NUTRITIOUS ATTEMPTS TO DETOXIFY AFLATOXIC DIETS OF TILAPIA FISH: 1. FISH PERFORMANCE, FEED AND NUTRIENTS UTILIZATION, ORGANS INDICES, RESIDUES AND BLOOD PARAMETERS

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(Received 19/10/2006, accepted 25/1/2007)

SUMMARY

This study was conducted to investigate the toxic effects of aflatoxin B_1 (AFB₁) on mono-sex Nile tilapia *Oreochromis niloticus* fingerlings and attempting to detoxify these drastic effects by using some nutritious agents. Therefore, half percent of each of these nutritious agents namely; Bio-Buds-2x, chamomile flowers, aspirin and ginger were added to aflatoxic (100 ppb aflatoxin B_1) diet for fingerlings. These diets were offered 6 days a week at 3% daily of actual biomass in glass aquaria in duplicate/treatment in an indoor feeding experiment lasted 14 weeks. The aflatoxic diet led to the worst fish growth performance and survival rate, feed and protein utilization, internal organs indices, carcass composition and residues of AFB₁ (ppb) in the whole body of fish and the tested parameters of blood haematology and biochemistry of the experimented fish. Dietary ginger inclusion alleviated aflatoxicosis symptoms by fish, since it improved all the above tested parameters of aflatoixcated fish. Generally, the obtained results in the present study indicated that the ginger was the best detoxifying agent of aflatoxin, followed by aspirin and chamomile flowers respectively.

Keywords: Nile tilapia, ginger, aspirin, chamomile flowers, aflatoxin

INTRODUCTION

Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in foods as a result of fungal growth. They cause a toxic response, termed a mycotoxicosis, when ingested by higher vertebrates and other animals.

Consumption of mycotoxin contaminated foods has been associated with several cases of human poisoning, or mycotoxicosis, sometimes in death (Sweeney and Dobson, 1998 and Bathnagar and Garcia, 2001).

The Food and Agricultural Organisation (FAO) estimated that 25% of the world's crops are affected by

mycotoxins. of which the notorious are aflatoxins. Aflatoxins are considered the most carcinogenic. mutagenic and teratogenic poisonous by-products of the growth of the molds Aspergillus flayus and Aspergillus parasiticus. and are important contaminants of certain foods and animal feeds because of their ability to produce aflatoxins (Farr et al., 1989). As well as, Gradelet et al., (1997) suggested that these metabolites cause liver damage to humans and to most experimental animal species tested. Also, aflatoxins caused economic and health problems because of their ability to contaminate human food and animal feeds, in particular cereals, nuts and oilseeds (Arim 1995 and Njapau et al.," 1998). Aflatoxin losses to livestock and poultry producers from aflatoxincontaminated feeds include death and more subtle effects of immune system suppression, reduced growth rates, and losses in feed efficiency (Vincelli et al., 1995 and FAO 1997 & 2002).

Therefore, some scientific efforts were conducted to use the herbs or natural plants which, detoxification the drastic effects of mycotoxins or aflatoxins on some animals such as, glucomannan (Karaman et al., 2005) or yeast cell wall mannanoligosaccharide (MOS) (Devegowda et al., 1998), or Saccharomyces cerevisiae which were found to have beneficial effects in poultry during mycotoxicosis (Raju and Devegowda 2000), chamomile (Abdelhamid et al., 1985; Soliman and Badeaa 2002 and Ibrahim, 2004), ginger (Vimala et al., 1999 and Abdelhamid et al., 2002e).

Nile tilapia Oreochromis niloticus may represent a model (as a sensitive

model for mycotoxicosis), since this fish is highly susceptible to nutritional deficits and is extremely vulnerable to toxic insult from various chemicals and poisons including aflatoxin B₁ (AFB₁). Therefore, the present work aims to study the drastic effects of AFB, on growth performance and survival, feed and nutrients utilization, some organs indices, carcass composition, residues of AFB₁ and some parameters of blood hematology and biochemistry of the experimented fish O. niloticus. Also, this study conducted to evaluate the ability of some nutritious agents namely. Bio-Buds-2x. chamomile flowers, aspirin and ginger (at a level of 0.5%) to detoxify the drastic effects of this dangerous toxic AFB, on the Nile tilapia fish for 14 weeks.

MATERIALS AND METHODS

This study was conducted to evaluate the ability of some nutritious agents namely Bio-Buds-2x (T₃), chamomile flowers (T_4) , aspirin (T_5) , and ginger (T₆), (at a level of 0.5%), to detoxify the drastic effects of this dangerous toxic AFB₁ on the Nile tilapia fish for 14 weeks. A group of 180 of mono-sex Nile tilapia O. niloticus fingerlings (obtained from the private fish farm at Tolombat 7, Kafr El-Sheikh), with an average initial body weights of 10g were used in this study. Fish were maintained in the aquaria for one month before the beginning of the experiment for acclimatization purpose. The fish in all experiments were distributed into the aquaria at stocking rate of 15 fish per aquarium. The

experimental treatments were tested in two aquaria for each.

A basal diet (30.38% crude protein, 8.79% ether extract, 4.40% crude fiber. 6.24% ash, 478.4 Kcal/100g DM gross energy and 63.5mg cp/Kcal GE, P/E ratio) was formulated from commercial ingredients (fish meal 10%, soybean meal 38%, yellow corn 35.5%, sun flower oil 4%, wheat bran 12% and vit. & min.0.5%). The basal diet was considered as a control (T₁). These ingredients were pressed manufacturing machine (pellets size 1mm), they were milled and toxin AFB₁ was added at a concentration of 100ppb to all diets (T2,T3,T4,T5,T6), except control (T₁). Anti-toxin was added at a concentration of 0.5%. The ingredients and supplements were bought from the local market, aflatoxin B1 was produced through pellets fermentation using Aspergillus parasiticus NRRL 2999 according to the method described by Abdelhamid and Mahmoud (1996). Concentration of the produced aflatoxin B₁ was calculated and incorporated into the experimental diets at a rate of 100 ppb.

The experiment continued for 14 weeks. During the experimental period the fish were fed the experimental diets at a rate of 3% of the live body weight daily, six days a week. The diets were introduced twice daily, at 8 a.m. and 2 p.m.. The amount of the feed was adjusted bi-weekly based on the actual body weight changes. Light was controlled by a timer to provide a 14h light: 10h dark as a daily photoperiod.

At the end of the experiment, one fish from each aquarium was taken immediately to determin the residues of AFB₁ in the whole fish body. Also, the

remained fish were sampled from each aquarium and kept frozen for chemical analysis. The chemical analyses of the basal diet and whole fish body were carried out according to the AOAC (2000). Aflatoxin B₁ determinations in the media extract and the basal diet were determined as described by Abdelhamid (1996). Water quality parameters were measured weekly (Abdelhamid, 1996) including temperature (via a thermometer), pH (using Jenway Ltd., Model 350-pHmeter) and dissolved oxygen (using Jenway Ltd., Model 970- dissolved oxygen meter).

Body weight of individual fish was measured biweekly to point feed quantity and to calculate growth performance and feed utilization in form of: Average weight gain (g/fish) AWG = Average final weight (g) -Average initial weight (g), Average daily gain, (g/fish/day) ADG = AWG (g)/Experimental period (days), Specific growth rate (SGR, %/day) = [In final weight - In initial weight] x 100/Experimental period (d), Feed conversion ratio (FCR) = Feed intake (g)/Live weight gain (g), Protein efficiency ratio (PER) = Live weight gain (g)/Protein intake (g), Protein productive value (PPV %) = Retained protein (g)/Protein intake (g) x 100, and Survival rate (SR%) = End number of the alive fish/The beginning number of the fish x 100. Total tissue residues of aflatoxin B, were estimated by TLC (Thin Layer Chromatography) method described by Abdelhamid (1981). At the end of the experiment, the liver, spleen, kidneys and gonads were removed and weighted individually. The liver, spleen, kidneys and gonads indices were calculated, where: Hepatosomatic index (HSI) = Liver weight (g) x 100/Gutted fish weight (g) (Jangaard et al., 1967), Spleeno-somatic index (SSI) = Spleen weight (g) x 100/fish weight (g), Kidney - somatic index (KSI)=Kidneys weight (g) x 100/fish weight (g) (Alabaster and Lioyd, 1982) and Gonado-somatic index (GSI) = Gonads weight (g) x 100/fish weight (g) (Tseng and Chan, 1982).

Blood samples from the different groups were collected from the caudal fish peduncle. Adequate amounts of whole blood in small plastic vials containing heparin were used for the determination of hemoglobin (Hb) by using commercial kits (Diamond total Diagnostic, Egypt). Also, erythrocytes count (RBCs) and total leucocytes count (WBCs) measured on an Ao Bright -Line Haemocytometer model (Neubauer imroved, Precicolor HBG, Germany). Other blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of total protein according to Gornall et al., (1949), according albumin Weichsebum (1946). globulin difference according to Doumas and Biggs (1977), cholesterol according to Richmond (1973), uric acid according to Barham (1972), alkaline phosphatase (AIP) according to Belfield and (1971),aspartate Goldberg aminotransferase (AST) and alanine aminotransferase (ALT) according to (1976)using а Varley (model spectrophotometer 5010. Germany) and commercial kits.

The data collected statistically analyzed using general linear models procedure adapted by SAS (1997) for

users guide, with a one-way ANOVA. Means were statistically compared for the significance (p \leq 0.05) using Duncan (1955) multiple range test.

RESULTS AND DISCUSSION

1- Quality parameters of rearing water:

All tested water quality criteria were suitable for rearing mono-sex Nile tilapia O. niloticus fingerlings as cited by Abdelhamid (2000b) and Abd El-Hakim et al. (2002). Since water temperature ranged between 22 and 25°C, pH values 6.9 - 7.8 and dissolved oxygen 6.71 - 8.77 mg/l. Also, Abdelhamid et al. (2002c) suggested that these values are suitable for rearing Nile tilapia O. niloticus. In the same trend, Abdelhamid et al. (2004 b&c) found that all the tested water quality (temperature °C, pH value, conductivity mg/l and dissolved oxygen mg/l) criteria were suitable for rearing Nile tilapia fish Omiloticus.

2- Growth performance and survival rate:

Data presented in Table (1) showed that there were no significant ($P \ge 0.05$) differences among the initial body weights and survival rate of the different dietary groups of fish. Yet, were significant $(P \le 0.05)$ differences among various group of fish concerning final body weight, AWG, ADG and SGR of the experimented fish. Being the best values in favor of T₆ (aflatoxin-contaminated diet plus 0.5% ginger), which seem even more better than the control (uncontaminated diet, T_1) and significantly (P ≤ 0.05)

Table (1): Means* ± standard errors of the growth performance of the experimented tilapia fish as affected by the dietary treatment for 14 weeks.

Treat. No.	Body weight (g/fish)		Body gain		SGR	SR %
	Initial	Final	AWG (g/fish)	ADG (g/fish/)	(%/day)	3K 76
Tı	10.00	25.34ab	15.34 ab	0.15 ab	0.94 ab	100.00
	± 0.10	±1.13	±1.03	±0.01	±0.03	±0.00
T_2	10.00	23.45 b	13.45 b	0.13 Ъ	0.86 b	93.33
	±0.00	±0.05	±0.05	±0.00	±0.00	00,0±
T_3	9, 95	22.35 b	12.40 b	0.12 b	0.82 Ъ	100.00
	±0.05	±1.65	±1.60	±0.01	±0.07	±0.00
T ₄	10.10	23.82 b	13.72 b	0,13 ხ	0.87 Ь	86.66
	±0.10	±0.28	±0.37	±0.00	±0.02	±13.33
T,	10.00	25.38ab	15.38 ab	0.15 ab	0.95 ab	100.00
	±0.00	±0.62	±0.62	±0.00	±0.02	±0,00
T_6	10.00	29.25 a	19.25 a	0.19 a	1,09 a	89.99
	±0.00	±1.97	±1.97	±0.02	±0.06	±3.34

^{*}Means (in the same column) superscripted with different letters significantly ($P \le 0.05$) differ. $T_1 = \text{Control diet}$, $T_2 = \text{Diet}_1 + \text{AFB}_1$ (100ppb), $T_3 = \text{Diet}_1 + \text{AFB}_1$ (100ppb) + 0.5% Bio-Buds-2x, $T_4 = \text{Diet}_1 + \text{AFB}_1$ (100ppb) + 0.5%Chamomile flowers, $T_5 = \text{Diet}_1 + \text{AFB}_1$ (100 ppb) + 0.5% Aspirin, $T_6 = \text{Diet}_1 + \text{AFB}_1$ (100 ppb) + 0.5% Ginger.

Table (2): Feed intake and conversion as well as protein utilization in the experimented tilapia fish $(X^* \pm SE)$ as affected by the dietary treatments during the 14 weeks experiment.

Treat.			Protein utilization		
No.	FI (g/fish)	FCR -	PER	PPV %	
T_1	37.26 ^{ab} ±0.46	2. 43 ^{ab} ±0.13	1.35 b±0.07	24.18 ab±1.69	
T_2 T_3	33.39°±0.62 33.53°±1.35	2.48 ^{ab} ±0.03 2.73 ^a ±0.24	1.32 ^b ±0.01 1.21 ^b ±0.10	21.97 b±0.26 21.28 b±1.01	
T ₄ T ₅	33.74°±1.01 34.93°°±0.78	$\substack{2.46^{ab}\pm0.01\\2.27^{ab}\pm0.03}$	1.34 b±0.00 1.45ab±0.02	$23.15^{b}\pm1.13$ $24.48^{ab}\pm1.00$	
T_6	38.44°±0.81	2.01 ^b ±0.16	1.65 a±0.12	28.48 a±1.92	

^{*} Means (in the same column) superscripted with different letters significantly (P≤0.05) differ. T₁= Control diet, T₂= Diet₁ + AFB₁ (100ppb), T₃= Diet₁ + AFB₁ (100ppb) + 0.5% Bio-Buds-2x, T₄= Diet₁ + AFB₁ (100ppb) + 0.5% Chamomile flowers, T₃= Diet₁ + AFB₁ (100 ppb) + 0.5% Aspirin, T₀= Diet₁ + AFB₁ (100 ppb) + 0.5% Ginger. FI= Feed intake, FCR= Feed conversion ratio, PER= Protein efficiency ratio, PPV= Protein productive value.

better than the aflatoxin-contaminated diet (T_2) and aflatoxin-contaminated diets plus 0.5% Bio-Buds-2x (T_3) or 0.5% chamomile flowers (T_4) . The aflatoxin-contaminated diet plus 0.5% aspirin gave performance values not significantly $(P \ge 0.05)$ differ than those given by T_1 and T_6 groups. But, T_3 was the worst one, even than T_2 .

In this context, similar negative effects of AFB₁ on different growth performance parameters and survival rate of tilapia fish were recorded by other authors (Hussein et al., 2000; Abdelhamid et al., 2002 b&c and 2004 b&c; Nguyen et al., 2002 and Shehata et al., 2003). Recently, also Abdelhamid et al., (2004 b&c) found that the effects of aflatoxins B₁ (AFB₁) were significant decreases on growth performance and survival rate of O. niloticus fish. Yet, the effects of mycotoxins on fish depend on potency of mycotoxin, dose, species and strain of the fish, state of health, stage of life, temperature of the water and presence or absence of substances that can modify the toxicity (El-Said, 1997 and Abdelhamid, 2000a).

The positive effects to alleviate the toxic effects of AFB₁ on growth performance and survival rate of tilapia fish by dietary addition of Nigella sativa seeds was recorded by Hussein et al. (2000). As well as, Shehata et al. (2003) found that adding the adsorbent agents significantly ($P \le 0.05$) reduced the toxic effect of aflatoxin on growth performance and mortality rate of Nile tilapia fish. Recently, Abdelhamid et al. (2004 b&c) found that the best feed additives led to significant overcoming the aflatoxic symptoms on growth performance and mortality were egg

shell and clay, respectively. Also, they added that the effects of either adsorbents namely, egg shells and shrimp wastes at levels of 1 and 2%, respectively, were useful to reduce the toxic effects of AFB₁ on *O. niloticus* fish via adsorbing the toxin from the fish diets. On the other side, Abdelhamid *et al.* (2002e) suggested that no one of the tested medicinal herbs (thyme, safflower, ginger, black cumin and/ or garlic) completely overcome the effects of foodborne aflatoxicosis.

Meanwhile, in the present study useful effects of some nutritious agents which, used to detoxify aflatoxic diets of tilapia fish, namely ginger and aspirin due to their chemical and physical properties and/or their positive effects on immune system. Ginger stimulates digestion as it influences positively on the terminal enzymes of digestive process (Ahmed and Sharma, 1997 and Platel and Srinivasan, 2000). However, aspirin (acetylsalicylic acid) is known to cyclooxygenases inhibit the enhancement of cellular immune response, or induction of apoptosis (Shiff and Rigas, 1999 and Subongkot et al., 2003).

3- Feed and Protein Utilization:

All criteria studied and presented in Table (2) showed that once again, T_6 was the best ($P \le 0.05$) treatment (even than the control, T_1) concerning FI, FCR, PER and PPV in tilapia fish; then followed by T_5 . There were no significant ($P \ge 0.05$) differences among T_1 , T_5 and T_6 for FCR and PPV. While, there were no significances between T_6 and T_5 in data of FCR, PER and PPV. Again, T_3 was the worst one, even than the aflatoxin-contaminated diet without additives (T_2).

Similar negative effects of AFB₁ feed and protein utilization parameters of tilapia fish were recorded by Hussein et al. (2000), Abdelhamid et al. (2002 b&c and 2004 b&c), Nguyen et al. (2002) and Salem (2002). This negative effect of AFB₁ may be attributed to its causative pathological alterations in the gastro-intestinal tract (Murjani, 2003). Also, the present results agree with the finding of Nguyen et al. (2002) who suggested that fish fed diets containing 10 and 100 mg AFB₁/kg were observed to expel feed after ingestion. As well as, the authors added that because fish fed the highest levels of aflatoxin-B₁did not consume all of the feed, the AFB, dose was not directly related to the concentration in the feed. Therefore, fish fed the 100 mg AFB₁/kg diet consumed a total of 59 mg AFB₁/kg of body weight, which was only three times than the amount for fish fed the 10 mg AFB₁/kg.Yet, Salem (2002) indicated that feed and protein parameters significantly utilization (P<0.05) reduced by O. niloticus fed the dietary AFB₁. Similar results were obtained by Abdelhamid et al. (2002 b&d).

Moreover, Kasper et al. (2002) found that Betafin did not significantly change feed efficiency and protein utilization. Also, Abdelhamid et al., (2002b) found that dietary Biogen® supplementation was not useful in AFB₁ detoxification. As well as, Abdelhamid al.. (2002a) mentioned that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce the aflatoxicity. On the other side, recently Abdelhamid et al. (2004 b& c) found that the best feed additives led to significant overcoming the aflatoxic symptoms on growth, mortality, feed

and protein utilization were egg shell and clay, respectively. However, in the present results, better results of ginger and aspirin used may be due to their chemical and physical properties and/or its positive effects on immune system. Ginger stimulates digestion as it influences positively the terminal enzymes of digestive process (Ahmed and Sharma, 1997 and Platel and Srinivasan, 2000). However, aspirin (acetylsalicylic acid) is known to inhibit the cyclooxygenases and enhancement of cellular immune response, or induction of apoptosis (Shiff and Rigas, 1999 and Subongkot et al., 2003).

4- Internal organs indices:

The only significant ($P \le 0.05$) differences were found among the dietary treatments for gonado-somatic index (GSI), but not ($P \ge 0.05$) for hepato (HSI), kidney (KSI), and spleen-somatic index (SSI) as presented in Table (3). Treatment No.5 (aflatoxin-contaminated diet plus 0.5% aspirin) reflected significantly ($P \le 0.05$) higher value for GSI than those of the contaminated diet (T_2) and contaminated diet plus 0.5% Bio-Buds-2x (T_3).

Generally, from these results in the present study, the aflatoxic diets caused negative effects on the internal organs indices significantly ($P \le 0.05$) in GSI or not significantly ($P \ge 0.05$) in other indices (HSI, KSI and SSI) comparing with the control diet (T_1). This means that AFB₁ not only reduced growth performance of the tested fish, but also negatively altered internal organs function as a consequence of increasing their relative weights, which may be due to increasing their cells number or volume or elevating their water and/or blood contents (Glaister, 1986). The

Table (3): Means $* \pm$ standard errors of the internal organs indices of the tilapia fish at the end of the 14-weeks period as affected by the experimental diets.

Treat.	HSI%	KSI%	SSI%	GSI%
T_1	3.85±1.25	0.51±0.02	0.26±0.08	0.85 ab±0.20
T_2	4.34±0.11	0.41 ± 0.06	0.47±0.15	0.59 b±0.12
T_3	3.32±0.71	0.38±0.03	0.24±0.05	0.79 b±0.04
T_4	3.68±0.85	0.35 ± 0.03	1.44±1.04	1.37 ab±0.47
T_5	3.72±0.16	0.39±0.07	0.25±0.03	1.69 a±0.27
T_6	3.02±0.09	0.50 ± 0.11	0.25±0.01	0.91 ab±0.07

^{*}Means (in the same column) superscripted with different letters significantly ($P\le0.05$) differ. $T_1=$ Control diet, $T_2=$ Diet₁ + AFB₁ (100ppb), $T_3=$ Diet₁ + AFB₁ (100ppb) + 0.5% Bio-Buds-2x, $T_4=$ Diet₁ + AFB₁ (100ppb) + 0.5% Chamomile flowers, $T_3=$ Diet₁ + AFB₁ (100 ppb) + 0.5% Aspirin, $T_6=$ Diet₁ + AFB₁ (100 ppb) + 0.5% Ginger.

Table (4): Proximate chemical analysis and energetic value of the whole tilapia body as affected by the experimental diets $(X^* \pm SE)$.

Treat.	- · · · · · · · · · · · · · · · · · · ·	Chemical composition % (On Dry matter basis					
No.	DM %	СР	EE	Ash	EC** (Kcal/100g)		
At the star	rt of the expe	riment:					
	24.80	54.30	8.70	22.10	388.38		
At the end	of the exper	iment:					
T_1	26.55	60.61 ab	15.26 e	24.09 a	486.05 c		
	± 0.18	± 0.17	± 0.07	<u>+</u> 0.17	± 0.86		
T_2	25.68	59.37 d	16.82 a	23.85 ab	493.71 a		
_	± 0.12	± 0.07	± 0.06	± 0.03	± 0.35		
T_3	26.19	60.05 c	16.49 b	23.61 b	494.35 a		
	± 0.21	± 0.18	<u>+</u> 0.13	± 0.07	± 0.78		
T_4	25.95	60.37 bc	15.99 c	23.71 ab	491.43 b		
	± 0.43	± 0.25	± 0.12	± 0.11	<u>+</u> 0.37		
T_5	26.11	60.26 bc	15.66 d	24.09 a	487.72 c		
-	± 0.30	± 0.10	± 0.05	± 0.10	± 0.67		
T_6	26.18	60.99 a	15.24 e	23.83 ab	487.21 c		
-	± 0.35	± 0.15	± 0.09	± 0.23	± 1.05		

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

^{**} Calculated after Macdonald et al. (1973). T_1 = Control diet, T_2 = Diet₁ + AFB₁ (100ppb), T_3 = Diet₁ + AFB₁ (100ppb) + 0.5% Bio-Buds-2x, T_4 = Diet₁ + AFB₁ (100ppb) + 0.5% Chamomile flowers, T_5 = Diet₁ + AFB₁ (100 ppb) + 0.5% Aspirin, T_6 = Diet₁ + AFB₁ (100 ppb) + 0.5% Ginger.

same negative effects of AFB₁ on internal organs indices of *O. niloticus* were recorded too by Hussein *et al.* (2000) and Abdelhamid *et al.* (2002c and 2004 a, b & d).

In this context, recently, Abdelhamid et al. (2004a) suggested that the aflatoxic diets increased obviously relative weights of all tested organs (liver, kidneys, spleen, testes, heart and lungs) of rats comparing with the aflatoxin free diets. As well as, Abdelhamid et al. (2004 b &d) reported that the aflatoxic diet (100 ppb AFB₁) led to significant increases (P<0.05) all organs indices comparing with the control diet (zero ppb AFB₁).

Anyhow, AFB₁ effects are variable depending on level and exposure time of/ to the toxin as well as animal species, age, sex and physiological and nutritional states (Abdelhamid. 2000a). Moreover, although AFB₁ is a strong hepatic mycotoxin (Hussein et al., 2000 and Nguyen et al., 2002), it has also nephritic (Abdelhamid and Saleh, 1996) as well as sexual negative effects (Constantini et al., 1999), therefore, it affected either of the tested indices.

Hussein et al. (2000) reported that Nigella sativa seeds reduced the negative effect of 1.0µg AFB₁/kg BW on internal organs indices of O. niloticus fish. Also, Abdelhamid et al. (2004 b &d) reported that the best feed additives led to significant overcoming the aflatoxic symptoms on organs indices were egg shell and clay, respectively. Also, they added that the effects of either adsorbents namely, egg shells and shrimp wastes at levels of 1 and 2%, respectively, were useful to reduce the toxic effects of AFB₁ on O. niloticus fish via adsorbing the toxin

from the fish diets. On the other side, Abdelhamid et al. (2002a) found that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce aflatoxicosis symptoms. As well as, Abdelhamid et al. (2002b) reported that dietary Biogen® supplementation was not useful in AFB, detoxification. Also, Abdelhamid et al. (2004a) added that the additives (tafla, ammonia and hydrogen peroxide) did not alter the organs weight; yet, they slightly diminished- to some extent- the negative effect of dietary aflatoxin inclusion on the relative weights of all tested organs. However, in the present study, the effects of ginger and aspirin may be due to their adsorbative characteristics as mentioned before, so prevented or reduced the absorption of AFB₁ and hence hide its negative effects on internal organs indices of fish.

5- Biochemical analysis and AFB₁ residues of the whole body:

Data of the proximate chemical analysis of the whole body of the tested tilapia fish are given in Table (4). Dry matter (DM) content increased by age advance, but not significantly (P≥0.05) affected by the dietary treatments. Also, crude protein (CP), ether extract (EE), and ash contents increased by aging, but reflected significant (P < 0.05)differences among dietary the treatments. The highest CP and the lowest EE and energy contents were determined in the fish group of T₆, which was even more better than the uncontaminated control group (T_1) . The best treatment (T₆) did not differ significantly (P≥0.05) in ash content of the fish than in the other dietary treatments. The fish group of T₃

reflected the lowest CP and the highest energy contents among the dietary treatments tested.

Similar results were recorded by Hussein et al. (2000) and Abdelhamid et al. (2002 b&c) concerning fish carcass analyses. The same adverse effects of AFB₁ on carcass composition of O. niloticus were recorded too by Abdelhamid et al. (2004 b & c) and Salem (2002).

The present results agree with the findings of Salem (2002) who found that the control group of fish had the highest (P < 0.05) DM and CP values and the lowest (P < 0.05) EE percentage. The latest author added that percentages of DM and CP decreased as the levels of the dietary aflatoxin B₁ increased, while the values of EE and ash increased with increasing the levels of AFB₁. Also, Hussein et al. (2000) found that AFB1 administration led to reduce fish muscular protein, but it was improved by feeding fish on 1-2% Nigella sativa seeds. Yet, Abdelhamid et al. (2004 b&c) found that atlatoxin B₁ significantly reduced DM and CP contents of the O. niloticus fish carcass but, it significantly increased EE and ash contents of the fish. Meanwhile, they added that dietary addition of clay or egg shell and shrimp wastes to the AFB₁ including diets improved this picture.

In accordance with the present findings, Abdelhamid et al. (2002b) reported that the aflatoxic diets significantly (P < 0.01) reduced the fish flesh crude protein content but increased its fat and ash contents proportional to the dietary levels of the aflatoxin. The opposite trends were recorded with the Biogen inclusion, since it increased

crude protein and decreased fat contents (P < 0.01) of the whole fish body. without significant (P > 0.05) effect on the ash percentage. However, the dietary Biogen® addition improved these parameters. Anyhow, Abdelhamid et al. (2002 c) confirmed that adsorbents still neither obstacle nor sufficient mean for removing AFB, and its toxic effects. Yet, the positive effects of ginger and aspirin used in the present study may be due to their adsorbative characteristics as mentioned before, so prevent or reduce absorption of AFB, and hence hide its negative effects on carcass composition of O. niloticus fish.

However, the control fish were free from the aflatoxin residues; whereas, T_2 reflected the highest level being 28.95 ppb aflatoxin B_1 , followed by T_3 , T_4 , T_5 and T_6 , being 27.50, 26.72, 21.21 and 15.64 ppb respectively. So, T_6 was the best treatment in reducing the level of the residues, followed by T_5 .

In this respect, similar results were recorded by (Soliman et al., 1998 and 2000). Yet, Abdelhamid et al. (1998) recorded high level of aflatoxin-B1 (246-303 ppb) in whole body of Nile tilapia fish. As well as, Abdelhamid et al. (2004d) found that residues of AFB1 in the whole body of the aflatoxicated O. niloticus fish directly at the end of the experiment were high and tended to decrease after freezing periods. On the other hand, Abdelhamid et al. (2002 b&c and 2004 b) reported that there were no AF- residues in O. niloticus body. They added that the absence of AFB₁ residue may be attributed to the lost appetite of fish to feed. Thus, AF may be mobilized or excreted from the fish. These variable results may be due to AF level and exposure time as well as to sensitivity variation among fish species to AF.

Moreover, Soliman et al. (1998) found that the presence of Fix-A-tox in the contaminated diet led to a significant decreases in aflatoxin residue (p < 0.05) in O. niloticus fish. Also, recently Abdelhamid et al. (2004d) found that AFB, levels were reduced by going on the freezing time of the fish samples in all treatments of aflatoxicated fish. As well as, they reported that addition of 1% egg shell and 2% shrimp wastes to aflatoxicated diets led to adsorptive effects of the dietary aflatoxin and reduced its residue in fish carcass. However, in the present results the effects of ginger and aspirin may be due to their adsorbative characteristics as mentioned before, so prevent or reduce absorption of AFB₁ and hence there were no AFB, residues in the fish body and muscles.

6- Blood analysis:

Data of some hematological parameters are illustrated in Table (5) which presented significant ($P \le 0.05$) differences among the tested dietary treatments. The ginger diet (T_6) led to higher ($P \le 0.05$) hemoglobin content (Hb), red blood cells (RBCs) count and white blood cells (WBCs) count at all, followed by the aspirin diet (T_5). The first three treatments (T_1, T_2 and T_3) did not differ significantly ($P \ge 0.05$) in these hematological parameters tested.

Table (6) presents some biochemical parameters in fish plasma known as kidney function indicators. There were significant $(P \le 0.05)$ differences among dietary the treatments for concentrations of uric acid, total protein, albumine and

globuline, but not $(P \ge 0.05)$ for albumine/ globuline ratio. The lowest values were recorded for T_6 followed by T_5 .

Liver function indicators were studied in the treated fish plasma. Data of these plasma biochemical criteria are given in Table (7), All toxic diets with different additives (T₁, T₄, T₅ and T₆) used elevated the values of the tested parameters faspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP). cholesterol] than in the toxic diet without additives (T2). Yet, there were no significant (P>0.05) differences among T₆ and T₅ on one side and T₁, on the other side concerning activity of AST and ALT and concentration of cholesterol.

Similar negative effects of AFB₁ on blood parameters of tilapia fish were recorded also (Soliman et al., 2000; Abdelhamid et al. 2002 c, d &e and Nguyen et al., 2002). Moreover, the present findings confirm those reported by Abdelhamid et al. (2002b) who mentioned that the aflatoxin contaminated diets reduced the blood values of PCV, Hb and total protein (P <0.01). Also, the authors added that the dietary addition of Biogen® did not improve this blood picture of O. niloticus fish.

Moreover, Hussein et al. (2000) suggested that AF negatively affected blood parameters of tilapia fish. Also, Shehata et al. (2003) found that the activity of AST and ALT enzymes increased significantly ($P \le 0.05$) in the fish fed aflatoxin-B₁ contaminated diet. Also, in the present results; ALP, AST and ALT activity increased by the AFB₁ treatments indicating a damage of the

Table (5): Means* and standard errors of some hematological parameters of the tilapia fish at the end of the 14- weeks experimental feeding.

Treat. No.	Hb (g/dl)	RBCs (x10 ⁶ /mm)	WBCs (x10 ³ /mm)
T_1	7.70 b±0.10	28.50 bc±0.50	200.00 b±0.00
T_2	7.60 b±0.10	27.50 c±0.50	200.00 b±0.00
T ₃	7.89 b±0.02	30.50 abc±0.50	200.00 b±0.00
T_4	$6.60 c \pm 0.30$	27.50 c±1.50	300.00 ab±99.99
T ₅	8.45 b±0.45	31.00 ab±1.00	400.00 ab±0.00
T ₆	9.70 a±0.20	33.50 a±0.50	500.00 a±99.99

^{*} Means (in the same column) superscripted with different letters significantly (P≤0.05) differ. T₁= Control diet, T₂= Diet₁ + AFB₁ (100ppb), T₃= Diet₁ + AFB₁ (100ppb) + 0.5% Bio-Buds-2x, T₄= Diet₁ + AFB₁ (100ppb) + 0.5% Chamomile flowers, T₅= Diet₁ + AFB₁ (100 ppb) + 0.5% Aspirin, T₆= Diet₁ + AFB₁ (100 ppb) + 0.5% Ginger.

Table (6): Means* and standard errors of some plasma biochemical (kidney function) parameters at the end of the 14-weeks experimental feeding of the tilapia fish.

Treat. No.	Uric acid mg/dl	Total protein g/dl	Albumine g/dl	Globuline g/dl	Al/Gl ratio
$\overline{T_1}$	2.85 a	4.50 a	2.26 a	2.25 a	1.11
	±0.05	±0.00	±0.05	±0.05	±0.35
T_2	2.75 ab	4.20 a	2.20 a	2.00 ab	1.11
	±0.15	± 0.20	±0.00	±0.20	±0.11
T_3	2.43 bc	3.60 b	1.70 b	1.90 b	0.90
	± 0.13	±0.20	±0.20	±0.00	±0.11
T_4	2.15 с	3.33 bc	1.44 bc	1.89 b	0.77
	±0.15	±0.08	±0.04	±0.04	±0.01
T_5	1.65 d	3.18 bc	1.40 bc	1.78 b	0.80
	±0.50	±0.03	±0.05	±0.03	±0.04
T_6	1.40 d	3.14 c	1.33 c	1.81 b	0.74
	±0.10	±0.04	±0.03	±0.01	±0.02

^{*} Means (in the same column) superscripted with different letters significantly (P≤0.05) differ. T₁= Control diet, T₂= Diet₁ + AFB₁ (100ppb), T₃= Diet₁ + AFB₁ (100ppb) + 0.5% Bio-Buds-2x, T₄= Diet₁ + AFB₁ (100ppb) + 0.5%Chamomile flowers, T₅= Diet₁ + AFB₁ (100 ppb) + 0.5% Aspirin, T₆= Diet₁ + AFB₁ (100 ppb) + 0.5% Ginger.

Table (7): Means* and standard errors of some plasma biochemical (liver function) parameters at the end of the 14-weeks experimental feeding of the tilapia fish.

Treat. No.	AST UL	ALT U/L	ALP U/L	Cholest. mg/dl
TL	26.00 b	20.50 b	17.00 c	104.50 ab
	±1.00	±0.50	±2.00	±25.50
T_2	29.50 ab	27.00 ab	23.00 bc	87.50 b
_	±1.50	±3.00	±3.00	±7.50
T_3	34.00 ab	35.00 a	27.50 ab	99.50 ab
	± 4.00	±1.00	±1.00	±9.50
T_4	37.00 a	34.00 a	34.00 a	115.50 ab
•	±3.00	±5.00	±2.00	±3.50
T ₅	34.00 ab	29.00 ab	29.50 ab	122.00 ab
-	±2.00	±1.00	±2.50	±2.00
T_6	34.00 ab	31.00 a	32.00 a	132.50 a
·	±2.00	±1.00	±1.00	±2.50

^{*} Means (in the same column) superscripted with different letters significantly ($P \le 0.05$) differ. $T_1 = C$ ontrol diet, $T_2 = D$ iet₁ + AFB₁ (100ppb), $T_3 = D$ iet₁ + AFB₁ (100ppb) + 0.5% Bio-Buds-2x, $T_4 = D$ iet₁ + AFB₁ (100ppb) + 0.5% Chamomile flowers, $T_5 = D$ iet₁ + AFB₁ (100 ppb) + 0.5% Aspirin, $T_6 = D$ iet₁ + AFB₁ (100 ppb) + 0.5% Ginger.

liver and probably also the kidney. Evidence for acute aflatoxin B₁ nephrotoxicity was provided by distended gall bladder indicating disrupte osmoregulation (i.e. water retention) as reported by Carpenter *et al.* (1995).

In the same trend, recently Abdelhamid et al., (2004 b&d) found that AFB₁ caused significant decrease in hemoglobin concentration, red blood cells count and uric acid and significantly increase in white blood cells count and alkaline phosphatase and transaminases activity of aflatoxicated O. niloticus fish.

Yet, Hussein et al. (2000) added that Nigella sativa seeds (1 and 2%) alleviated the negative effects of aflatoxicosis by fish. Recently. Abdelhamid et al. (2004 b&d) found that the best feed additives led to significant overcoming the aflatoxic symptoms on blood hematological and biochemical parameters were egg shell and clay, respectively. As well as, the positive effects of some nutritious additives used in the present study, chamomile namely Bio-Buds-2x. flowers, aspirin and ginger may be due to their adsorbative characteristics as mentioned before, so prevent or reduce absorption of AFB, and hence hide its negative effects on blood parameters of O. niloticus fish.

This positive effects of the best nutritious additives [namely 0.5% ginger (T₆) and 0.5% aspirin (T₅)], may be due to their chemicals and physicals properties and/or its positive effects on immune system. Ginger stimulates digestion as it influences positively the terminal enzymes of digestive process. (Ahmed and Sharma, 1997 and Platel

and Srinivasan, 2000). However, aspirin (acetylsalicylic acid) is known to inhibit the cyclooxygenases and enhancement of cellular immune response, or induction of apoptosis (Shiff and Rigas, 1999 and Subongkot *et al.*, 2003).

CONCLUSION

From the foregoing results it could concluded that aflatoxin be contamination of fish diets caused many drastic effects on all the tested parameters. Also, AFB₁ is very dangerous from the view point of fish production and public health. It could be recommended for the beneficial using of 0.5% ginger and/or 0.5% aspirin to alleviate the toxic effects of AFB: contaminated fish diets. Also, it is a must to conduct a lot of scientific efforts in this trend to use the medical herbs and other natural agents to detoxify the aflatoxic diets of fish. But, the wisdom still right, that prophylaxis, from toxic effects of mycotoxins especially AFB₁, is more useful than treatments.

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محاولات غذائية لإزالة سمية علائق اسماك البلطي الملوثة بالأفلاتوكسين: 1- أداء الأسماك واستفادتها من الغذاء والمغذيات، دلائل الأعضاء، المتيقيات، و قياسات الدم

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أ قسم إنتاج الحيوان- كلية الزراعة- جامعة المنصورة ، المنصورة- ج.م.ع.
 المعمل المركزي لبحوث الثروة المسمكية بالعباسة- شرقية- وحدة بحوث الأسماك بسخا- ج.م.ع.

أجريت هذه الدراسة للكشف عن التأثيرات السامة للأفلاتوكسين ب على أصبعيات البلطي النيلي وحيد الجنس ، وكذا لمحاولة إزالة تلك الأثار السيئة باستخدام بعض الإضافات الغذائية. لذلك تم إضافة 0.5 % من كل من هذه المواد و هي مادة ال Bio-Buds-2x ، زهر البابونج ، الأسبرين و الجنزبيل لعلائق أسماك البلطي النيلي الملوثة بالأفلاتوكسين (100 جزء في البليون أفلا توكسين ب إ). قدمت هذه العلائق على مدار 6 أيام في الأسبوع بمعدل 3% من الكتلة الحيوية الحقيقية للأسماك في الأحواض الزجاجية ، حيث مُثلت كل معامله في مكررتين (حوضين) ، وتم تغذية الأسماك على هذه العلائق لمدة 14 أسبوعا. حيث أوضحت النتائج أن العلائق الملوثة بالأفلاتوكسين أدت إلى تأثيرات سيئة على كل من معدل النمو و الإعاشة للأسماك ، الاستفادة من الغذاء والبروتين ، ودلائل الأعضاء الداخلية ، والتحليل الكيماوي لجسم الأسماك ، وكذا سجلت النتائج وجود متبقيات من الأفلاتوكسين ب و دلائل الأعضاء الداخلية المعاملة ، كما أثر هذا السم تأثيرات مديئة على قياسات الدم المختلفة للأسماك ، كذلك أظهرت النتائج أن العليقة المحتوية على الجنزبين بعد ألماماك المعاملة بالأفلاتوكسين ب على الأمماك المعاملة بالأفلاتوكسين ب يليها الأسبرين ثم زهر البابونج على أن الجنزبين بعد أفضل مادة مستخدمة لإزالة التأثيرات السيئة للأفلاتوكسين ب يليها الأسبرين ثم زهر البابونج على التوالي.