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Is It Possible to Detoxify Aflatoxic Aquafeed?

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ABSTRACT

The aim of this experiment was to investigate the effect of aflatoxin B (AFB) in the diets of Nile tilapia (*Oreochromis niloticus*) and to examine the detoxification activity of a commercial anti-mycotoxin ARCAVIT® Bioacid Forte. The experimental period lasted 70 days during September - December 2018. Sixty fish were chosen with an average initial weight of about 40 g then randomly distributed into three treatments in six glass aquaria (30 × 60 × 40 cm); each treatment was applied in two aquaria (replicates). Dietary AFB was added at a concentration of 5 ng / g diet. The anti-mycotoxin ARCAVIT® (pro- and prebiotics) is one of the commercial anti-mycotoxins in the local market. It is the first test for it in aquaculture, which was added at a concentration of 0.5 g/Kg diet. The experimental diets of the three treatments were; the control treatment (T_1): the basal diet (BD) without any additives. The second treatment (T_2): BD with an addition of 5 ng AFB / g diet. The third treatment (T_3): BD with an addition of 5 ng AFB / g diet and 0.5g ARCAVIT® / Kg diet. Fish fed the experimental diets at a daily rate of 3% of their live body weight, at two meals. The obtained results showed that AFB is very toxic, even at a low concentration as 5 ng / g diet, for Nile tilapia. It threats fish morphology, gross pathology, liver histology, growth performance, and internal organs indices, feed utilization, body composition, and properties, as well as hematological and plasma biochemistry parameters. Moreover, the anti-toxic agent (ARCAVIT®) at the tested level did not completely overcome the aflatoxicity symptoms. That leads to keep the old wisdom "Prophylaxis is better than cure".

INTRODUCTION

Dietary mycotoxin contamination is an area of great concern for different organisms (Abdelhamid, 2019 a & b). It negatively affects the immune system, gut development (Mass, 2018), digestion and performance (Abdelhamid *et al*., 1992), and may lead to death (Abdelhamid *et al*., 2006); so, leads to economic loss (Abdelhamid, 2000; 2004 and 2009). Particularly aflatoxins, since their producing fungi are detected in various food-and feedstuffs that found also in diseased fish and fish diets (Abdelhamid *et al*., 2006 & 2008; Abdelhamid, 2010 and Abdallah *et al*., 2017).

Therefore, many attempts were done to prevent growth of toxigenic fungi (Abdelhamid *et al*., 2008 and 2009) and mycotoxin production (Abdelhamid *et al*.,

1985 and Kumar *et al*., 2018) and to control mycotoxicoses (Abdelhamid and Mahmoud, 1996; Abdelhamid *et al*., 2002a, b &c; 2004 a, b &c and 2007). All these attempts concluded that exact overcome of mycotoxicoses is impossible, so recommended the prevention (Prophylaxes that is better than cure). Hence, the aim of the present research was to study the effects of a new commercial feed additive aiming in ameliorating fish aflatoxicosis.

MATERIALS AND METHODS

Experimental fish:

Unsexed Nile tilapia fingerlings collected from Lake Manzala in September 2018 and transferred to Elmataria Station for Aquatic Research, Dakahlia Governorate, National Institute of Oceanography and Fisheries, Egypt in order to investigate the effect of aflatoxin B (AFB) in the diets of Nile tilapia (*Oreochromis niloticus*) and to examine the detoxification activity of a commercial anti-mycotoxin ARCAVIT® Bioacid Forte (Italy Product) distributed in Egypt by ALLgaeuvet Egypt. The experimental period lasted 70 days (10 weeks). The experiment started on 15th of September 2018 and finished on the 2nd of December 2018. After 2 weeks adaptation period in a 3m^3 fiberglass tank; during that period, fish were fed a basal diet (BD) from Bio Feed Factory (Asafra Industrial zone, Dakahlia governorate, Egypt). Fish (40 g initial body weight) were randomly distributed into three treatments in six glass aquaria. Each aquarium had dimensions of 30 × 60 × 40 cm, filled with 60 L of dechlorinated tap water, supplied by air stone and electric glass heater to keep water temperature constant at 25-28ºC and stocked with 10 fingerlings. Each treatment was applied in two aquaria (replicates). One-third of the water in each aquarium was replaced daily and totally once weekly after removing the wastes by siphoning. Photoperiod was natural by the sunlight.

Experimental diets:

Formulation and composition (according to the manufacturer Bio feed factory) of BD used are illustrated in Table1. The experimental diets (treatments) were T_1: BD-free AFB (as a control), T_2: BD artificially contaminated with 5 ng AFB / g diet, and T_3: BD artificially contaminated with 5 ng AFB / g diet and included 0.5 g ARCAVIT® / Kg diet. AFB was produced by growing *Aspergillus parasiticus* (standard toxigenic strain, NRRL 2999 culture, Lyophilized strain, was kindly obtained from Vet. Med. Microbiology Dept., Iowa State University, USA) on rice fermentation. The moldy rice was steamed to kill the fungus, dried, milled and analyzed for aflatoxin determination using the AflaTest Fluorometer Procedure (Isaka *et al*., 2011). The AFB-extract of chloroform was sprayed on T_2 and T_3 diets to reach AFB level of 5 ng / g diet. Diets were let in a dry and dark place to chloroform volatilization, then the feed stored in a cool and dark place (just two days before the experiment starts, under the normal refrigerator temperature) till be offered to fish. The ARCAVIT® is one of the commercial anti-mycotoxins in the local market; it is the first test for it in aquaculture. ARCAVIT® composition according to the company's advertising brochure is cell wall of *Saccharomyces* extract 100%, β-glucans mannan oligosaccharide, lactic acid, propionic acid, calcium propionate, sodium citrate, potassium citrate, chestnut extract tannins, seaweed meal (*Ascophyllum nodosum*), silicon dioxide and sepiolite. One gram of ARCAVIT® was dissolved in one liter of distilled water then sprayed on two kilograms of feed, which

contains 5 ng AFB / g diet to be treatment 3 (T_3). All the feed of the experiment stored in a dark place under low temperature.

Table 1: Formulation and composition of the commercial basal diet used in the experiment

Ingredients	%
Soybean meal	45
Yellow corn	17.5
Wheat bran	12.5
Distiller's dried grains (DDGs)	6
Rice bran	6
Fish meal	5
Corn gluten	3
Fish oil	1
Dried yeast	1
Dicalcium Phosphate	1
Calcium carbonate	0.5
Salt	0.5
Potassium bicarbonate	0.5
Vit. & Min. & Premix (1)	0.4
Methionine	0.1
Chemical analysis as dry matter basis	
Dry Matter (DM, %)	89.59
Crude protein (CP, %)	30.87
Ether extract (EE, %)	6.15
Crude fiber (CF, %)	6.34
Nitrogen-free extract (NFE, %) (2)	48.21
Ash (%)	8.43
Gross energy (GE, Kcal / 100 g DM) (3)	435.99
Protein/Energy (P/E ratio) mg CP/Kcal GE (4)	70.8

(1) Vit & Min. Premix each 1 kg contains: Vit. A, 12.000.000 IU; Vit. D3, 3000.000 IU; Vit. E, 10.000 mg; Vit PtK3, 3000 mg;Vit. B1, 200 mg; Vit. B2, 5000 mg; Vit B6, 3000 mg; Vit. B12, 15 mg; Biotin, 50 mg; Folic acid, 1000 mg; Nicotinic acid,35000 mg; Pantothenic acid, 10.000 mg; Mn 80 mg; Cu 8.8 g; Zn 70 g; Fe 35 g; Co 0.15 g and Se 0.3 g. (2) Nitrogen-free extract (carbohydrate) content was calculated by subtraction of the total percentages of CP, EE, CF and ash from 100. (3) The gross energy contents of the experimental diets and fish samples were calculated using factors of 5.65, 9.45 and 4.22 Kcal/g of protein, lipid, and NFE, respectively (NRC, 1993). (4) P/E ratio (mg Protein/Kcal gross energy) = CP/GE × 1000.

Feeding regime:

All the experimental groups were fed the experimental diets at a daily rate of 3% of the live body weight of the fish, at two meals. The feed quantity was revalued monthly on the basis of the actual average biomass of the fish in each treatment. The feed residues were collected daily from each aquarium and then calculated as the average weight of the dry feed to calculate the actually feed consumed by the fish.

Water quality parameters:

Air stones connected by an electric air blower were used for aerating the aquarium; it was enough to reach the dissolved oxygen (DO) to the optimum range (6-8 mg/L), it was measured by HANNA HI 9146-04 – Romania. The water temperature was adjusted between 25 and 28º C by an electric glass heater. The pH values were in the optimum range in all treatments (7-8), it was measured by Consort C860 – Belgium. Because of changing and cleaning the water on each treatment daily there are no troubles came from water quality parameters.

Growth performance and feed utilization:

At the beginning of the experiment, randomly samples of fishes were measured for its weight and total length. At the end of the experiment, fish samples of each aquarium were weighted to calculate the growth performance and feed utilization parameters according to NRC (1993), Toguyeni *et al*. (1997) and Abdelhamid (1996 and 2009) in form of final weight (FW, g / fish), total weight gain (TWG, g / fish), average daily gain (ADG, g / fish / d), relative growth rate (RGR, %), specific growth rate (SGR, %/d), survival rate (SR, %), feed intake (FI, g / fish), feed conversion ratio (FCR), feed efficiency (FE, %), protein efficiency ratio (PER), protein productive value (PPV, %), energy utilization (EU, %), and lipid retention efficiency (LR, %**)**.

Internal organs indices and dressing and boneless meat:

At the end of the experiment, three fish per treatment were randomly taken, individually weighed and total length were measured; to calculate the condition factors (K_t); then the liver, gonads, and viscera with associated fat tissue were individually removed and weighed, to calculate the hepatosomatic index (HSI, %), the gonadosomatic index (GSI, %) and viscerosomatic index (VSI, %), respectively according to Clement and Lovell (1994) and Abdelhamid (2009). Dressing and boneless meat (BLM, fillet) percentages were calculated too (Clement and Lovell, 1994).

Proximate analysis, water holding capacity and lean meat of fish:

At the start of the experiment, 5 fish were taken and kept frozen (-24 º C) until carrying out the chemical analysis. At the end of the experiment, a random sample of five fish collected from each treatment was weighed and minced, then dried at 65°C for 12 hours, ground, then assayed to determine the moisture, crude protein, ether extract, and ash contents by using standers methods (AOAC, 1990). Gross energy was calculated according (NRC, 1993). Water holding capacity (WHC, %) was calculated as cited from Abdelhamid (1983). Lean meat (LM, %) was also estimated as cited from Pearson (1962) and Less (1968).

Blood sampling and estimated parameters:

Blood samples were collected from five fish each experiment under anesthesia using clove oil (1 ml/ 10 L) at the end of the experiment from the caudal peduncle by special syringe in small plastic vials containing EDTA as an anticoagulant and used to obtain the blood plasma by centrifuge at 3500 rpm for 15 min. Blood plasma samples were used for determination of cortisol (Zaki and Fawzy, 2012), uric acid and glucose (Elboshy *et al*., 2008 and Shalaby, 2009), creatinine (Tietz, 1986), triglycerides (McGowan *et al*., 1983), total protein (Henry, 1964 and Tietz, 1990), high density lipoprotein (HDL), low density lipoprotein (LDL) and albumin (Wotton and Freeman, 1982) concentrations, as well as the activity of enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using commercial test kits (Robonik Prietest touch, Biochemistry Analyzer, India). Globulin level was calculated by subtracting albumin from total protein. The other whole blood samples were used to determine the hematological parameters as hemoglobin (Hb), total erythrocytes (RBCs), total leukocytes (WBCs), and thrombocytes (PLT) (Natt and Herrick, 1952) and hematocrit (Hct) using (Horbia ABX micros 60 Auto blood hematology analyzer, USA) (Decie and Lewis, 2006). The other hematological parameters (MCV, MCH, and MCHC) were mathematically calculated according to Stoskopf (1993).

Histopathological examination:

At the end of the experiment, three fish were randomly selected from each treatment then sacrificed to take the liver samples. The liver samples were fixed at 10% neutralized formalin solution followed by washing with tap water, then dehydrated by different grades of alcohol. Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six microns. The sections were stained by hematoxylin and eosin and then subjected to a histological examination (Roberts, 2001).

Statistical analysis:

Data were statistically analyzed by one-way ANOVA using the Statistical Package for the Social Sciences (SPSS, 2017). Duncan's multiple range test (Duncan, 1955) was used to separate differences between treatment means at the probability level of 5%.

RESULTS

Morphology and gross pathology:

Normal morphology was observed for T_1 and T_3 fed fish concerning the healthy external appearance (shape, fins, color, eyes, gills, … etc.), revealing that ARCAVIT in T_3 succeeded in overcoming the AFB toxicity symptoms. Yet, fish fed the AFB-contaminated diet T_2 reflected bad appearance (discoloration, scattered-destructed fins, scales fall, dive eyes). Concerning the gross pathology, it showed normal post-mortem examination for fish fed either T_1 or T_3; whereas, AFB only fed fish (T_2) reflected pale internal organs, particularly liver.

Growth performance:

The initial body weight of the experimental fish was identical in all experimental groups (Table 2). The AFB-included diets (T_2 and T_3) were responsible for significantly ($P \leq 0.05$) lower values of all calculated measurements, where FW, TWG, ADG, RGR, or SGR, as well as significantly ($P \leq 0.05$) reduced SR of fish. Yet, dietary inclusion of ARCAVIT® in T_3 significantly ($P \leq 0.05$) improved all these parameters than in T_2.

Table 2: Growth performance parameters (mean ± standard errors) of the experimented fish throughout the whole experimental period.

Treatment	Control (T_1)	T_2	T_3
Initial weight	39.85	39.6	40.35
FW, g / fish	78.12^{a} ±0.53	61.90^{c} ±0.25	72.48^{b} ±0.51
TWG, g / fish	38.27^{a} ±0.53	22.30^{c} ±0.25	32.13^{b} ±0.51
ADG, g / fish /d	0.55^{a} ±0.01	0.32^{c} ±0.00	0.46^{b} ±0.01
RGR, %	96.04^{a} ±1.33	56.31^{c} ±0.64	79.62^{b} ±1.26
SGR, % / d	0.96^{a} ±0.01	0.64^{c} ±0.01	0.84^{b} ±0.01
SR, %	90.00	55.00	70.00

a – c: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Internal organs indices:

The internal organs indices including as a percentage of HSI, GSI, and VSI were calculated and presented in Table 3. It cleared the presence of significantly ($P \leq 0.05$) increases of both HSI and VSI percentages with AFB-included diets (T_2 and T_3) comparing with the control (T_1). Yet, ARCAVIT® (T_3) significantly ($P \leq 0.05$) alleviated the negative effects of AFB on these indices. On the other hand, AFB alone (T_2) significantly lowered the GSI %, but ARCAVIT® did not improve it.

Table 3: Internal organs' indices (mean ± standard errors) of the experimented fish at the end of the experiment

Items	Treatment		
	Control (T_1)	**T_2**	**T_3**
HSI, %	1.80^{c} ±0.03	2.19^{a} ±0.03	1.99^{b} ±0.02
GSI, %	1.92^{a} ±0.05	1.63^{b} ±0.03	1.85^{ab} ±0.02
VSI, %	7.10^{b} ±0.04	7.94^{ab} ±0.33	8.66^{a} ±0.20

a – c: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Feed utilization:

AFB alone included diet (T_2) significantly ($P \leq 0.05$) lowered FI, FCR, and FE than the control (T_1), but T_3 improved this picture (Table 4). The same negative effect of AFB-diet (T_2) was calculated for the nutrient's utilization criteria; since it reduced ($P \leq 0.05$) both of PER, PPV, EU, and LR than in T_1 and T_3. Yet, ARCAVIT® (T_3) significantly ($P \leq 0.05$) alleviated the negative effects of AFB on these parameters. Diets containing AFB reduced the appetite of fish, i.e. lowered the consumed feed and consequently also reduced all growth performance parameters (Table 2) and negatively affected all indices of the internal organs too (Table 3).

Table 4: Feed and nutrients utilization (mean ± standard errors) by the experimental fish throughout the whole experimental period

Items	Treatment		
	Control (T_1)	**T_2**	**T_3**
FI, g / fish	92.41^{b} ±0.32	89.64^{c} ±0.68	98.98^{a} ±0.58
FCR	2.41^{c} ±0.32	4.03^{a} ±0.05	3.09^{b} ±0.06
FE, %	41.52^{a} ±0.56	24.89^{c} ±0.34	32.49^{b} ±0.61
Protein utilization			
PER	1.34^{a} ±0.18	0.81^{c} ±0.01	1.05^{b} ±0.02
PPV, %	27.01^{a} ±0.32	17.84^{c} ±0.21	22.56^{b} ±0.37
Energy utilization			
EU, %	13.84^{a} ±0.19	9.58^{b} ±0.11	14.10^{a} ±0.17
Lipid utilization			
LR, %	41.29^{a} ±0.53	36.13^{b} ±0.41	40.59^{a} ±0.67

a – c: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Proximate chemical analysis of the fish:

Chemical analysis on DM basis of the fish body is given in Table 5, which cleared that DM content at the end of the experiment was higher than at the start, therefore and consequently also CP and energy contents were higher at the end of the experiment than at the start. However, DM, EE and ash percentages in T_2 and T_3 were higher ($P \leq 0.05$) than in T_1; yet, CP % was significantly lower, particularly in T_2 than T_3.

Table 5: Proximate chemical analysis (mean ± standard errors) of the whole fish body at start and end of the experiment

Items	Initial	Treatment		
		Control (T_1)	**T_2**	**T_3**
DM, %	25.83	28.46^{b} ±0.49	30.65^{a} ±0.41	30.20^{a} ±0.28
CP, %	57.86	61.36^{a} ±0.06	57.19^{c} ±0.36	59.01^{b} ±0.33
EE, %	21.32	20.40^{b} ±0.12	21.99^{a} ±0.36	21.43^{a} ±0.19
EC, Kcal/g DM	529.89	539.72^{a} ±1.43	532.74^{a} ±3.11	537.39^{a} ±2.89
Ash, %	20.26	17.98^{b} ±0.21	20.18^{a} ±0.44	19.01^{ab} ±0.34

a – c: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Physical properties:

At the end of the experiment, some physical properties were measured and calculated for the experimental fish (Table 6). This Table cleared the negative effects of the toxicated diet (T_2) on fish, although the very low level of AFB (5 ng / g) in this diet. Since it was responsible for significant ($P \leq 0.05$) decrease of K_t, BLM, and LM, besides high WHC. These refer to lower weight of each of the fish body, flesh, lean meat and dry matter of this group's fish. That refers to unsuitability of these group's fish for preservation or filleting nor processing, i.e. it has poor or bad properties and thus has low price and economic value. Dietary addition of ARCAVIT® (T_3) improved, to some extent, these fish flesh quality parameters.

Table 6: Physical properties (mean ± standard errors) of the experimental fish

Items	Treatment		
	Control (T_1)	T_2	T_3
K_t	1.90^{a} ±0.12	1.78^{b} ±0.19	1.94^{a} ±0.03
WHC, %	13.14^{c} ±0.41	17.68^{a} ±0.40	14.83^{b} ±0.32
BLM, %	35.32^{a} ±2.33	28.00^{b} ±0.58	31.67^{ab} ±0.33
LM, %	2.52^{a} ±0.00	2.35^{c} ±0.01	2.42^{b} ±0.01

K_t: condition factor based on total length, WHC: water holding capacity, BLM: boneless meat (fillet), LM: lean meat. a – c: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Blood picture:

Hematological parameters:

The aflatoxic diet (T_2) led to significant ($P \leq 0.05$) increase (Table 7) in values of Hb, RBCs and WBCs; but did not affect ($P > 0.05$) all other parameters [i.e. Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT) and all WBCs-differentiations]. Dietary addition of ARCAVIT® (T_3) improved, to some extent, these affected criteria particularly, Hb, RBCs, and WBCs. That means that the dietary treatments were of limited effect, perhaps for the low level of AFB tested.

Table 7: Hematological parameters (mean ± standard errors) of the experimental fish at the end of the experiment

Items	Treatment		
	Control (T_1)	T_2	T_3
Hb, g/dL	6.33^{b} ±0.34	7.47^{a} ±0.13	6.60^{b} ±0.12
RBCs, $\times 10^6/\mu L$	1.88^{b} ±0.13	2.29^{a} ±0.05	2.01^{b} ±0.03
Hct, %	26.77^{a} ±2.82	29.33^{a} ±1.32	27.90^{a} ±1.56
MCV, fl	141.47^{a} ±0.50	128.33^{a} ±3.04	138.95^{a} ±5.98
MCH, pg	33.70^{a} ±4.98	32.63^{a} ±0.13	32.95^{a} ±5.98
MCHC, %	23.90^{a} ±1.15	25.37^{a} ±0.72	23.80^{a} ±0.92
PLT, $\times 10^3/\mu L$	5.33^{a} ±2.40	9.00^{a} ±1.15	9.00^{a} ±1.73
WBCs, $\times 10^3/\mu L$	95.87^{b} ±2.18	120.37^{a} ±6.14	104.75^{b} ±0.03
Lymphocytes, %	90.00^{a} ±0.58	87.67^{a} ±0.88	89.00^{a} ±1.00
Monocytes, %	7.67^{a} ±0.67	9.67^{a} ±0.89	9.00^{a} ±1.00
Eosinocytes, %	1.00^{a} ±0.00	1.00^{a} ±0.00	1.00^{a} ±0.00

a – b: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Plasma biochemical parameters (hepatic function):

Data in Table 8 presents the mean values of some plasma biochemical parameters measured for experimental fish at the end of the experiment. AFB alone (T_2), and even T_3 (AFB + ARCAVIT®), increased ($P \leq 0.05$) the values of different parameters of the liver function enzymes (ALT, AST, and AST/ALT, referring to

liver damage), but decreased the albumin level ($P \leq 0.05$) and globulin ($P > 0.05$) too, which is important for good immunity responses parameters.

Table 8: Plasma biochemical parameters (hepatic function, as mean ± standard errors) of the experimental fish at the end of the experiment

Items	Treatment		
	Control (T_1)	T_2	T_3
Total Protein, g/dL	2.80^{a} ±0.58	3.40^{a} ±0.58	3.30^{a} ±.58
Albumin, g/dL	2.60^{a} ±0.06	1.90^{b} ±0.06	1.90^{b} ±0.06
Globulin, g/dL	2.00^{a} ±0.09	1.50^{a} ±0.06	1.40^{a} ±0.06
AL/GL	1.31^{a} ±0.08	1.27^{a} ±0.08	1.36^{a} ±0.09
ALT, U/L	72.00^{b} ±0.57	82.00^{a} ±0.57	82.00^{a} ±0.57
AST, U /L	257.00^{b} ±0.57	352^{a} ±0.57	352^{a} ±0.57
AST/ALT	3.57^{b} ±0.40	4.29^{a} ±0.03	4.23^{a} ±0.03

a – b: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Plasma biochemical parameters for liver and kidney functions and immunity:

Some other plasma biochemical parameters for liver function (triglyceride, cholesterol, HDL, LDL, and glucose), kidney function (creatinine and uric acid), and immunity (cortisol) were measured (Table 9) to clear to what extent these functions were affected by the dietary treatments. There were significant ($P \leq 0.05$) effects of the AFB diets (T_2 and T_3), although the very low AFB and ARCAVIT® concentrations in these experimental diets. Since T_2 increased triglyceride, cholesterol, LDL (bad cholesterol), glucose, and cortisol besides uric acid, but decreased HDL (sound or good cholesterol). That means this very low level of AFB is harmful for liver, kidney, and immunity. Since cortisol is an indicator for suffering from different stress factors. The dietary inclusion of ARCAVIT® alleviated one of the toxic effects of AFB (lowered significantly the triglyceride level than in T_1 and T_2 and LDL than T_3), but even strengthen the other toxic symptoms (increased uric acid, cholesterol and glucose and lower HDL levels significantly than in T_1 and T_2).

Table 9: Plasma biochemical parameters (mean ± standard errors) for liver and kidney functions, as well as immunity parameters of the experimental fish at the end of the experiment

Items	Treatment		
	Control (T_1)	T_2	T_3
Creatinine, mg/dL	0.10^{a} ±0.00	0.10^{a} ±0.00	0.10^{a} ±0.00
Triglyceride, mg/dL	127.00^{b} ±0.57	230.00^{a} ±0.57	104.00^{c} ±0.57
Cholesterol, mg/dL	168.00^{c} ±0.00	237.00^{a} ±0.00	209.00^{a} ±0.00
Uric acid, mg/dL	0.60^{c} ±0.00	2.10^{b} ±0.00	2.50^{a} ±0.00
HDL, mg/dL	34.20^{a} ±0.00	31.10^{b} ±0.00	30.70^{c} ±0.00
LDL, mg/dL	108.40^{c} ±0.00	159.90^{a} ±0.00	157.50^{b} ±0.00
Glucose, mg/dL	72.00^{c} ±0.58	74.80^{b} ±0.58	81.80^{a} ±0.58
Cortisol, ng/dL	1.90^{c} ±0.06	6.80^{b} ±0.06	8.80^{a} ±0.08

a – c: Mean in the same row superscripted with different letters differ significantly ($P \leq 0.05$).

Hepatic histopathology:

Histologically, *O. niloticus* fed free AFB-basal diet (BD, as a control group, T_1) showed intact hepatic lobular architecture with normal hepatocytes (h) arrangement around the central vein (CV), and with a normal nucleus (N) (Fig. 1 A and B). However, *O. niloticus* fed 5 ng AFB / g BD (T_2) showed enlargement and severe congestion of the portal blood vessel (PBV), severe degeneration of the hepatocytes, and severe congestion of the blood vessels (BVs), besides severe congestion and dilatation, thickening of BV (Fig. 1 C and D). From another side, *O. niloticus* fed 5

ng AFB / g BD supplemented with 0.5 g ARCAVIT® / Kg BD (T_3) showed intact hepatic lobular architecture with slight degenerative changes, moderate necrotic of the hepatocytes, moderate congestion of BV (Fig. 1 E and F).

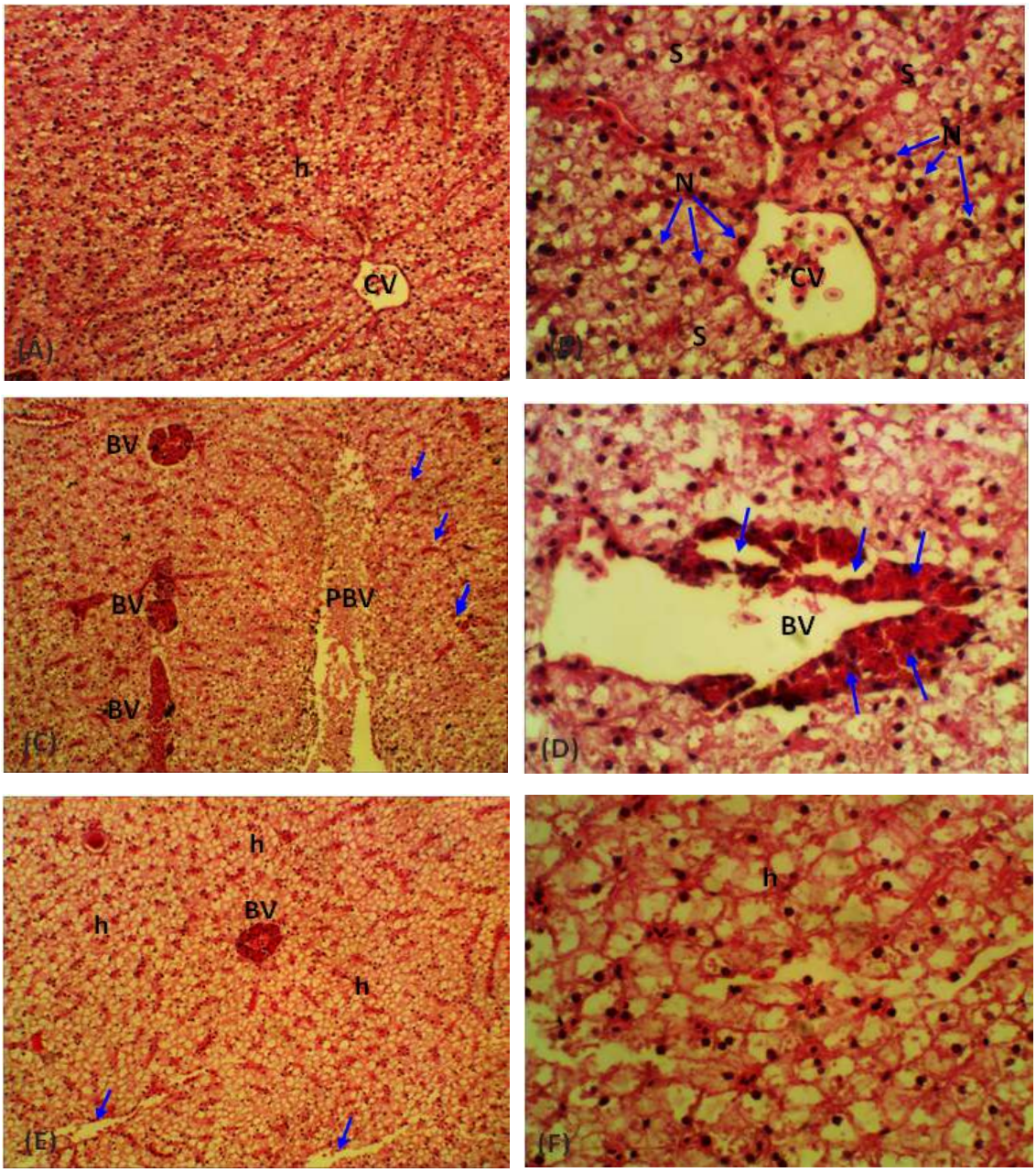

Fig. 1 (A - F): Light micrograph of cross sections in liver of O. niloticus fed (A) T1: zero ng AFB / g BD showing intact hepatic lobular architecture with normal hepatocytes (h) arrangement around the central vein (CV) (× 100, H&E stains); (B) High magnification in liver of T1 showing normal hepatocytes with normal nucleus (N) and intact hepatocyte arrangements arrangement around CV with intact blood sinusoids (s) (× 400, H&E stains); (C) T2: treated fish with 5 ng AFB / g BD showing enlargement and severe congestion of the portal blood vessel (PBV), adjacent with severe degeneration of the hepatocytes (arrows), and severe congestion of the blood vessels (BVs) (× 100, H&E stains); (D) High magnification in liver of T2 showing severe congestion and dilatation, thickening of BV (arrows) (× 400, H&E stains); (E) T3: fish fed 5 ng AFB / g BD supplemented with 0.5 g ARCAVIT® / Kg BD showing intact hepatic lobular architecture with slight degenerative changes (arrows), moderate necrotic of the hepatocytes (h), and moderate congestion of BV (× 100, H&E stains); (F) High magnification in liver of T3 showing nearly intact hepatocytes and hepatic lobular architecture (× 400, H&E stains).

DISCUSSION

From the foregoing results, it was clear (although the very low level of AFB used herein) that 5 ng AFB / g BD in tilapia diet (fed for 70 days) led to external disorders (bad appearance), post-mortem (gross pathological) alterations, lower growth performance, increased internal organs' indices, lower feed and nutrients utilization, lower fish carcass protein content, bad physical properties of the fish (K_t, BLM, LM, and WHC), blood profile changes (liver, kidney and immune-dysfunction). Such morphological and gross pathological alterations were reported too in aflatoxicated fish by Abdelhamid *et al.* (2002b & c, 2004b, and 2007). However, AFB is well known as of hepatorenal (Newberne *et al*., 1964), chromosomal and histological (Abdelhamid *et al.*, 2002c) and carcinogenic (Ahamad *et al.*, 2015) effects. It is a risk to human and animal health, and it is responsible for significant economic losses (Abdallah *et al.*, 2015 and Anater *et al*., 2016).

In a long-term trial (20 weeks), Deng *et al*. (2010) studied the toxic effects of 19-1641 ppb aflatoxin B_1 on tilapia, that was in a dose- and duration-dependent manner. It was of harmful effects with 245 µg / Kg diet or higher doses reducing the growth and lipid content, induced hepatic disorder, HSI, cytochrome P450 A1 activity, elevated plasma ALT activity, and abnormal hepatic morphology. They added that such dietary AFB_1 doses did not affect SR. The AFB_1 residue was only detected in the liver, in a dose-dependent manner, but not inedible flesh. No toxic effects of AFB_1 were found during the first 10 weeks, but by 20 weeks under good culture conditions, tilapia is a rather tolerant species for dietary AFB_1 exposure up to 1641 µg / Kg diet during 20 weeks. Yet, Abdelhamid *et al.* (2004c) found aflatoxin residues in fish muscles.

The obtained negative effects of AFB on fish skin, gut, liver, and kidney are reflecting the low immunity, since these organs belonging to the fish immune system (Press and Evesen, 1999) and blood proteins are non-specific immune factors (Shehata and Goda, 2000 and Hussein and Kobeisy, 2001). Moreover, Abdelhamid *et al.* (2004a, b, & c) evaluated the toxicity symptoms in *Oreochromis niloticus*, whether on growth performance, feed and nutrients utilization, clinical, blood, and histological signs. Abdelhamid *et al.* (2006) reported that stress factor (e.g. toxicants) negatively affect blood picture, i.e. led to decrease in Hb content, Hct %, RBCs, PLT count, hemolytic activity, and total protein, but increase blood glucose. Recently, Mehrim *et al*. (2016) reported that 150 µg AFB_1 / Kg diet negatively affected *O. niloticus* growth, feed efficiency, body composition, condition factor, HSI, blood profile, and liver histological parameters. AFB can carryover from food to fish tissues. Additionally, Hussain *et al*. (2017) described the toxicity symptoms of aflatoxicated (2-4 µg / Kg diet), which reduced weight gain, feed efficiency ratio, HSI, muscle ratio, whole-body crude lipid and protein retention efficiency of *O. niloticus*. Saei *et al*. (2017) registered some changes in the blood picture of aflatoxicated rainbow trout fingerlings than the control. Therefore, considerable investigations are being performed to diminish AFB harmful effects and to prevent its formation.

ARCAVIT® addition not often succeeded in overcoming or improving (to some extent alleviation) the AFB toxicity symptoms, but even strengthens some other toxic symptoms. In this field of study, i.e. attempts of prevention, control, and/ detoxification were lately conducted. Thus, Abdelhamid *et al*. (2002b) emphasized to hygienic control of *O. niloticus* diets to avoid toxigenic fungal invasion, since prophylaxis is better than medication. Also, Abdelhamid *et al*. (2004a) used egg shell,

betafin, clay and silica to detoxify AFB_1 effects on *O. niloticus*. They found egg shell and clay were the best for that purpose. In this respect, Mehrim *et al*. (2006) came to the conclusion that ginger was the best detoxifying agent of 100 μg AFB_1 / Kg diet by fish, followed by aspirin and chamomile flowers, respectively among the tested supplements. Perhaps the positive effects of ARCAVIT® are due to its containing on active yeast and probiotics, that has positive effects on fish performance, nutrient utilization, body composition, and blood constituents (Abdelhamid *et al*., 2000 & 2002b; El-Ebiary and Zaki, 2003; Khattab *et al*., 2004 and Aly *et al*., 2008, respectively). Moreover, Zychowski *et al*. (2012) used NovaSil® to overcome the aflatoxicosis by fish. Additionally, Mehrim *et al*. (2016) tested Glutathione-Enhancer™ against foodborne aflatoxicosis (150 μg AFB_1 / Kg diet) by *O. niloticus*. It ameliorated the aflatoxicosis severity. Magouz *et al*. (2016) reported that black pepper, Filofeed plus and cap T_2 could be used for aflatoxin detoxification. Also, Hussain *et al*. (2017) used clay-based binders (calcium bentonite clay) to adsorb 2-4 mg AFB_1 / Kg in *O. niloticus* food, which improved some of aflatoxic-fish responses. Furthermore, Saei *et al*. (2017) tried to bind dietary 1 mg AFB_1 / Kg diet (to prevent its absorption by fish) via 0.2% Biotoxin, which increased SR of fish. More recently, Ayyat *et al.* (2018) alleviated aflatoxin residues by *O. niloticus* fed 2 mg AFB_1 / Kg diet using some dietary additives (clay, coumarin, curcumin, vitamin C, probiotics and prebiotics). Kovač *et al.* (2018) also reached to nanoparticles capable to possess a great potential of modifying secondary metabolites biosynthesis of AFB_1 in *Aspergillus flavus*. Moreover, Neeratanaphan and Tengjaroenkul (2018) studied the protective effect of dietary bentonite on aflatoxicated *O. niloticus*.

Generally, the negative correlation between protein and fat percentages is a fact (Soltan *et al.*, 2006; Ali, 2008; Salem *et al*., 2008; Saad, 2010 and Farrag *et al*., 2013). Yet, other researchers found a positive relation between CP and EE contents of the fish body (Eweedah *et al*., 2006 and Soltan *et al.*, 2008). Others did not find the effect of dietary treatments on fish body composition (El-Dakar, 2004).

Histopathological alterations have been usually used as biologically markers in the assessment of the health status of fish exposed to pollutants or contaminants (Thophon *et al*., 2003). Especially, the liver is considered to be the primary target organ of AFB. From the obtained histological characteristics, it could be noted the adverse effects of AFB on liver tissue of treated *O. niloticus*. These findings are similarly obtained by Mehrim *et al.* (2006 & 2016); El-Barbary and Mehrim (2009); Mehrim and Salem (2013); Zychowski *et al*. (2013). Additionally, Mahfouz and Sherif (2015) reported the same histopathological effects of AFB_1 on the liver of *O. niloticus* treated fish. Also, they stated that the observed alterations in fish status, especially in the liver coincide well with the expected oxidative stress related to the toxicity of AFB_1. In a recent study, Shahafve *et al*. (2017) stated that *Cyprinus carpio* fed AFB -contaminated diet, even in low concentrations (≤ 1.4 mg / Kg diet) caused histopathological damages, especially for liver tissue and disturbed their physiological balance. In this regard, severely hepatic lesions in the AFB_1-injected *O. niloticus* were recently reported by El-Barbary (2018). Moreover, Anikuttan *et al*. (2018) also clearly indicated the strictly hepatotoxicity of AFB_1 on tropical estuarine teleost fish, *Etroplus suratensis*.

A number of studies are attentive on the prevention or detoxification of mycotoxins, especially AFB from food and feed. Consequently, it is of utmost importance to develop a safe and suitable detoxification technique without conceding the nutritional value of food (Aiko and Mehta, 2015). In the present findings, the addition of 0.5g ARCAVIT® / Kg of AFB- contaminated diet (T_3) as an anti-AFB

agent led to protective effects against the aflatoxicosis of *O. niloticus* compared with those fed AFB- contaminated BD diet (5 ng AFB / g diet, T_2). These currently promising effects of ARCAVIT® against the toxicity of AFB on treated fish are comparable with those previously well documented by Mehrim *et al*. (2006 & 2016); El-Barbary and Mohamed (2014). Recently, Abdel Rahman *et al*. (2017) found that the aflatoxicated *O. niloticus* treated with Fennel essential oil (FEO) or *Saccharomyces cerevisiae* or their mixture revealed significant improvement of mostly measured parameters, as well as, they reported that FEO can successfully relieve AFB_1 noxious effects compared with *S. cerevisiae*. In this respect, a degree of protection of aflatoxiced *O. niloticus* fed garlic and curcumin supplemented diets was recently detected by El-Barbary (2018). Inversely with the obtained findings herein, Abdelhamid *et al*. (2002a) suggested that no significant effects of dietary Biogen of detoxification of AFB_1 of treated *O. niloticus*. These differences may be related with the concentration of AFs, the exposure time, type of the detoxification method and experimental management.

REFERENCES

Abdallah, M.F.; G. Girgin and T. Baydar (2015). Occurrence, Prevention and Limitation of Mycotoxins in Feeds. Animal Nutrition and Feed Technology, 15: 471-490.

Abdallah, M.F.; G. Girgin, T. Baydar, R. Krskaa and M. Sulyoka (2017). Occurrence of multiplemycotoxins and other fungal metabolites in animal feed and maize samples from Egypt using LC-MS/MS. Published online in Wiley Online Library: (wileyonlinelibrary.com) DOI 10.1002/jsfa.8293.

Abdel Rahman, A. N.; S. A. Abdellatief and H. H. H. Mahboub (2017). Protection of Nile tilapia, *Oreochromis niloticus* from aflatoxin B1 toxicity by dietary supplementation with Fennel essential oil and *Saccharomyces cerevisiae*. The Egyptian Journal of Aquatic Research, 43 (3): 235-240.

Abdelhamid, A. M. (1983). Mykotoxin-Nachweis in Lebens-und Futtermitteln des subtropischen Klimas. In: Kurzfassungen der Vorträge zur 37. Tag. Ges. Ernährungsphysiol. Haustiere, Gőttingen, 5. Z. Tierphysiol., Tierernährug u Futtermittelkde., 50: 4-5 (Egyptian Agricultural Bibliography, IX, 1986, 85-100438).

Abdelhamid, A.M. (1996). Field and Laboratorial Analysis in Animal Production. Dar Al-Nashr for the Egyptian Universities. Cairo, 680p., 977-5526-47-7, Deposition No. 11318/1996.

Abdelhamid, A. M. (2000). Pollution of aquatic environment. Proc. Symposium "Fish Wealth Development-Principles and Limitations", Mansoura Univ., May 9, PP: 225-233.

Abdelhamid, A. M. (2004). Aquafeed Quality: Mycotoxins and Mycotoxicoses in Fish. Nutrition in the Management of Aquaculture System, 14-24 June, World Fish Center/ FAO. World Fish Center, Abbassa- Abou Hammad- Sharkia- Egypt.

Abdelhamid, A. M. (2009). Thirty years (1978-2008) of mycotoxins research at Faculty of Agriculture, Mansoura University, Egypt. Egyptian Journal of Nutrition and Feeds, 12 (1): 1-14.

Abdelhamid, A. M. (2010). Thirty-two years (1978 – 2010) of mycotoxins research at Faculty of Agriculture, Mansoura University, Egypt. Abstracts book of MycoRed Workshop on "Mycotoxicological risks in Mediterranean countries: economic impact, prevention, management and control", 25-27 Oct., Cairo, Egypt.

Abdelhamid, A.M. (2019a). Husbandry, Breeding, Physiology and Diseases of Fish. 625p, Deposition No. 25440/2018.

Abdelhamid, A.M. (2019b). Fish Nutrition. 469p, Deposition No. 25442/2018.

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

Abdelhamid, A. M.; A. E. Abdel- Khalek, A.I. Mehrim and F. F. Khalil (2004a). An attempt to alleviate aflatoxicosis on Nile tilapia fish by dietary supplementation with chicken-hatchery by-products (egg shells) and shrimp processing wastes (shrimp shells) on: 1- Fish performance and feed and nutrients utilization. J. Agric. Sci. Mansoura Univ., 29: 6157 – 6173.

Abdelhamid, A. M.; A. E. Abdel- Khalek, A. I. Mehrim and F.F. Khalil (2004b). An attempt to alleviate aflatoxicosis on Nile tilapia fish by dietary supplementation with chicken-hatchery by-products (eggshells) and shrimp Processing wastes (shrimp shells) on: 2 - Clinical, blood and histological parameters. J. Agric. Sci. Mansoura Univ., 29: 6175 – 6196.

Abdelhamid, A. M.; A. I. Mehrim and F. F. Khalil (2004c). Detoxification of aflatoxin-contaminated diet of tilapia fish using dietary supplementation with eggshell, Betafin®, clay or silica. J. Agric. Sci. Mansoura Univ., 29: 3163 – 3174.

Abdelhamid, M. A.; A. M. Ahmed and Kh. M. El-Meleigy (2002a). Detoxification of aflatoxins - contaminated diet by some physical and chemical means. J. Agric. Sci. Mansoura Univ., 27: 8213 - 8224.

Abdelhamid, A. M.; E. A. Sadik and E. A. Fayzalla (1985). Preserving power of some additives against fungal invasion and mycotoxin Production in stored-crushed-corn containing different levels of moisture. Acta Phytopathologica Academca Scientiarum Hungaricae, 20: 309-320.

Abdelhamid, A. M.; F. F. M. Khalil and M.A.A. Seden (2000). Possibility of using dried live yeast and lacto sacc in Nile tilapia fingerlings' diets. J. Agric. Sci. Mansoura Univ., 25 (8): 4905-4911.

Abdelhamid, A. M.; F. F. M. Khalil, M. I. El-Barbary, V. H. Zaki, and H. S. Husien (2002b). Feeding Nile tilapia on Biogen® to detoxify aflatoxic diets. Proc. 1st Ann. Sc. Conf. Anim. & Fish Prod., Mansoura Fac. Agric., 24 & 25 Sep., PP: 207-230.

Abdelhamid, A. M.; F. I. Magouz, M. F. E. Salem, A. A. Mohamed, and M. K. Mohsen (2002c). Effect of dietary graded levels of aflatoxin B1 on growth performance and biochemical, chromosomal and histological behavior of Nile tilapia, *Oreochromis niloticus*. Proc. 1st Ann. Sc. Conf. Anim. & Fish Prod., Mansoura Fac. Agric., 24 & 25 Sep., PP: 231-250.

Abdelhamid, A. M.; M. F. I. Salem, A. I. Mehrim, and M. A. M. El-Sharawy (2007). Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 1-Fish performance, feed and nutrients utilization, organs indices, residues and blood parameters. Egypt. J. Nutr. Feeds, 10, 205-223.

Abdelhamid, A. M.; S. A. El-Ayoty and H. H. Elsaadany (1992). The influence of contamination with separate mycotoxins (aflatoxins, ochratoxin A, citrinin, patulin, penicillic acid or sterigmatocystin) on the dry matter and organic matter digestibility of some roughage (berseem hay and wheat straw) using *in vitro* rumen fermentation. Arch. Anim. Nutr., 42:179-185.

Abdelhamid, A. M.; Y. M. Shabana and S. S. A. Gomaa (2006). Aquatic fungi and fish production in Egypt. The 2nd Inter. Sci. Con. For Environment "Recent Environmental Problems and Social Sharement", 28-30 March, South Valley University. PP: 488 – 523.

Abdelhamid, A. M.; Y. M. Shabana and S. S. A. Gomaa (2008). Aquatic fungi and fish production in Egypt, I- *in vitro* studies. J. Agric. Sci. Mans. Univ., 33: 4887 – 4899.

Abdelhamid, A. M.; Y. M. Shabana and S. S. A. Gomaa (2009). Aaquatic fungi and fish Production in Egypt, II- *in vivo* studies. J. Agric. Sci. Man. Univ., 34: 7675 – 7686.

Ahamad, D.B.; A.K. Sharma and P. Dwivedi (2015). Aflatoxin B_1 induced carcinogenicity in Wistar rats: Clinical signs and growth performance. Shanlax International Journal of Veterinary Science, 3 (2): 2321-6387.

Aiko, V. and A. Mehta (2015). Occurrence, detection and detoxification of mycotoxins. J. Biosci., 40: 943–954.

Ali, B.A. (2008). Effect of total replacement of fish meal by plant protein source and amecozyme on growth performance and feed utilization of monosex Nile tilapia (*Oreochromis niloticus*) fingerlings. Egypt. J. of Appl. Sci., 23 (7): 13-24.

Aly, S.M.; F.M. Mohamed and G. John (2008). Effect of probiotics on the survival, growth and challenge infection in tilapia nilotica (*Oreochromis niloticus*). Aquaculture Research, 39: 647-656.

Anater, A.; L. Manyes, G. Meca, E. Ferrer, F.B. Luciano, C.T. Pimpão and G. Font (2016). Mycotoxins and their consequences in aquaculture: A review Aquaculture, 451: 1–10.

Anikuttan, K. K.; K. C. George, N. K. Sanil and K. S. Sobhana (2018). Hepatic lesions associated with induced aflatoxicosis in the estuarine teleost *Etroplus suratensis* (Bloch, 1790). Indian J. Fish., 65 (3): 57-65.

AOAC. (1990). Official Methods of Analysis Chemists. AOAC International, Washington DC, USA.

Ayyat, M. S.; A. M. N. Ayyat, A. A. Al-Sagheerand and A. E. A. M. El-Hais (2018). Effect of some safe feed additives on growth Performance, blood biochemistry, and bioaccumulation of aflatoxin residues of Nile tilapia fed aflatoxin-B1 contaminated diet. Aquaculture, 495: 27-34.

Clement, S. and R. T. Lovell (1994). Comparison of processing yield and nutrient composition of cultured Nile tilapia (*Oreochromis niloticus*) and channel catfish (*Ictalurus punctatus*). Aquaculture, 119 (2-3): 299-310.

Decie, S. I. V. and S. M. Lewis (2006). Practical Hematology. 10th Edn., Churchill Livingstone, London. ISBN: 13: 978- 443, PP: 736.

Deng, S. X.; L. X. Tian, F. J., Liu, S. J., Jin, G. Y., Liang, H. J. Yang and Y. J. Liu (2010). Toxic effects and residue of aflatoxin B_1 in tilapia (*Oreochromis niloticus* × *O. aureus*) during long-term dietary exposure. Aquaculture, 307 (3-4): 233-240.

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

El-Barbary, M.I. (2018). Impact of garlic and curcumin on the hepatic histology and cytochrome P450 gene expression of aflatoxicosis *Oreochromis niloticus* using RT-PCR. Turkish Journal of Fisheries and Aquatic Sciences, 18: 405-415.

El-Barbary, M. I. and A.I. Mehrim (2009). Protective Effect of Antioxidant Medicinal Herbs, Rosemary and Parsley, on Subacute Aflatoxicosis in *Oreochromis niloticus*. Journal of Fisheries and Aquatic Science, (4): 178-190.

El-Barbary, M.I. and M.H. Mohamed (2014). Chemoprevention and therapeutic efficacy of glutathione against aflatoxicosis in Nile tilapia (*Oreochromis niloticus*). Global Vetrinaria, 13: 1111-1121.

El-Boshy, M. E., A. M. M., El-Ashram and N. A. A. El-Ghany (2008). Effect of dietary beta-1, 3 glucan on immunomodulation on diseased *Oreochromis niloticus* experimentally infected with aflatoxin B1. In 8th international symposium on Tilapia in aquaculture (PP. 1109-1127).

El-Dakar, A.Y. (2004). Growth response of hybrid tilapia, *Oreochromis niloticus* × *Oreochromis auraus*, fingerlings to diets supplemented with different levels of caraway seeds. J. Agric. Sci. Mansoura Univ., 29 (11): 6083-6094.

El-Ebiary, E.H. and M.A. Zaki (2003). Effect of supplementing active yeast to the diets on growth performance and blood constituents of mono-sex tilapia (*Oreochromis niloticus*). Egypt. J. Aquat. Biol. & Fish., 7 (1): 127-139.

Eweedah, N.M.; E.M. Abd El-Raouf, M.F.I. Salem, M.M.E. Khalafalla and B.S. Abd El-Aty (2006). Replacement of fish meal by fresh water crayfish meal (*Procombrus clarkii*) in practical diets for Nile tilapia (*Oreochromis niloticus*). Egypt. J. Agric. Res., 84 (*1B*): 325-338.

Farrag, F.H.; F.F. Khalil, A.I. Mehrim and M.M.A. Refaey (2013). Pawpaw (*Carica papaya*) seeds powder in Nile tilapia (*Oreochromis niloticus*) diet. 1- Growth performance, survival, feed utilization, carcass composition of fry and fingerlings. J. Animal and Poultry Prod., Mansoura Univ., 4 (6): 363-379.

Henry, R.J. (1964). Colorimetric determination of total protein. Clinical Chemistry. Harper and Row Publ., New York, USA, P 18.

Hussain, D.; A. Mateen and D. M. Gatlin III (2017). Alleviation of aflatoxin B_1 (AFB_1) toxicity by calcium bentonite clay: Effects on growth performance, condition indices and bioaccumulation of AFB_1 residues in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 475: 8-15.

Hussein S.Y. and M.A. Kobeisy (2001). Influence of dietary zinc and vitamin A levels on growth performance, blood constituents and immune competenceof Nile tilapia, *Oreohromis niloticus* under Upper Egypt conditions. Ass. Vet. Med. J., 45 (90): 55-74.

Isaka, M.; M. Sappan, J. Jennifer Luangsaard, N. L., Hywel-Jones, S. Mongkolsamrit and S. Chunhametha (2011). Chemical taxonomy of Torrubiella s. lat.: zeorin as a marker of Conoideocrella. Fungal Biology, 115 (4-5), 401–405.

Khattab, Y.A.E; A.M.E. Shalaby, S.M. Sharaf, H.I. El-Marakby and E.H. RizkAlla (2004). The physiological changes and growth performance of the Nile tilapia *Oreochromis niloticus* after feeding with Biogen® as growth promoter. Egypt. J. Aquat. Biol. & Fish., 8 (2): 145-158.

Kovač, T.; I. Borišev, B. Crevar, F. Č. Kenjerić1, M. Kovač, I. Strelec, C. N. Ezekiel, M. Sulyok, R. Krska and B. Šarkanj (2018). Fullerol C60(OH)24 nanoparticles modulate aflatoxin B_1 biosynthesis in *Aspergillus flavus*. Scientific Report, 8:12855 | DOI:10.1038/s41598-018-31305-9, 8pp.

Kumar, S.; A. Sinha, S. Meshram, M. K. Singh, V. Singh and K.S. Hooda (2018). Myco-toxins Monitoring Device and their Management Strategies through Detoxifying Agents in Feed. Int. J. Curr. Microbiol. App. Sci., 7 (1): 3410-3426.

Less, R. (1968). The Laboratory Handbook of Methods of Food Analysis.Leonard Hill Books, London.

Magouz F. I.; Eweedah, N. M., Salem, M. F. E. and A. A. Amer (2016). Detoxification of aflatoxin contaminated ration by chemical, biological and spices methods in Nile tilapia (*Oreochromis niloticus*) diets. J. Agric. Res., Kafr El-Sheikh Univ., 42 (4): 102-119.

Mahfouz, M. E. and A. H. Sherif (2015). A multiparameter investigation into adverse effects of aflatoxin on *Oreochromis niloticus* health status. The Journal of Basic and Applied Zoology, 71: 48-59.

Mass, J. (2018). Effect of mycotoxins on gut development. Gut Health, pp. 71-72.

McGowan, M.W.; J.D. Artiss, D.R. Standbergh and B.A. Zak (1983). Peroxidasecoupled method for colorimetric determination of serum triglycerides. Clin. Chem., 29: 538.

Mehrim, A.I. and M.F. Salem (2013). Medicinal herbs against aflatoxicosis by Nile tilapia (*Oreochromis niloticus*): Clinical, postmortem signs and liver histological patterns. Egypt. J. Aquac., 3 (1): 13-25.

Mehrim, A.I.; M.M. Refaey, and Kh. M. Elmeleigy (2016). Glutathione-enhancer™ against foodborne aflatoxicosis of *Oreochromis niloticus* (Linnaeus, 1758). Journal of Fisheries and Aquatic Science, 11 (2): 131 – 146.

Mehrim, A.I.; A.M. Abdelhamid, A. Abou-Shousha, M.F.I. Salem and M.A.M.M. El-Sharawy (2006). Nutritious attempts to detoxify aflatoxic diets of tilapia fish: 2-Clinical, biochemical and histological parameters. Journal of the Arabian Aquaculture Society, 1 (2): 69-90.

Natt, M.P. and C.A. Herrick (1952). A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poultry Science, 31: 735–738.

Neeratanaphan, L. and B. Tengjaroenkul (2018). Protective effects of Thai bentonite on aflatoxin B1 contaminated in diet of tilapia fish. Livestock Research for Rural Development, 30 (8) Article #152, 14pp http://www.lrrd.org/lrrd30/8/teng30152.html

Newberne, P.M.; W.W. Carlton and G.N. Wogan (1964). Hepatomas in rats and hepatorenal injury in ducklings fed peanut meal or Aspergillus flavus extract. Path. Vet., 1: 105-132.

NRC (National Research Council) (1993). Nutrient Requirements. National Academy Press. Washington. D. C., USA.

Pearson, D. (1962). The Chemical Analysis of Food. J. & A. Churchill LTD, London.

Press, C. McL. and Ø. Evensen (1999). The morphology of the immune system in teleost fishes. Fish & Shellfish immunology, 9: 309 – 318.

Roberts, R.J., 2001. Fish Pathology. 3rd Edn., Churchill Livingstone, London, ISBN-13: 978-0702025631, Pages: 492.

Saad, A.S. (2010). Effect of fermented diets on growth, body composition and survival of Nile tilapia "*Oreochromis niloticus*". J. Egypt. Ger. Soc. Zool., 61A: Comparative Physiology, 49-62.

Saei, M.M.; H. M. Taee, S. Siahpoust and M. Taheri (2017). Effects of Toxin Binder Biotox on Growth Performance Survival, Enzymatic Activity, Hematologichal and Biochemical Parameters of Fingerlings Rainbow Trout (*Oncorhynchus mykiss*) Fed Diets- Contaminated with Aflatoxin. J. Aquac. Res. Development, S2-013. doi:10.4172/2155-9546.S2-013.

Salem, M.F.I.; M.M.E. Khalafalla, I.A.I. Saad and A.M.A. El-Hais (2008). Replacement of fish meal by silkworm, *Bombyx mori* pupae meal, in Nile tilapia, *Oreochromis niloticus* diets. Egytptian J. Nutrition and Feeds, 11 (3): 611-624.

Shahafve, S.; M. Banaee, B. N. Haghi and M. Mohiseni (2017). Histopathological study of common carp (*Cyprinus carpio*) fed aflatoxin-contaminated diets. Int. J. Aquat. Biol., 5(2): 63-70.

Shalaby, A. M. (2009). The opposing effect of ascorbic acid (vitamin C) on ochratoxin toxicity in Nile tilapia (*Oreochromis niloticus*). Acta Polenica, 2: 18-22.

Shehata, T.M. and A.M. Goda (2000). Effect of dietary lipids levels and sources on immune response of Nile tilapia *Oreochromis niloticus* broodstock in winter season. Egypt. J. Aquat. & Fish., 4 (2): 1-25.

Soltan, M.A.; M.A. Hanafy and M.I.A. Wafa (2008). An evaluation of fermented silage made from fish by-products as a feed ingredient for African catfish (*Clarias gariepinus*). Global Veterinaria, 2 (2): 80-86.

Soltan, M.A.; K.A. Mohamed and A.H. Eid (2006). Effect of protein to energy ratio on growth performance and body composition of red tilapia reared in freshwater. Journal of the Egyptian Aquaculture Society, 1: 57-68.

SPSS (2017). Statistical Package for Social Science (for Windows). Release 25 Copyright ©, SPSS Inc., Chicago, USA.

Stoskopf, M.K., 1993. Fish Medicine. 1st Edn., W.B. Saunders Co., Philadelphia, ISBN-13: 9780721626291, Pages: 882.

Thophon, S.; M. Kruatrachue, E.S. Upatham, P. Pokethitiyook, S. Sahaphong and S. Jaritkhuan (2003). Histopathological alterations of white sea bass, *Lates calcarifer* in acute and subchronic cadmium exposure. Environ. Pollut., 121: 307–320.

Tietz, N.W. (1986). Textbook of clinical chemistry. W.B. Saunders, Philadelphia, 1271.

Tietz, N.W. (1990). Clinical Guide to Laboratory Tests 2nd ed. Philadelphia.

Toguyeni, A.; B. Fauconneau, T. Boujard, A. Fostier, E.R. Kuhn, K.A. Mol and J.F. Baroiller (1997). Feeding behaviour and food utilization in tilapia, *Oreochromis niloticus*: effect of sex ratio and relationship with the endocrine status. Physiology and behavior, 62 (2): 273-279.

Wotton, I.D. and H. Freeman (1982). Microanalysis in Medical Biochemistry. Churchill, New York, USA.

Zaki, M. S. and O. Fawzy (2012). Effect of Afla-Toxins B_1 on Endocrine Status in Cat fish (*Clarious lazera*). Life Science Journal-Acta Zhen. Univ. overseas. 9 (1): 419-422.

Zychowski, K.E.; A.R. Hoffmann, H.J. Ly, C. Pohlenz, A. Buentello, A. Romoser, D. M. Gatlin and T.D. Phillips (2013). The effect of aflatoxin-B_1 on Red Drum (*Sciaenops ocellatus*) and assessment of dietary supplementation of NovaSil for the prevention of aflatoxicosis. Toxins, 5: 1555–1573.

Zychowski, K.E.; C. Pohlenz, T. Mays, A.H. Romoser, B.A. Michael, D.M. Gatlin III and T.D. Phillips (2012). The effect of NovaSil dietary supplementation on the growth and health performance of Nile tilapia (*Oreochromis niloticus*) fed aflatoxin-B1 contaminated feed. Aquaculture 376, 117-123, doi: 10.1016/j.aquaculture.2012.11.020.

ARABIC SUMMARY

هل من الممكن إزالة سُمية علائق الكائنات المائية الملوثة بالأفلاتوكسين؟

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تم إجراء تجربة تغذية على أسماك بلطى نيلى غير مُجنسة لبحث تأثيرات تلوث علائق الأسماك بالسم الفطرى "أفلاتوكسين ب"، وكذا بحث نشاط إزالة السمية لمضاد السموم الفطرية "أركافت بيوأسد فورت". استمرت التجربة ٧٠ يوما خلال سبتمبر- ديسمبر ٢٠١٨م. متوسط وزن الأسماك الأولى ٤٠ جم، أجريت التجربة على ٣ معاملات فى ٦ أحواض زجاجية، بمعدل حوضين (مكررتين) لكل معاملة. مستوى التلوث بأفلاتوكسين ب كان ٥ جزء فى البليون (٥ ميكروجرام فى الكيلو علف), ومستوى مضاد السموم الفطرية المستخدم أركافيت (لأول مرة يختبر فى الاستزراع السمكى) كان نصف جرام فى كيلو علف، وكانت العلائق التجريبية للمعاملات الثلاثة: المعاملة الأولى مقارنة تغذت على العليقة الأساسية بدون إضافات، المعاملة الثانية تغذت على العليقة الأساسية مضافا لها الأفلاتوكسين ب، المعاملة الثالثة تغذت على العليقة الأساسية مضافا لها الأفلاتوكسين ب ومضاد السموم الفطرية. تغذت مجاميع المعاملات على العلائق التجريبية بمعدل ٣% من الوزن الحى للأسماك، على وجبتين يوميا.

أظهرت النتائج تفوق واضح لمعاملة المقارنة وكذلك معلمة مضاد السموم المغذاه على أركافيت تفوقا واضحا عن المعلمة الثانية والمغذاه على العليقة المضاف إليها الأفلاتوكسين ب من حيث المظهر الخارجي والصفة التشريحية. بينما تفوقت معاملة المقارنة على المعاملتين الثانية والثالثة من حيث مقاييس النمو والوزن النهائي وأوزان الكبد والأحشاء. وفي حين تفوقت معاملة المقارنة على المعاملتين الثانية والثالثة من حيث استهلاك العلف والكفاءة الغذائية حيث قلل الأفلاتوكسين من شهية الأسماك إلا أن أركافيت قد خفض معنويا الأثر السلبي للأفلاتوكيسين ب من حيث قياسات الاستفادة الغذائية. كما أظهرت نتائج التحليل الكيماوي انخفاض المحتوى البروتيني في جسم الاسماك في كلا من المعاملة الثانية والثالثة عن معاملة المقارنة.

انخفض معامل الحالة والفليه واللحم الخالي من الدهن وكذا قدرة العضلات على الاحتفاظ بالماء في المعاملتين الثانية والثالثة مقارنة بمعاملة المقارنة وكذلك أدت عليقة الأفلاتوكسين للمعاملة الثانية الى زيادة معنوية في تركيز الهيموجلوبين، عدد كرات الدم الحمراء والبيضاء، ولم تؤثر على باقى قياسات الدم ولقد حسّنت لحد ما إضافة الأركافيت من قيم القياسات المتأثرة بالأفلاتوكسين. وعليه فكانت تأثيرات المعاملات الغذائية محدودة، ربما لانخفاض تركيز التوكسين جدا. كما أدى إضافة التوكسين في المعاملتين الثانية والثالثة لزيادة معنوية في قيم قياسات وظائف الكبد (إنزيمات نقل الأمين) مشيرا لتلف الكبد، وانخفاض تركيزات الألبيومين والجلوبيولين الهام للمناعة؛ أدى ذلك أيضا لارتفاع تركيزات كل من الجليسريدات الثلاثية، الكوليستيرول، الليبوبروتينات منخفضة الكثافة (الممرضة)، الجلوكوز، والكورتيزول، بجانب حمض اليوريك، لكن خفضت من تركيزات الليبوبروتينات عالية الكثافة (الصحية).

أدى إضافة الأفلاتوكسين إلى تضخم واحتقان شديد فى الوريد البابى، اضمحلال شديد فى خلايا الكبد، احتقان الأوعية الدموية بشدة، بجانب احتقان شديد وتمدد زيادة سُمك الأوعية الدموية. مع تلاشي ذلك الأثر عند إضافة أركافيت.

الخلاصة: أفلاتوكسينات ب سامة جدا، حتى على المستوى المنخفض لحد ٥ جزء في البليون (نانوجرام لكل جرام)، للبلطي النيلي. تسئ لمظهر الأسماك، وصفتها التشريحية، وتركيب أكبادها النسيجي، وأداء النمو، ودلائل الأعضاء الداخلية، والاستفادة الغذائية، وتركيب الجسم وخصائصه، وكيمياء الدم. أكثر من ذلك، مضاد السموم المستخدم بمستواه المنخفض لم يقضى تماما على آثار سمية الأفلاتوكسين. وهذا يقودنا للتمسك بالحكمة القديمة " الوقاية خير من العلاج".