into the experimental aquaria for the adaptation to the experimental conditions until starting the experiment. Fish were fed the control diet for two weeks, during this period, healthy fish at the same weight replaced the died one.

2. Experimental design of rearing fish :

A group of 408 Nile tilapia fingerlings *(O. niloticus)* fish with an average initial body weight 20 g were randomly allotted into 24 glass aquaria (80x35x40cm), with 17 fish in each aquarium and each treatment was applied in three aquaria. Fresh tap water was stored in fiberglass tanks for 24 h under aeration for dechlorination. One third of the water in each aquarium was replaced daily and totally changed once every week after removing the wastes. 24 air stones were used for aerating the water aquaria. Water temperature ranged between 24 and 29 °C. Photoperiod was adjusted to be 14 h light and 10h darkness using florescent light. Fish feces and feed residue were removed daily by siphoning.

3- Experimental diets and feeding regime:

Two weeks before the beginning of the experimental trial, about the fishes were adapted to a basal diet containing 30% crude protein and consisted of fish meal, soybean meal, yellow corn, wheat bran, corn gluten meal, di – ca - phosphate, sunflower oil and Vit.& Min mixture. Toxin AFB_1 was added at a concentration of 150 ppb to all the experimental diets except the control. Anti-toxin was added at a concentration of 0.5 and 1g / kg to the diets T3, T4, T5, T6, T7 and T8 as shown in Table 1. These ingredients were mixed well and pressed through manufacturing machine (pellets size 1mm).The ingredients and supplements were bought from the local market, but aflatoxin B_1 was produced through pellets fermentation according to the method described by **Abdelhamid and Mahmoud (1996).**

4- Experimental dietary additives:

Formulation of the control and experimental diets are shown in Table 1.

The required amount of the diet was prepared every two weeks and stored in a refrigerator until use. The pellets were dried under room temperature for 24 h before use.

Table 1: Formulation of the control and experimental diets %

Ingredients	T_1	T_2	T3	T4	T5	T6	T7	T8
Fish meal	11	11	11	11	11	11	11	11
Soybean meal	35	35	35	35	35	35	35	35
Yellow corn	32	32	32	32	32	32	32	32
Wheat bran	12	12	12	12	12	12	12	12
Corn glutein meal	5	5	5	5	5	5	5	5
Sunflower oil	3	3	3	3	3	3	3	3
Ca phosphate	1	1	1	1	1	1	1	1
Vit.&Min. mixture*	1	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100	100
Aflatoxin B1(ppb)	-	150	150	150	150	150	150	150
Black pepper (g)	-	-	0.5	1	-	-	-	-
Filo feed plus (g)	-	-	-	-	0.5	1	-	-
Cap T2 (g)	-	-	-	-	-	-	0.5	1

*Vitamins and minerals premix (product of Victoir) each 3kg contain: 12.00.000 IU Vit. A ; 3.00.000 IU Vit. D3; 700 mg Vit. E; 500 mg Vit. K3; 500 mg Vit. B1; 200 mg Vit. B2; 600 mg Vit. B6; 3000 µg Vit. B12; 450 mg Vit. C; 3000 µg Niacin; 3000 mg Methionine; 10000 mg Choline Chloride; 600 µg Biotin; 300 mg Folic acid; 670 mg Pantothenic acid; 3000 mg Magnesium Sulphate; 1800 mg Zinc Sulphate; 10000 mg Iron Sulphate; 3000 mg Copper Sulphate; 300 µg Cobalt Sulphate.

Fish in all treatments were daily fed the experimental diets at a level of 3% of the body weight, the amount of the feed was given six days a week at two times daily (8a.m and 2p.m) for 14 weeks. Fish were weighed bi-weekly and feed amounts were adjusted according to the new weight.

-Analytical methods:

4.1-Water quality analysis:

Water samples from each aquarium were taken bi-weekly to determine the values of pH, dissolved oxygen, nitrite, ammonia concentration and temperature.

4.1.1-Temperature:

Water temperature in degree centigrade was recorded every day by using a thermometer.

4.1.2- PH value:

PH value of water was monitored using pH meter (Model Digit-Sense, Cole – Parmer Instruments Co. Vernon Hills, IL. USA.).

4.1.3- Dissolved oxygen:

Dissolved oxygen was measured digitally using an oxygen meter (Model FE 247, EDT Instruments LTD. Dover Kent, and UK).

4.1.4-Ammonia (NH_3) and Nitrite (NO_2):

Ammonia and nitrite were estimated using test kits by Diamond, Diagnostic, Egypt (Model NI, Cat. NO. 22669-00, Hach CO.).

4.2- Proximate analysis of the experimental diets and fish body:

Chemical composition of the control, experimental diets and fish body was at the start and at the end of the experiment carried out according to the methods described by **A.OA.C. (1995).**

At the end of the experiment, five fish were derived from each group for drying at 60°C for 48 hours and then milled through electrical mill and stored in deep freezer until analysis.

5-Growth performance and efficiency of feed and protein utilization:

The growth performance and feed utilization parameters were calculated according to the following equations:

- Total weight gain (TWG):

TWG = final weight (g) - initial weight (g)

- Average daily gain (ADG):

ADG (g) = TWG (g) / Time (days)

- Survival rate (SR %):

SR % = Total number of fish at the end of the experimental × 100 / total number of fish at the start of the experiment.

- Specific growth rate (SGR, % / day):

SGR = 100 × [In wt1- In wt0/T]

Whereas:

In: Natural log.
Wt1: Final weight (g)
Wt0: Initial weight (g)
T: Time in days

- Feed conversion ratio (FCR):

FCR = Total feed consumption (g) / Weight gain (g)

- **Feed efficiency** (**FE**):

FE= Body weight gain (g) / Feed intake (g)

- Protein efficiency ratio (PER):

PER = Body weight gain (g) / protein intake (g)

- Productive protein value (PPV %)

PPV% = 100 × [Retained protein (g)/protein intake]

6- Internal Organs Indices:

All fish were killed at the end of the experiment, and soon the abdominal cavity was opened to remove liver, kidney and spleen which were weighed individually. Hepatic somatic index (HSI), kidney somatic index (KSI), and spleen somatic index (SSI) were calculated as follow:

-**HSI**=Liver weight ×100 / fish weight **(Jangaard *et al.,* 1967).**

-**KSI** = kidney weight ×100 / fish weight **(Alabaster and Lioyd, 1982).**

-**SSI** = spleen weight ×100 / fish weight **(Jangaard *et al.,* 1967).**

7- 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).**

8- Residues of aflatoxin in the liver and muscle of Tilapia niloticus:

Liver and muscles samples of different groups of fish under the current study were examined for aflatoxin residues **(AOAC, 1995).** AFB_1 residues was extracted and analyzed by the method of **Jemmali and Murthy(1976)** using **HPLC.**

9- Statistical analysis:

The data collected were analyzed using statistical package social science procedure by **SPSS (2006)** for users guide; Means were statistically compared for the significance (P≤ 0.05), using Duncan's multiple rang test.

RESULTS AND DISCUSSION

1-Chemical composion of the experimental diet:

Chemical composition of the different experimental diets is shown in Table 2.

Table 2: Chemical Proximate analysis (% DM basis) of the experimental diets

Ingredients	Diet No.							
	1	2	3	4	5	6	7	8
Dry matter	91.92	91.17	91.54	91.27	90.92	91.15	91.45	90.97
Crude protein	29.52	29.86	30.39	30.16	29.22	30.14	29.67	30.19
Ether extract	5.91	5.87	6.04	6.51	5.89	6.01	6.19	5.94
Crude fiber	3.39	3.14	3.06	3.62	3.44	3.51	3.48	3.89
Ash	6.61	6.79	6.73	6.54	6.59	6.47	6.75	6.56
Nitrogen free extract	54.57	54.34	53.78	53.17	54.86	53.87	53.91	53.42
Calculated energy value								
GE (Kcal/100g)*	452.92	453.49	455.73	456.30	452.26	454.41	453.63	452.13
DE (Kcal/100g)**	309.93	310.81	313.87	315.73	308.83	312.53	311.88	311.25
P/GE, mg/kcal***	65.17	65.84	66.68	66.09	64.60	66.32	65.40	66.77

* Gross energy was calculated according to ***NRC (1993)*** by using factors of 5.65, 9.45 and 4.22 Kcal per gram of protein, EE and NFE, respectively.

** DE (Digestible energy) (Kcal/ 100g), based on 5.0 Kcal/g protein 9.0 Kcal/g EE, 2.0 Kcal/g NFE. According to ***Wee and Shu. (1989).***

*** P/GE (protein to energy ratio) = crude protein (mg) / GE (Kcal)

Chemical analysis revealed that no differences were observed among all diets. The diets a were approximately isonitrogenous and isoenergetic. The CP content was between 29.22 to 30.39 % on DM basis, and the GE values ranged between 452.13 and 456.30 Kcal/100g, 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).**

2-Water quality parameters of rearing fish:

Results of water quality parameters of the experimental aquaria are shown in Table 3, which were suitable for rearing Nile tilapia fingerlings as cited by **Abdel -Hakim, *et al.* (2002) and Abdelhamid (2000b).** In general, the water temperature of the different treatments were ranged between 26.2 and 27.3C°. pH values between 6.9 and 7.8 and dissolved oxygen between 5.1 and 6.3 mg / L.

Similar results were reported by **Abdelhamid *et al.* (2004) and Salem (2012)**. Average of nitrite (NO_2) and ammonia (NH_3) concentration ranged from 0.37 to 0.46 mg /L and from 0.04 to 0.06 mg / L, respectively. Which were suitable for rearing Nile tilapia as reported by **Khalafalla and Mohsen (2007)**.

Table 3: Averages of some physic- chemical parameters of water

Treat No.	Water parameters				
	Temperature °C	pH value	DO mg/l	NH_3 mg/l	NO_2, mg/l
T1	27.0	7.1	6.3	0.04	0.39
T2	26.4	7.6	5.1	0.05	0.44
T3	26.7	7.3	5.3	0.06	0.46
T4	27.1	7.8	5.7	0.04	0.39
T5	26.2	7.4	5.9	0.04	0.41
T6	27.3	6.9	6.1	0.05	0.37
T7	26.9	7.7	5.7	0.04	0.40
T8	26.5	7.3	6.0	0.04	0.42

3- Growth performance and survival rate:

Data presented in Table 4 showed that aflatoxin B1 (AFB1) had negative effects ($P \leq 0.05$) on fish growth performance. Even though, there were no significant ($P \geq 0.05$) differences in the initial body weights among all treatment. Average weight gains (AWG), average daily gain (ADG) and specific growth rate (SGR) of the experimental fish were the best for the fish fed T1 (control). However, T8 (aflatoxin-contaminated diet plus 1 g Cap T2) and T6 (aflatoxin-contaminated diet plus 1g Filofed) were better than T2 (contaminated with aflatoxin B_1 150 ppb). In general, the fish of T2 recorded the lowest values for AWG, ADG, SGR and SR when compared to the other treatment. On the other hand, there were no significant differences between either fish fed diets T3, T4, T5 and T7. These results were in agreement with the finding of **Abdelhamid (2008)** who found that AFB_1 at levels 100ppb and 150ppb in the diet of fish fingerlings without adding antitoxins caused a significant growth depression. This poor growth might be a result of expelling the feed from the mouth of fish **(Nguyen *et al.*, 2002).** Also**,** the same results reported by **Salem *et al.* (2009)** who found a significant reduction in growth performance and survival rate of *O. niloticus* fish as affected by

aflatoxin B_1 (AFB1) in the diet. On the other hand, adding some medicinal plants and some spices to the contaminated diet reduced the toxic effect of the AFB1 and stimulated growth performance of the fish **(El-Dakar *et al.* 2005 and Russo *et al.*, 2013).**

These results agreed with the findings reported by **Salem *et. al.* (2010) and Salem (2012)** who mentioned that AFB1 at levels of 100ppb and 150ppb significantly increased the mortality rate in tilapia. The ability of Rotamin and Power Top to decrease the mortality rate may be due to its constituents that stimulate the immune system **(Piraret *et al.*, 2006).**

Table 4: The growth performance parameters and survival rates of the tilapia fish as affected by the dietary treatments (Mean*+ SE)

Treat.	Initial weight g/fish	Final weight g/fish	Total weight gain (TWG) g/fish	Average daily gain (ADG) g/fish/day	SGR %/ day	Survival rate (SR) %
T1	20.03±0.04a	49.22±0.25a	29.19±0.20a	0.29±0.00a	0.91±0.00a	98.03±1.96a
T2	20.09±0.02a	37.28±0.19e	17.19±0.20e	0.17±0.00e	0.62±0.00e	60.78±1.96d
T3	20.05±0.03a	40.48±0.22d	20.42±0.20d	0.20±0.00d	0.71±0.00d	78.43±1.96c
T4	19.99±0.03a	40.85±0.34d	20.86±0.34d	0.21±0.00d	0.72±0.00d	90.19±1.96b
T5	19.99±0.05a	41.00±0.28d	21.01±0.26d	0.21±0.00d	0.72±0.00d	90.19±1.96b
T6	20.07±0.02a	43.77±0.78c	23.70±0.76c	0.24±0.00c	0.79±0.01c	92.15±1.96ab
T7	20.05±0.03a	43.03±0.48c	22.97±0.50c	0.23±0.00c	0.77±0.01c	94.11±3.39ab
T8	20.05±0.03a	45.46±0.20b	25.41±0.21b	0.25±0.00b	0.83±0.00b	98.03±1.96a

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

4-Feed intake and protein utilization:

All criteria studied and presented in Table 5 showed that T1, T8and T6 were better (P≤0.05) in comparison with the T2 group (containing AFB1) concerning FI, FCR, PER, and PPV% in tilapia fish. On the other side, there was no significance difference between T1, T8 and T6 in data of FCR, PER, and PPV%. The addition of Cap t2 was more effective compared to the other treatment. While, the addition of AFB1 (T2) had negative effect on all fish performance parameters (FI, FCR, PER, and PPV%). Black pepper and coriandrum stimulates digestion and influences positively the terminal enzymes of digestive process **(Abdel-Wahab *et al.*, 2007, Salem *et al.*, 2009 and Salem, 2012).**

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.* Those authors added that the high levels of aflatoxin B1 (10 and 100 mg AFB1/kg) led to decrease feed intake. On the other hand, **Svobodova *et al.* (1982)** proved that AFB1 at doses of 20 to 200 mg/Kg of feed did not show any effects on feed and protein utilization. Also, **Aly and Mohamed**

(2010) concluded that, garlic supplemented diets has immuno - stimulation for tilapia (*O. niloticus*) and improved feed intake, body weight and body weight gain than the groups fed diet with aflatoxin.

Table 5: Feed intake and feed and protein utilization of Nile tilapia fish as affected by the dietary treatments (Mean*+ SE)

Treat.	Feed Intake (g)	FCR	PER	PPV%
T1	74.48±0.32a	2.55±0.00e	1.32±0.00a	36.68±1.92a
T2	64.32±0.19e	3.74±0.03a	0.89±0.00e	17.05±1.33d
T3	66.56±0.22d	3.25±0.02b	1.00±0.00d	20.25±3.08bc
T4	67.51±0.08c	3.23±0.05b	1.02±0.01d	22.62±2.33bc
T5	67.91±0.17c	3.23±0.04b	1.05±0.01cd	26.22±0.70b
T6	69.80±0.22b	2.95±0.09cd	1.12±0.03bc	18.10±1.23cd
T7	69.77±0.23b	3.03±0.06c	1.10±0.02bc	39.25±2.40a
T8	71.88±0.18b	2.82±0.02d	1.17±0.00b	23.73±2.11bc

a, b, c, d and e means in the same column bearing different letters differ significantly at 0.05 level.

5- proximate chemical analysis of the whole fish body:

The proximate chemical analysis of the whole body of the tested tilapia fish is given in Table 6. The control and T8 diets had the higher (P≤0.05) DM content compared with the other treatment. while, the fish fed T2 had the lowest DM content. There were significant differences among the dietary treatments for CP contents. The highest CP was observed in the fish groups T7, T5, and T8 and lowest values were in groups T2, T3, T4, and T6. The differences were significant (P≤0.05) for EE and ash in the all treatments. Generally, the addition of black pepper, filofeed plus and cap t_2 improved proximate analysis of fish carcass.

Table 6: Proximate Chemical analysis (% DM basis) and energetic value of the whole tilapia body as affected by the experimental diets (Mean*+ SE)

Treat. No		% On Dry matter basis			
	DM%	CP%	EE%	Ash%	EC** (Kcal/100g)
At the start of the experiment					
	23.61±0.01e	59.61±0.23e	10.33±0.02e	22.55±0.22a	423.15±1.13d
At the end of the experiment					
T1	27.82±0.07a	67.68±0.25a	17.44±0.31c	16.21±0.09e	535.90±4.39a
T2	25.33±0.14d	62.89±0.20d	21.22±0.24a	16.38±0.20e	544.61±2.77a
T3	25.68±0.16cd	63.71±0.37cd	19.32±0.34b	20.40±0.23b	531.30±3.96ab
T4	27.61±0.33ab	64.22±0.30bc	20.25±0.48a	19.16±0.24b	542.92±3.54a
T5	26.16±0.04c	64.82±0.10b	16.07±0.39d	17.14±0.16cd	506.79±3.49c
T6	27.40±0.07bc	63.42±0.46cd	17.30±0.62c	17.26±0.25c	510.56±5.14c
T7	27.22±0.02bc	67.74±0.45a	15.75±0.63d	16.48±0.33d	520.30±8.21bc
T8	27.98±0.22a	64.31±0.46b	17.37±0.17c	17.49±0.19c	518.84±3.04bc

* Means superscripted (in the same column) with different letters significantly (P≤0.05) differ.
**EC= Calculated energy content.

Similar results were reported by **Salem (2002)** who found that the percentages of DM and CP decreased as the levels of the aflatoxin B1 increased, while the values of EE and ash increased with increasing the levels of AFB1. Additionally, **Abdelhamid *et al.* (2004 and 2006) and Mehrim *et al.* (2006)** concluded that aflatoxin B1 significantly reduced DM and CP content of the *O. niloticus* fish carcass, but it significantly increased EE and ash content of the fish. Also, **Salem (2012)** found high significant (P≤0.05) differences among the dietary treatments in CP, EE and ash contents. The highest CP was observed in the fish groups of T1, T3, T4, T5 and T6 and the lowest values were found in groups T2. The highest EE was observed in the fish group of T2.

6-Internal organ indices:

Significant (P≤0.05) differences were found among the dietary treatments for kidney- somatic index **(KSI)**, hepato –somatic index **(HSI)**, and spleen –somatic index **(SSI)** as presented in Table 7.

Generally, from the results in the present study, treatment (T2) caused negative effects on the internal organs indices comparing with the control diet (T1) and the other treatments (T3,T4, T5, T6 ,T7 and T8). This means that AFB1 not only reduced growth performance of the tested fish, but also negatively altered internal organs function as a consequence of affecting their relative weights, which may be due to changing in their cells number or volume or elevating their water and / or blood contents **(Glaister, 1986, Abdelhamid *et al.,* 2006 and Mehrim *et al.*, 2006).**

Hussein *et al.* (2000) observed that hepato – somatic index **(HSI)** was increased by increasing AFB1 level in the diet of *O. niloticus* but gonado – somatic index **(GSI)** decreased. **Abdelhamid *et al.* (2006) , Salem *et al.* (2009) and Salem (2012)** reported that the aflatoxic diet at a level of 100 ppb AFB1 led to significant increases (P≤0.05) in all organs indices comparing with the control diet (zero ppb AFB1). However, **Zychowski *et al.* (2013)** and **El-Barbary** and **Mohamed (2014)**. found that HSI was decreased significantly in groups exposed to AFB1.

On the other hand, **Abdelhamid *et al.* (2006)** found that the additives (tafla, ammonia and hydrogen peroxide) did not alter the organs weigh; yet, they slightly diminished –to some extent – the negative effect of dietary aflatoxin inclusion on the relative weights of all tested organs. Also, **Shehab el-din *et al.* (2013**) recently added that the additives of (0.2% Rotamin and 0.3% Power top) as antimycotoxins for Nile tilapia rations caused decrease in hepato – somatic indices comparing with the aflatoxin B1 contaminated diet. In the present study, the effects of biological(Cap T2) and chemical

(Filofed plus) additives may be due to increase of immunity of fish, reduced the effect of the toxin of AFB1 and hence hide its negative effects on indices of fish. Additionally, **Zychowski *et al.* (2013)** and **El-Barbary** and **Mohamed, (2014)** found that the HSI was decreased significantly in groups exposed to AFB1.

Table 7: Internal organs indices of the tilapia fish at the end of experimental period as affected by the experimental diets (Means * ± SE)

Treat.	HSI%	KSI%	SSI%
T1	2.94±0.02e	0.31±0.01e	0.29±0.00d
T2	4.12±0.01a	0.53±0.02a	0.51±0.02a
T3	3.44±0.00b	0.43±0.01bc	0.45±0.02b
T4	3.39±0.01bc	0.45±0.01b	0.28±0.01d
T5	3.36±0.01c	0.43±0.02bc	0.40±0.00bc
T6	3.14±0.01d	0.41±0.00bc	0.29±0.01d
T7	3.19±0.03d	0.39±0.01cd	0.39±0.03c
T8	3.00±0.00e	0.34±0.01d	0.36±0.00c

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

7-Residues of aflatoxin in the fish body:

The residues of aflatoxin (AFB1) in the viscera and muscles of fish in the different experimental groups are shown in Table 8. The group fed AFB1 contaminated diet (150 ppb) T2 without any feed additives, showed the highest level (18.0 ppb aflatoxin B1) in viscera, followed by the group fed AFB1 contaminated with Black pepper, Filofed plus and Cap T2 which were 5.2, 7.2, 4.6, 5.4, 2.6 and 3.1 ppb for T3, T4, T5, T6, T7 and T8, respectively in viscera. So, T7 was the best treatment in reducing these residues. However, in muscles, the residues was detected only in the group fed on AFB1 contaminated diet (150 ppb) T2 without any feed additives (4.2 ppb) and did not detect in the other groups.

In this respect, **Abdelhamid *et al.* (2004)** reported that AFB1 residues in the *O. niloticus* flesh showed a cumulative effect related to the 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* fish after 6 months. **Abdelhamid *et al.* (2004) , Salem *et al.* (2009) and Salem (2012)** recorded residues of AFB1 in the whole body of *O. niloticus* at the end of the experiment and tended to decrease after a freezing period.

The residual AFB1 level in the liver was much higher than that in muscle fed the same contaminated diet with aflatoxin B1**(Bintvihok *et al.*, 2002 and El- Sayed and Khalil, 2009).** Similar result was concluded by **Arafa *et al.* (2014)**

No AFB1 residue in muscles was found in tilapia that treated by spices (black pepper), chemicals (Fiofed-plus) and biological (Cap T2) in the present study. This agreed with the results reported by **Lim *et al.* (2001) and Usanno *et al.* (2005).**

Table 8: The residues of aflatoxin B1in the tilapia fish wet weight basis as affected by the dietary treatments (Means * ± SE)

AFB1 in whole body fish (ppb)	Treatment							
	T1	T2	T3	T4	T5	T6	T7	T8
Muscles	ND	4.2	ND	ND	ND	ND	ND	ND
Viscera	ND	18	5.2	7.2	4.6	5.4	2.6	3.1

8-Musculature and abdominal areas:

The variation among the tested fish treatments, concerning musculature and abdominal areas are presented in Table 9 and Figs.1, 2, 3, 4, 5, 6, 7, and 8. The fish fed T2 (AFB1 without any additives) had the lowest musculature and abdominal area while the control and all the diets supplemented with feed additives had wider musculature and abdominal area and the control, T7 and T8 were more effective. These results agreed with the finding reported by **Salem *et al.* (2009) and Salem (2012)** who mentioned that AFB1 at levels of 100 ppb and 150 ppb increased significantly the abdominal cavity (black area) and decreased the musculature (white area) in tilapia.

Table 9: Musculature and abdominal area

Items.	Treat No.							
	T1	T2	T3	T4	T5	T6	T7	T8
Musculature area mm	34.3	25.4	27.2	27.2	28.3	29.0	32.7	33.6

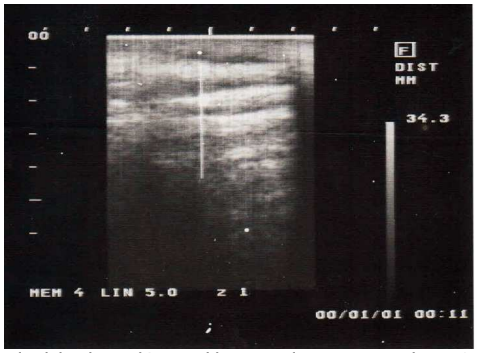

Fig.(1) Diet 1 (Control) Musculature area about 34.3

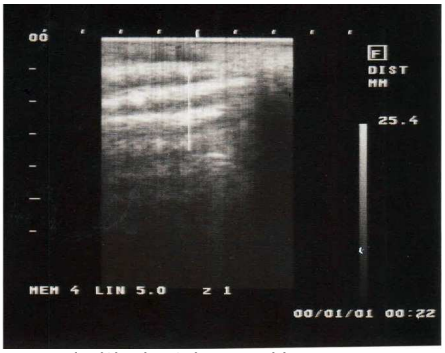

Fig.(2) Diet 2 (AFB1 without any additives) Musculature area about 25.4

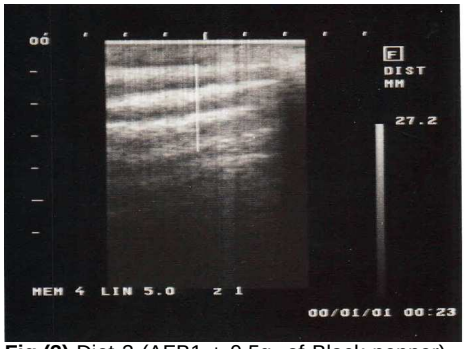

Fig.(3) Diet 3 (AFB1 + 0.5g of Black pepper)

Musculature area about 27.2

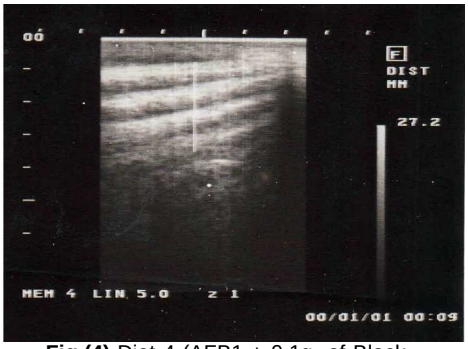

Fig.(4) Diet 4 (AFB1 + 0.1g of Black pepper)

Musculature area about 27.2

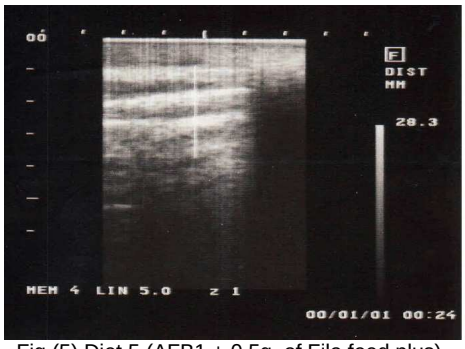

Fig.(5) Diet 5 (AFB1 + 0.5g of Filo feed plus)

Musculature area about 28.3

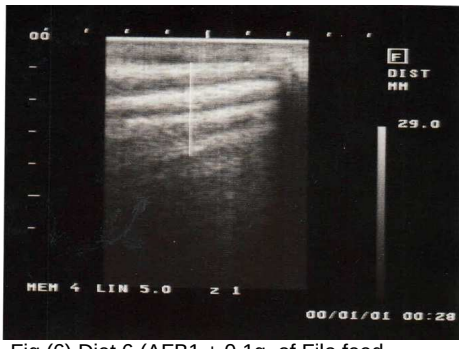

Fig.(6) Diet 6 (AFB1 + 0.1g of Filo feed plus)

Musculature area about 29.0

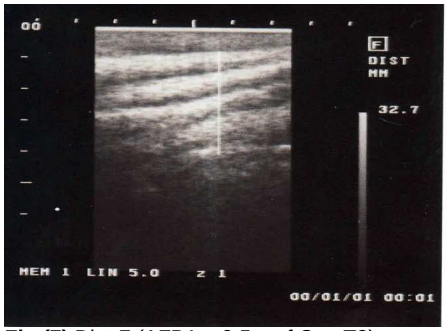

Fig.(7) Diet 7 (AFB1 + 0.5g of Cap T2)

Musculature area about 32.7

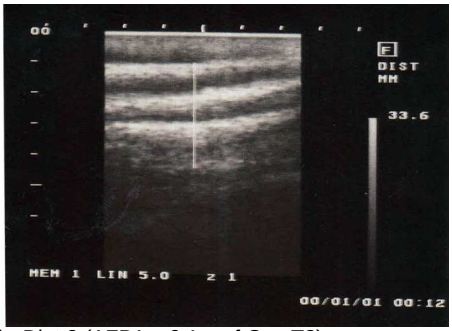

Fig.(8) Diet 8 (AFB1 + 0.1g of Cap T2)

Musculature area about 33.6

CONCLUSIONS

From the foregoing results it could be concluded that aflatoxin contaminated diets caused many drastic effects in all tested parameters. Adding Black pepper (spices), Filofeed plus (chemicals) and Cap T2 (biological) at both levels (0.5 and 1g) to the diets of Nile tilapia showed positive effects on all fish performance parameters as well as alleviate the toxic effects of AFB1 contaminated diets. Moreover, it is needed a lot of scientific efforts in this trend to use of the natural agents to detoxify mycotoxin (particularly aflatoxin) in diets of fish.

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الملخص العربي

إزالة التلوث بالسموم الفطرية الافلاتوكسين بواسطة الطرق الكيماوية ، والبيولوجية والتوابل في علائق اسماك البلطي النيلى

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1- قسم الانتاج الحيواني ,كلية الزراعة ,جامعة كفر الشيخ ,مصر .2- المعمل المركزي لبحوث الثروة السمكية بالعباسة -وحدة بحوث الثروة السمكية بسخا.

أجريت هذه الدراسة للكشف عن التأثيرات السامة للأفلاتوكسين ب 1 على إصبعيات البلطي النيلى ، وكذا محاولة إزالة تلك الآثار السيئة باستخدام بعض الإضافات الغذائية. لذلك تم إضافة 0.5، 1جرام من كل من هذه المواد وهى مادة (الفلفل الاسود، الفلو فيد بلس والكاب تي 2) لعلائق أسماك البلطي النيلى الملوثة بالأفلاتوكسين 150 جزء في البليون أفلا توكسين ب1 قدمت هذه العلائق على مدار 6 أيام فى الأسبوع بمعدل 3 % من الكتلة الحيوية الحقيقية للأسماك فى الأحواض الزجاجية ، حيث مُثلت كل معامله بثلاث مكررات في ثلاث أحواض كل حوض يحتوى على 17 سمكه من اصبعيات البلطى النيلى وتم تغذية الأسماك على هذه العلائق لمدة 14 أسبوع. أوضحت النتائج أن العلائق الملوثة بالأفلاتوكسين أدت إلى تأثيرات سيئة على كل من معدل النمو والإعاشة للأسماك ، الاستفادة من الغذاء والبروتين ، ودلائل الأعضاء الداخلية ، والتحليل الكيماوي لجسم الأسماك ، وكذا سجلت النتائج وجود متبقيات من الأفلاتوكسين ب1فى جسم الأسماك المعاملة ، وأيضا أظهرت النتائج انخفاض مساحة العضلات التى تغذت على علائق ملوثة بالأفلاتوكسين ب 1 وزياده في مساحة التجويف البطني نتيجة لزيادة نسبة الارتشاحات. كذلك أظهرت النتائج أن العليقة المحتوية على الفلفل الاسود ، الفلو فيد بلس والكاب تي 2 قد خففت من تلك التأثيرات السيئة للأفلاتوكسين على الأسماك ،حيث تحسنت كل القياسات السابقة الذكر للأسماك المعاملة بالأفلاتوكسين. بصفة عامة أوضحت النتائج المتحصل عليها في هذه الدراسة الحالية أن الكاب تي2يعد أفضل مادة مستخدمة لإزالة التأثيرات السيئة للأفلاتوكسين ب1 يليها الفلو فيد بلس والفلفل الأسمر على التوالي.