Effect of some mycotoxin on growth performance and feed utilization of Nile tilapia *(Oreochromis niloticus)*

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Abstract

The present study was conducted in the fish lab, Sakha Aquaculture Research Unit, Central laboratory for Aquaculture Research-Abbassa, during season 2015, Feeding experiment was conducted for 16 weeks to study the effect of dietary contamination with aflatoxin B_1 (150 ppb, AFB_1) with and without the dietary supplementation of (1gm/kg) of Liquorice (medicinal plants), (2g) of MTB-100 (chemicals) and (0.5g) of Gromin-plus (chemicals) on growth performance and feed utilization of Nile tilapia *(Oreochromis niloticus)* fingerlings. A group of 150 Nile Tilapia fingerlings (O. *niloticus)* with an average initial body weight 13.50 gm were randomly allotted into 5 treatments in 10 glasses aquaria (80x35x40cm). Each treatment was applied in two aquaria. Fresh tap water was stored in fiberglass tanks for 24h under aeration for dechlorination. Prior to the start of the experiment, the fishes were adapted to a basal commercial diet (control diet) containing 31% crude protein and consisted of herring fish meal, soybean meal, yellow corn, wheat bran, sunflower oil and vitamins and minerals mixture for two weeks. These ingredients were pressed by manufactured machine (pellets size 1mm), milled and toxin AFB1 was added at a concentration of 150 ppb except the control. Fish in all treatments were daily fed the experimental diets at level of 3% of the fish biomass. The feed amount was given at two times daily, six days a week for 16 weeks. Fish were weighed weekly and the feed amounts were adjusted on the basis of the new weight. The aflatoxic dite has adversely affected the growth performance and survival rate, feed utilization and carcass composition in fish and residues of AFB_1 in the whole body of fish and indices organs and blood biochemistry of the experimented fish. Dietary MTB-100, Liquorice and Gromin-plus inclusion alleviated aflatoxiccosis semptoms by fish, since it improved all the above tested parameters of aflatoxicated fish. Generally, obtained results in the present study indicated that the additives all the above could be used as detoxifying agents for aflatoxins.

Keywords: Nile tilapia, Liquorice, MTB-100, Gromin-plus, aflatoxin B1
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تأثير بعض السموم الفطرية على أداء النمو وكفاءة الغذاء في اسماك البلطى النيلى

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الخلاصة

أجريت هذه الدراسة بمعمل بحوث الاسماك، وحدة بحوث الثروة السمكية بسخا، المعمل المركزي لبحوث الثروة السمكية بالعباسة خلال موسم صيف ٢٠١٥م ولمدة ١٦ اسبوعا. لدراسة تأثير تلوث العليقة بالأفلاتوكسين ب١ (١٥٠ جزء في البليون) بدون أو مع الإضافات الغذائية المستخدمة كمضادات لهذه السموم (١، ٢ و٠,٥جرام / كيلو جرام عليقة) من كل من هذه المواد وهى (العرق سوس، ام تى بى ١٠٠ و الجرومين بلس) على معدل النمو و البقاء للأسماك، الاستفادة من الغذاء والبروتين، ودلائل الأعضاء الداخلية ، والتحليل الكيماوي لجسم الأسماك، وكذا دراسة المتبقي من الأفلاتوكسين ب١ في جسم الأسماك المعاملة، وبعض القياسات للدم، وبعض الاختبارات البيوكيميائية لأسماك البلطي النيلي. استخدمت ١٥٠ سمكة بمتوسط وزن ابتدائى ١٣,٥ غم / سمكة وقسمت عشوائيا في ١٠ احوض

زجاجيه (٨٠ × ٣٥ × ٤٠ سم) ممثلة في ٥ معاملات. وزعت كل معاملة في حوضين بمعدل ١٥ سمكة لكل حوض. استخدمت المياه العذبة المخزنة فى خزانات من الفيبرجلاس لمدة ٢٤ ساعة تحت التهوية لإزالة الكلور، كما تم استبدال ثلث المياه فى أحواض التربية يوميا وتم استبدالها تماما مرة واحدة كل أسبوع لإزالة المخلفات كما استخدمت مضخات هوائية لتزويد مياه الاحواض بالتهوية اللازمة. قبل البدء في التجربة تم أقلمة الأسماك لمدة أسبوعين في الأحواض بالمعمل وتغذيته علي عليقة السيطرة والتي تحتوي علي٣١٪ بروتين خام وتتكون من مسحوق السمك، وفول الصويا، الذرة الصفراء، نخالة القمح، رجيع ذرة صفراء و زيت زهرة الشمس و مخلوط الفيتامين والأملاح المعدنية. هذه المكونات تم كبسها باستخدام ماكينة خاصة (لعمل العلف المحبب بقطر ١مم). وتم اضافة الأفلاتوكسين ب١ للعلائق بتركيز ١٥٠ جزء في البليون ما عدا المجموعة الضابطة السيطرة. غذيت جميع الأسماك علي العلائق المختبرة بمعدل تغذيه ٣٪ من وزن الأسماك يوميا لمدة ١٦ أسبوع مع توزيع الغذاء علي مرتين في اليوم (في الثامنة صباحا والواحده بعد الظهر) وتم تعديل كمية الغذاء علي أساس الوزن الجديد كل أسبوع خلال مدة التجربة. وعموما فإن نتائج هذا البحث دلت على أن بعض المواد المضافة يمكن أن تستخدم لتخفيف أعراض السموم الفطرية.

Introduction

Mycotoxins are highly toxic secondary products of the metabolism of some fungi mainly belonging to *Aspergilus, penicillium, and Fusarium spp.* Toxic syndromes caused by mycotoxin ingestion by humans and animals are indicated as mycotoxicosis (1). Since some mycotoxins (i.e., AFB1. FB1. OTA. and T2 toxin) are known to produce membrane damage through increased lipid peroxidation (2-6), the protective properties of antioxidant substances have been extensively investigated.

Many specialists are of the opinion that the best approach for decontamination should be degradation by biological matters giving a possibility for removal of AF under mild condition, without using harmful chemicals and without significant losses in nutritive value and palatability of detoxified feed and feedstuffs (7). 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 (8-10). Practically, it is not possible to destroy the contaminated feed, therefore to prevent aflatoxicosis in fish by increasing animal immunity (11,12) and detoxification chemically (13) or biologically (14).

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 especially in Egypt (15). *A. flavus* and *A. parasiticus* are abundant in the soil and are a common contaminant of feed crops in warm and humid environments (1,16).

Tilapia grows and reproduces in a wide range of environmental conditions and tolerates stress induced by transmission (17). Currently, Egypt is one of the countries where, aquaculture is growing fast with *Oreochromis niloticus*, which is the most widely farmed species. Fisheries production in Egypt was recorded to be 1,481,882/ ton Year 2016 according to GAFRD (18).

Nile tilapia is still the most widely cultured species of tilapia in Africa. Nile tilapia is an economically important cultured species in several areas of the world (19,20).

Nile tilapia is the most familiar and popular fishes in Egypt, as well as, in the Middle East and warm climate countries. Tilapia are widely cultured in the tropical, subtropical, and temperate regions of the world and represent the third largest productive group of farmed finfish species, only after carps and salmonids, with annual growth rate of about 12.2% (21).

Therefore, the present research aimed to study the drastic effect of AFB1 on growth performance and survival rate, feed utilization, body composition, indices organs, residues of the AFB_1 in fish body and blood biochemistry as well as on the biochemical studies and histological examination of Nile tilapia *(Oreochromis niloticus)* fingerlings. Also, this study was conducted to evaluate the ability of some nutritional agents, namely Liquorice (as medicinal plants), MTB-100 (as chemicals) and Gromin-plus (as chemicals) at levels of 1, 2 and 0.5g /kg feed from each to detoxify the drastic effects of this dangerous toxin AFB1 on Nile tilapia.

Materials and methods

Experimental fish

The experimental fish, *(Oreochromis niloticus)*, were collected from a private farm in Tolombat 7 EL-Riyadh, Kafr EL-Sheikh governorate. The experimental started in October 2015 and lasted up to January 2016. The fingerlings were placed in a fiberglass tank and then randomly distributed into experimental aquaria for the acclimatization purpose on the experimental conditions until starting the diet. Fish were fed the diet for two weeks, during this period, healthy fish at the same weight replaced the dead fish.

Experimental design of rearing fish

A group of 150 Nile Tilapia fingerlings (O. *niloticus)* fish with an average initial body weight 13.50 g were randomly allotted into 5 treatments in 10 glasses aquaria (80x35x40cm). Each treatment was applied in two aquaria. Fresh tap water was stored in fiberglass tanks for 24h under aeration for dechlorination. One third of the water in each aquarium was replaced daily and totally once every week after removing the wastes. Ten air stones were used for aerating the aquaria. Water temperature ranged between 25 and 28 ^{o}C. Photoperiod was adjusted to be 14h light and 10h darkness using florescent light. Fish feces and feed residue were removed daily by siphoning.

Experimental diets and regime

Before the beginning of the experimental trail about two weeks the fishes were adapted for about two weeks on the basal diet containing 31% crude protein. A basal diet was formulated from the commercial ingredients (herring meal, soybean meal, yellow corn, wheat bran, sunflower oil and Vit. & Min mixture). These ingredients were pressed by manufactured machine (pellets size 1mm), milled and toxin AFB1 was added at a concentration of 150 ppb except the control. Anti-mycotoxin was added at a concentration of 1g Liquorice, 0.5g MTB-100 and 2g Gromin plus / kg diet. Aflatoxin B1 was produced through pellets fermentation (22). The estimated amount of oil was gradually added (few drops gradually) and mixing operation was continued for 20 minutes. After homogenous mixture was obtained, certain amount of water was slowly added to the mixture before pressing processes according to Shimeino *et al.* (23).

Experimental dietary additives

Aflatoxin B1 (150ppb) 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 Barley Fermentation. The moldy Barley was steamed to kill the fungus, dried, milled and analyzed for aflatoxin determination (24). Medicinal plants (Liquorice) was gifted from the local market**. C**hemicals (MTB-100) International free trade from-USE, GUARANTEED ANALYSIS: Glucan Min. 14%, INGREDIENTS: Inner cell wall extract derived from dried brewer's yeast, Dried fermented corn, Dried Saccharomyces cerevisiae fermentation solubles and Silicon dioxide. Chemicals (Gromin-plus) produced by Varsha Multitech- India. Each 1 Kg contains: Breweries dried yeast (saccharomyces cervicea) 2.5×10^{9} CFU, Manan oligosacchhrides 200 gram, Hydrated sodium calcium aluminosilicate (carrier) up to 1000 gram.

The composition and Chemical analysis of the basal and experimental diets were shown in Table 1.

The required amount of the diet was prepared every two weeks and stored in a refrigerator until the beginning of the experiment. The pellets were dried under room temperature for 48 h before use. Chemical analysis revealed that no differences were observed among all diets. The diets were approximately isonitrogenous and isoenergetic. The CP content was 30.90 % on DM basis, and the NFE values 51.61%, such level was within the range suggested by NRC (25).

Table 1: composition of the control and the experimental diets %

Ingredients	T1	T2	T3	T4	T5
Fish meal	10	10	10	10	10
Soybean meal	39	39	39	39	39
Yellow corn	28	28	28	28	28
Wheat bran	18	18	18	18	18
Sunflower oil	4	4	4	4	4
*Vit.&Min. mixture	1	1	1	1	1
Total	100	100	100	100	100
Aflatoxin B1(ppb)	-	150	150	150	150
Liquorice (g)	-	-	0.1	-	-
MTB-100 (g)	-	-	-	0.2	-
Gromin plus (g)	-	-	-	-	0.05
Chemical proximate analysis (% DM basis).					
Dry matter (DM %)			91.29		
Crude protein (CP %)			30.92		
Ether extract (EE %)			6.97		
Ash %			6.30		
Crude fiber (CF %)			4.20		
** NFE %			51.61		
Calculated energy Values:					
***GE (kcal/100g)			458.35		
****DE (kcal/g)			320.55		
*****P/Er (g/kcal)			67.45		

*Vitamins and minerals mixture (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; 500mg Vit.B1; 200 mg Vit. B2; 600 mg Vit. B6; 3000 mg Vit. B12; 450 mg Vit.C; 3000 mg Niacin; 3000 mg Methionine; 10000 mg Choline Chloride; 600 mg Biotin; 300 mg Folic acid; 670 mg Pantothenic acid; 3000 mg Magnesium Sulphat; 1800 mg Zinc Solphat; 10000 mg Iron Solphat; 3000 mg Copper Solphat; 300mg Cobalt Sulphate. ** NFE (Nitrogen free extract**)** calculated by differences [NFE = 100 - (CP+ EE+ CF+ Ash)]. *** Gross energy was calculated according to NRC (25) 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 (26). ***** P/Er (protein energy ratio) = crude protein x 1000/ Gross energy.

Proximate analysis of the experimental diets and fish body

Proximate analysis for the basal diets and fish body at the start and the end of the experiment for different groups were carried out according to the method described by AOAC (27).

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

Growth performance and efficiency of feed and protein utilization

The growth performance and feed utilization parameters were calculated according to the following equations as described by De Silva (28): a-Average weight gain (AWG): = final weight (g) - initial weight (g). b-Average daily gain (ADG): = AWG (g) / Time (days). c-Specific growth rate (SGR): = 100 × [In final weight (g) - In initial weight (g)/ Experimental period (d)] (day). d-Survival rate (SR): SR (%) =100×[Total number of fish at the end of the experimental/ Total number of fish at the start of the experimental]. e-Feed conversion ratio (FCR): FCR =Feed Intake (g) /Weight gain (g). f-Protein efficiency ratio (PER): PER =live weight gain (g) / protein intake (g). g-Productive protein value (PPV): PPV (%) =100 × [Retained protein(g)/ protein intake (g)].

Blood parameters determination

At the end of the experimental period, blood samples from the different groups were taken from the caudal vein for serological analysis. Adequate amounts of whole blood were taken in small plastic vials containing heparin.

Hematological parameters

The blood were diluted with appropriate diluting fluids for RBC and WBC counts and were determined using improved Neubauwr haemocytometer and calculated (3). Hemoglobin Concentration (Hb mg/dl) was estimated according to the method of Zinkl (29). by using commercial kit (Diamond Diagnostic, Egypt). Packed Cell Volume (PCV %) was estimated by the microhaematocrite (30).

Biochemical parameters

Total proteins (TP) were measured using reagent kits obtained from Diamond Diagnostic Company, Egypt (5). Albumin was further assessed electrophoretically in serum (31). Globulin was calculated by mathematical subtraction of albumin value from total proteins (31). Albumin /Globulin (A/G) ratio was calculated according to the equation described by Zhou *et al.* (32). Alanine Amino transferees (ALT) and Aspirate Amino transferees (AST) activities were assayed (33) using reagent kits purchased from Randox Company (UK).

Internal organs indices

All fish were killed at the end of the experiment, and soon the abdominal cavity was opened to remove (liver, Kidney, Gonads and Spleen) which were weighed individually. Hepatic somatic index (HIS), kidney somatic index (KSI), gonads somatic index (GSI), and spleen somatic index (SSI) were calculated as follow: HSI= Liver weight × 100 / fish weight (34). KSI= kidney weight × 100 / fish weight (35). GSI= gonads weight × 100 / fish weight (36). SSI= spleen weight × 100 /fish weight (34).

Residues of aflatoxin in fish body

AFB_1 residues was extracted and analyzed by the method of Jantrarotai *et al.* (34) using HPLC. analyzed by the method of using Fluorometer (VICAM), series-4EX**.**

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 ($P\leq 0.05$), using Duncan multiple rang test (37).

Results and discussion

Growth performance and survival rate

Data presented in Table 2 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 treatments. Average weight gain (AWG), average daily gain (ADG) and specific growth rate (SGR) and survival rate (SR) of the experimental fish were the best for the fish fed T1 (control). However, the addition of all antitoxin additives improved all growth performance parameters compared to T2 (contaminated with aflatoxin B1 150 ppb) which was the lowest values for AWG, ADG, SGR and SR. On the other hand, there were no significant differences among fish fed diets T4 and control while significant differences were found between T4 and T5. AFB_1 at levels of 100ppb and 150ppb in the diet of fish fingerlings without adding antitoxins caused a significant growth depression (38). This poor growth might be a result of expelling the feed from the mouth of fish (39).

Also**,** the same results were reported by Salem *et al.* (40), 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 and shrimp (41,42).

These results also agreed with the findings reported by Salem *et al.* (43) and Salem (44) who found 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 (45). Additionally, Zaki *et al.* (46) mentioned that Aflatoxicosis produce a significant decrease in body weight if compared with the control group. With decreased weight gain and lower feed efficiency, 3 ppm AFB1-exposed fish performed poorly compared to 1.5 ppm AFB1-exposed fish and the control. Reduction in the weight gain and decreases in feed efficiency at the higher dose confirmed the anti-nutritional effects of AFB1 recorded by Zychowski *et al.* (47).

Feed intake and protein utilization

All criteria studied which presented in Table 3 showed that T1, T4 and T3 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 significant differences between T1 and T4 in the values of FCR, PER, and PPV%. The addition of MTB-100 was more effective compared to the other treatments. While, the addition of AFB1 (T2) had a negative effect on feed and protein utilization parameters (FI, FCR, PER, and PPV%). Liquorice and coriandrum stimulates digestion and influences positively the terminal enzymes of the digestive processes (43,48,49).

The present results agreed with the findings (39), 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.* (50) concluded that AFB1 at doses of 20 to 200 mg/Kg of feed did not show any effects on feed and protein utilization. Garlic supplemented diets have immune -stimulation for tilapia (*O. niloticus*) and improved feed intake, body weight and body weight gain than the groups fed diet with aflatoxin (35).

Internal organ indices

Significant (P≤0.05) differences were found among the dietary treatments for kidney- somatic index (KSI), hepato –somatic index (HSI), gonads- somatic index (GSI) and spleen –somatic index (SSI) as presented in Table 4.

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 and T5). 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 (31, 51).

Hepato – somatic index (HSI) was increased by increasing AFB1 level in the diet of *O. niloticus* but gonado – somatic index (GSI) decreased (52). 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) (40,44,53). However, Zychowski *et al.* (47), HSI was decreased significantly in groups exposed to AFB1 (54).

Table 2: Growth performance parameters and survival rates of tilapia as affected by the dietary treatments (Mean*+ SE)

Treatments	Initial Weight (g/fish)	Final Weight (g/fish)	AWG (g/fish)	ADG (g/fish/day)	SGR (% /day)	SR (%)
T1	13.50±0.00^{a}	80.40±2.40^{a}	66.90±2.40^{a}	0.59±0.02^{a}	1.58±0.02^{a}	100.0±0.00^{a}
T2	13.35±0.15^{a}	63.00±0.20^{d}	49.65±0.35^{d}	0.44±0.00^{d}	1.38±0.01^{d}	69.99±3.33^{d}
T3	13.45±0.15^{a}	73.30±0.30^{bc}	59.85±0.45^{bc}	0.53±0.00^{bc}	1.51±0.01^{bc}	89.99±3.33^{b}
T4	13.30±0.10^{a}	77.40±2.60^{ab}	64.10±2.50^{ab}	0.57±0.02^{ab}	1.56±0.02^{ab}	96.66±3.33^{ab}
T5	13.40±0.00^{a}	68.90±0.70^{c}	55.50±0.70^{c}	0.49±0.01^{c}	1.46±0.01^{c}	80.00±0.00^{c}

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

Table 3: Feed intake and feed and protein utilization of Nile tilapia as affected by the dietary treatments (Mean*+ SE)

Treatment	Feed Intake (g)	FCR (g)	PER (g)	PPV (%)
T1	101.02±3.07^{b}	1.51±0.10^{d}	2.14±0.14^{a}	33.65±0.03^{a}
T2	124.95±2.55^{a}	2.51±0.03^{a}	1.28±0.02^{d}	19.84±0.82^{c}
T3	108.82±4.12^{b}	1.81±0.08^{c}	1.78±0.08^{bc}	28.88±2.37^{b}
T4	104.10±0.45	1.62±0.07^{cd}	1.98±0.08^{ab}	31.07±0.38^{ab}
T5	11842±1.12^{a}	2.13±0.05^{b}	1.51±0.03^{cd}	23.16±0.72^{c}

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

On the other hand, the additives (tafla, ammonia and hydrogen peroxide) did not alter the organs weights; yet, they slightly diminished –to some extent – the negative effect of dietary aflatoxin inclusion on the relative weights of all tested organs (53). Also, Shehab El-din *et al.* (15) found that the additives of 0.2% Rotamin and 0.3% Power top as antimycotoxins for Nile tilapia rations caused a decrease in hepato –somatic indices comparing with the aflatoxin B1 contaminated diet. In the present study, the effects of chemicals (MTB-100) and medicinal plants (Liquorice) additives may be due to the increase of fish immunity, reduced the effect of the toxin of AFB1 and hence hide its negative effects on indices of fish. Additionally, the HSI was decreased significantly in groups exposed to AFB1 (47,54-56).

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 5. The control, T4 and T5 diets had the highest DM content compared with the other treatments, 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 fed T1 (control), T4 and T3 and the lowest values were in groups T2, and T5. The differences were significant (P≤0.05) for EE and ash among all treatments. Generally, the addition of MTB-100 and Liquorice improved proximate analysis of fish carcass.

Similar results were reported by Salem (49), who concluded 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, 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 (53,57,58). Also, Salem (44) 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 and T5 and the lowest values were found in groups T2. The highest EE was observed in the fish group of T2.

Residues of aflatoxin in the whole fish body

Data concerning aflatoxin (AFB1) residues in the whole fish body in the different experimental groups are shown in Table 6. The group fed AFB1 contaminated diet (150 ppb) T2 without any feed additives, showed the highest level (12.0 ppb aflatoxin B1) in fish body, followed by the group fed AFB1 contaminated with Liquorice, MTB-100 and Gromin plus which were 3, 3 and 8 ppb for T3, T4, and T5, respectively, So, T3 and T4 was the best treatments in reducing these residues.

Table 4: Internal organs indices of the tilapia fish at the end of experimental period as affected by the experimental diets (Means * ± SE)

Treatment	HSI (%)	KSI (%)	GSI (%)		SSI (%)
			Females	Males	
T1	3.44±0.11^{b}	0.47±0.00^{d}	1.73±0.20^{a}	0.88±0.05^{a}	0.30±0.01^{d}
T2	5.08±0.18^{a}	2.04±0.05^{a}	0.98±0.13^{b}	0.25±0.10^{d}	0.68±0.00^{a}
T3	3.85±0.28^{b}	0.72±0.06^{c}	1.51±0.23^{ab}	0.77±0.07^{ab}	0.38±0.00^{c}
T4	3.93±0.20^{b}	0.66±0.01^{c}	1.56±0.08^{ab}	0.45±0.05^{cd}	0.40±0.01^{c}
T5	4.82±0.05^{a}	1.60±0.07^{d}	1.16±0.19^{ab}	0.58±0.03^{bc}	0.51±0.00^{b}

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

Table 5: Proximate chemical analysis (% DM basis) and energetic value of the whole tilapia body as affected by the experimental diets (Mean*+ SE)

Treatment	% On Dry matter basis			
	DM %	CP %	EE %	Ash %
At the start of the experiment				
Initial	26.45	65.58	17.51	16.89
At the end				
T1	27.54±0.13^{a}	65.81±0.31^{a}	16.56±0.47^{c}	17.21±0.25^{a}
T2	26.39±0.02^{a}	62.96±0.16^{b}	19.68±0.05^{a}	17.35±0.11^{a}
T3	26.85±0.05^{a}	64.99±0.43^{a}	17.20±0.20^{bc}	17.34±1.07^{a}
T4	27.00±0.95^{a}	65.30±0.08^{a}	16.93±0.09^{c}	17.77±0.17^{a}
T5	27.35±0.81^{a}	63.78±0.34^{b}	18.24±0.38^{b}	17.82±0.02a

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

In this respect, AFB1 residues in the *O. niloticus* flesh showed a cumulative effect related to the levels of dietary AFB1 and feeding period (57). Also, Soliman *et al.* (59) found that the significant increase of aflatoxin residues was observed in *O. niloticus* fish after 6 months. Residues of AFB1 in the whole body of *O. niloticus* at the end of the experiment tended to decrease after a freezing period (40,44,57).

Table 6: The residues of aflatoxin B1 in the tilapia fish (wet weight basis) as affected by the dietary treatments (Means)

Treatment	T1	T2	T3	T4	T5
AFB1 in whole Body fish (ppb)	Zero	12	3	3	8

Blood analysis

Serum total protein, albumin and globulin concentrations were significantly lower in *O. niloicus* fed on AFB1 containing rations comparing with the control, but addition of antimycotoxins the serum protein level, albumin and globulin were improved (Table 7).

Nearly similar results were recorded in *O. niloticus* (27,60). Several authors also reported a reduction in plasma protein concentration in fresh water fish *(O. niloticus* and *rainbow trout)* exposed to mycotoxins (16,61). Additionally, a decrease in total plasma protein and albumin blood levels was reported in *Labeo rohita* exposed to AFB1 (62). Selim *et al.* (43) observed a decrease in plasma protein and globulin in *Oreochromis niloticus* fed aflatoxin B1 containing ration. Moreover, aflatoxin hepatotoxicity leads to alterations in protein synthesis and cellular integrity of the liver (63). Serum globulin reduction may be related to hemopoietic toxicity (anterior kidney and spleen) and lymphocytolysis (64). Aflatoxin B1 also proved to be immunosuppressive in fish (62).

The activities of ALT and AST were significantly ($P \leq 0.05$) elevated in the serum of *O. niloicus* fed ration containing aflatoxin B1 comparing with the control one. The results indicated that the antimycotoxins improved the enzymes ALT and AST activities against aflatoxins through lowering ALT and AST activities levels than that of AFB1 treated group but still higher than that in the control group (Table 8).

The present results were in similar to results in *O. niloicus* and *lates niloticus,* respectively (60,65**)**. Pepeljnjak *et al.* (61) reported elevation in liver transaminase enzymes in *Cyprinus carpio* fed 5.0 mg AFB1/kg body weight for 42 days. Also, an elevation was observed in liver transaminase of *O. niloticus* that had been intoxicated with AFB1 (52). Nearly similar results were obtained (66), who observed an increase in serum transaminase and alkaline phosphatase activities in sea bass exposed to prolonged oral administration of aflatoxins. Also, Selim *et al.* (67) recorded increases in serum AST and ALT in *O. niloticus* fed aflatoxin B1 containing ration. However, the exposure of *gibel carp* fish to aflatoxin B1 in the diet had no significant effect on serum AST and ALT enzymes activities (68).

Saad *et al.* (69) stated that the groups treated with black seed, garlic and onion had a higher total protein, albumin, globulin and albumin globulin ratio than the other treated groups. Also, aflatoxin causes a decrease in the serum protein level but by the addition of natural feeds as black seed, garlic and onion which improved the serum protein level.

The results showed a significant decrease in RBCs counts, Hb concentration and Packed cell volume (PCV) (normocytic norm-chromic anemia), however increase in WBCs concentration in the blood of *O. niloticus* fed on AFB1 containing ration were recorded as compared with the control group (Table 8).

The results indicated that the antimycotoxins improved the fish erythrogram picture against aflatoxins through improving RBCs, Hb, PCV and WBCs level than that of AFB1 treated group (T3) followed by T4 and T5, respectively but still lower than that of the control group.

The main hemopiotic tissue in *O. niloticus* is anterior kidney (59). Renal damage of AFB1 treated channel catfish was reported (70,71), as well as in common carp (61). Similarly, anemia in *O. niloticus* exposed to 0.5 µg or 1.0 µg AFB1 / Kg B.W. for 10 days (16). Also, anemia noticed in *O. niloticus* and *O. aureus* fed on ration supplemented with aflatoxins (33). Additionally, normocytic normochromic anemia in AFB1 treated group (15,60,69).

The present results concerning AST and ALT activity had widely differences among the different treatment (Table 7) indicating a damage of the liver and probably also the kidney. Moreover, Abdelhamid *et al*. (53) found that the activity of AST and ALT enzymes increased significantly ($P \leq 0.05$) in the fish fed aflatoxin-B1 contaminated died. These finding agreed with evidence for acute aflatoxin-B1 nephrotoxicity which was provided by distended gall bladder indicating disrupted osmoregulation (i.e. water retention) as reported by Mehrim *et al.* (58).

Recently In the same trend, Abdelhamid *et al*. (53) and Mehrim *et al.* (58) 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 MTB-100, Liquorice and Gromin-plus may be due to the increased of immunity and hence hide its negative effects on blood parameters of *O. niloticus* fish.

Table 7: Effect of dietary supplementation with AFB1 and anti-mycotoxin on some plasma biochemical parameters (Mean* ± S.E) in Nile tilapia *(Oreochromis. niloticus)* fed the different experimental diets

Treatment	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)	A/G ratio (g/100 ml)	ALT (m/l)	AST (m/l)
T1	6.93±0.27^{a}	3.75±0.05^{a}	3.18±0.28^{a}	0.84±0.04^{a}	7.14±0.31^{c}	89.50±0.50^{a}
T2	5.07±0.03^{c}	3.15±0.05^{c}	1.92±0.09^{b}	0.60±0.03^{a}	13.05±0.21^{a}	117.69±5.64^{a}
T3	6.12±0.22^{ab}	3.60±0.05^{ab}	2.51±0.27^{b}	0.69±0.08^{a}	12.86±0.53^{a}	104.84±5.54^{a}
T4	5.97±0.22^{bc}	3.58±0.01^{b}	2.39±0.23^{b}	0.66±0.06^{a}	8.71±1.16^{bc}	96.09±5.21^{a}
T5	5.65±0.35^{bc}	3.49±0.04^{b}	2.16±0.31^{b}	0.61±0.08^{a}	11.84±1.60^{ab}	93.45±4.88^{a}

Table 8: Effect of dietary supplementation with AFB1 and Anti-mycotoxin on erythrogram picture (Mean* ± S.E) in Nile tilapia *(Oreochromis niloticus)* fed the different experimental diets

Treatment	RBC ($\times10^3$/ mm^3)	Hb (g/100ml)	PCV (%)	WBCs ($\times10^3$/mm^3)
T1	2.35±0.04^{a}	6.94±0.06^{a}	20.50±0.50^{a}	53.98±3.65^{c}
T2	1.19±0.09^{c}	3.95±0.06^{d}	14.50±0.50^{c}	68.01±1.01^{a}
T3	2.18±0.21^{a}	5.06±0.05^{c}	17.50±1.50^{abc}	59.00±0.30^{bc}
T4	2.04±0.16^{ab}	6.10±0.20^{b}	19.50±1.50^{ab}	58.51±0.62^{bc}
T5	1.69±0.01^{b}	4.98±0.04^{c}	16.50±0.50^{bc}	61.27±0.71^{b}

Conclusion

From the foregoing results it could be concluded that aflatoxin contaminated diets caused many drastic effects in all tested parameters. Adding MTB-100 (chemicals) and Liquorice (medicinal plants) at level (1gm and 2gm /kg feed) 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 detoxify mycotoxin (particularly aflatoxin) in diets of fish.

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