*J. Agric. Sci. Mansoura Univ., 29 (6): 3163 - 3174, 2004*

# DETOXIFICATION OF AFLATOXIN – CONTAMINATED DIET OF TILAPIA FISH USING DIETARY SUPPLEMENTATION WITH EGG SHELL, BETAFIN, CLAY OR SILICA

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## ABSTRACT

Nile tilapia fingerlings were fed on a basal diet (control) containing 25% crude protein for 8 weeks at a level of 3% daily of the fish biomass for 6 days a week. Six fish groups were offered either the control diet, control plus 100 ppb aflatoxin $B_1$ ($AFB_1$) or control plus 100 ppb $AFB_1$ plus 1% of either egg shell, betafin, clay or silica. The aflatoxic diet led to reductions in final body weight, body gain, daily gain, specific growth rate, survival rate, feed and nutrients utilization, carcass protein and blood hemoglobin and uric acid. But, it increased mortality rate, internal organs indices, carcass fat and ash as well as blood activity of some enzymes. The best feed additives led to significant overcoming these aflatoxic symptoms were egg shell and clay.

Keywords: Nile tilapia – aflatoxin $B_1$ – clay – egg shell – betaine.

## INTRODUCTION

Mycotoxins are the silent enemy. Mycotoxins affect as much as 25% of the world's feed crops each year (Yegany *et al.*, 2002). The most common mycotoxin is aflatoxin. It is firstly identified after the deaths of large number of fish (Linsell, 1979). Aflatoxin $B_1$ ($AFB_1$) is a wide spreeding hepato carcinogen in fish diets. It is the most toxic mycotoxin which very often occurs all over the world in various commodities causing foodborne intoxications named aflatoxicosis by different animal species (Abdelhamid *et al.*, 1990, 1995 a, b&c and 1996 and Abdelhamid, 2000a) and human being (Fink-Gremmels, 1999). Therefore, it is a major contaminant in aquafeeds 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 (Hussain *et al.*, 1993; Abdelhamid and Saleh, 1996; El-Fiky and Zaki, 1997 and Abdelhamid *et al.*, 1998 a and 1999).

Abdelhamid (1985) reported that 90.48% of various Egyptian feedstuffs investigated were contaminated with less than 100 ppb total aflatoxins. Moreover, $AFB_1$ was found with high levels in commercial fish-feeds (749 – 3388 ppb) used in Egypt and in some of the local aquatic fauna, i.e. tilapia fish (246 – 303 ppb), crabs (298 ppb) and shrimp (185 – 372 ppb) (Abdelhamid *et al.*, 1998a). Tilapia fishes particularly *Oreochromis niloticus* are sensitive to aflatoxin (Essa, 1993; Marzouk *et al.*, 1994; El-Fiky and Zaki, 1997; Abdelhamid *et al.*, 1998a and Hemeda, 1999). Different works were attempted to reduce the aflatoxin content in contaminated feeds to the safety levels for animal species using numerous physical, chemical and/or biological

techniques (Abdelhamid *et al.*, 1998b, 2002 b,d&e; Soliman *et al.*, 1999; Ellis *et al.*, 2000 and Hussain *et al.*, 2000).

The aim of the present study was investigating the best adsorbent material, which can detoxify aflatoxin-$B_1$ contamination of *O. niloticus* diet fed for 8 weeks.

## MATERIALS AND METHODS

**1- Fish and Management:**

The present research was carried out in-door during summer season 2002 in a private fish hatchery, Tolmbat 7, Kafr El-Sheikh Governorate, to decide the best adsorbent material, which can detoxify aflatoxin-$B_1$ contamination of *O. niloticus* diet fed for 8 weeks. A total number of 180 healthy *O. niloticus* fingerlings were gifted from the same hatchery with an average initial body weight of about 13.00 gram. After an adaptation period of one week, the fishes were randomly divided into 6 treatments, each treatment at three replicates (each contained 10 fingerlings in a 40 L cylindric plastic aquarium). Each aquarium was supplied with 35 L dechlorinated tap water and an air-stone connected with an compressor and covered with fishing net. The replacement of the aquaria water was done partially every 2 days to re-new the water and to remove the wastes. Electric light was used to complete the day light to 14 hours.

**2- Experimental Diet and Feeding Regimes:**

The experimental fishes received the tested diets twice daily at 9.00 a.m. and 3 p.m. six days a week for 8 weeks. The daily feeding rate was 3% of the live body weight of the fish. The feed quantity was readjusted biweekly on the basis of the actual average biomass of the fish in each replicate. The ground basal floating diet was obtained from Joe Trade Company, Cairo Governorate. It consisted of fish meal, soybean meal, meat meal, wheat bran, rice bran, yellow corn, fish oil, dicalcium phosphate and vitamins and minerals mixture. Proximate analysis of the experimental diet is shown in Table (2). The diet was supplemented with aflatoxin $B_1$ at concentration of 0.0 and 100 ppb without or with additives (egg shell, Betafin, clay and silica) at a rate of 1% (10 g/kg diet). Egg shell, clay and silica were purchased from the local market. However, Betafin was gifted from the local distributor of the product produced by Danisco Animal Nut., Finland. Aflatoxin $B_1$ was extracted from potato dextrose-liquid (PDL) of aflatoxigenic fungal strain of *Aspergillus parasiticus* (NRRL 2999) after 6-days incubation period (Karunaratne *et al.*, 1990).

**3- Criteria Studied:**

Body weight of individual fish was measured biweekly to point feed quantity and to calculate growth performance and feed utilization (Abdelhamid, 2000b) in form of:

- Average weight gain (g/fish) AWG = Average final weight (g) – Average initial weight (g).
- Average daily gain, (mg/fish/day) ADG = AWG (g)/Experimental period (days) x 1000.

- Specific growth rate (SGR, %/day) = ln final weight – ln 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.
- Energy utilization (EU%) = Retained energy (Kcal)/Energy intake (Kcal) x 100.
- Survival rate (SR%) = End number of the alive fish/The beginning number of the fish x 100.
- Corrected mortality rate (CMR%) (Abbot, 1925) = Mortality rate in each treatment – Mortality rate in the control group x 100/100 – Mortality rate in the control group.
- At the end of the experiment, fish were sampled from each aquarium and kept frozen for chemical analysis. Blood samples from each fish of the different groups were collected from the caudal 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 Diagnostic, Egypt).

Other blood samples were collected and transferred for centrifugation at 3000 rpm for 20 min. Plasma was stored in plastic vials at –20°C until biochemical analysis. Uric acid, alkaline phosphatase (ALP, U/L), aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT/ U/L) were determined colorimeterically using commercial kits supplied by Diamond, Diagnostic, Egypt.

Thereafter, for all fish, the liver, spleen, kidneys and gonads were removed and weighed at once. The liver, spleen, kidneys and gonads indices were calculated, where:

- Hepato-somatic 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).
- Gonado-somatic index (GSI) = Gonads weight (g) x 100/fish weight (g) (Tseng and Chan, 1982).

Water quality parameters were measured weekly (Abdelhamid, 1996) including temperature (via a thermometer), pH (using Jenway Ltd, Model 350 – pH-meter), dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter), and conductivity (using Jenway Ltd., Model 470-portable conductivity meter). Proximate analysis of the basal diet and the whole fish body was carried out (A.O.A.C., 1990), besides $AFB_1$ in the basal diet and fish tissues by thin layer chromatography technique (Abdelhamid, 1981, 1985 and 1996).

**4- Statistical Analysis:**

The obtained data were statistically analysed using SAS (1996) procedures for personal computer. When F-test was positive, least significant difference (Duncan, 1955) was calculated for the comparisons among means.

## RESULTS AND DISCUSSION

**1- Rearing water quality and diet analysis:**

As shown from Table (1), all tested water quality criteria were suitable for rearing Nile tilapia (*O. niloticus*) fingerlings as cited by Abdelhamid (2000b) and Abd El-Hakim *et al.* (2002). Since water temperature ranged between 25 and 29°C, pH values 7.1 – 7.7, conductivity 243 – 350 mg/l and dissolved oxygen 5.19 – 15.86 mg/l.

**Table (1): Means of water quality parameters during (8) weeks of the fish rearing in aquaria.**

| Weeks | Temperature °C | pH value | Conductivity ms/cm | Dissolved oxygen mg/L |
|---|---|---|---|---|
| Week 1 | 28.5 | 7.7 | 243.00 | 5.28 |
| Week 2 | 29.00 | 7.4 | 309.20 | 5.19 |
| Week 3 | 28.50 | 7.6 | 350.00 | 5.32 |
| Week 4 | 26.45 | 7.5 | 336.43 | 12.66 |
| Week 5 | 26.80 | 7.7 | 330.82 | 10.52 |
| Week 6 | 26.40 | 7.6 | 320.25 | 14.22 |
| Week 7 | 25.00 | 7.5 | 340.33 | 15.17 |
| Week 8 | 25.32 | 7.4 | 340.67 | 15.86 |

The used basal floating diet contained 25% crude protein (CP), 13% ether extract, 490 Kcal gross energy (GE)/100 g and 51 mg CP/Kcal GE (Table 2). This means that the used diet was sufficient to cover the fish nutrient requirements according to El-Sayed and Teshima (1991) and Abdelhamid *et al.*, (1995 a&b and 1998a). Yet, Soltan *et al.* (2002) reported that the diet containing 30% CP, 300 Kcal/100 g metabolizable energy and 99 mg CP/Kcal was the best.

**Table (2): Chemical analysis (% dry matter basis) of the experimental diet.**

| Nutrients Composition | % |
|---|---|
| Dry matter (DM%) | 89.52 |
| Crude protein (CP%) | 25.14 |
| Ether extract (EE%) | 13.09 |
| Ash % | 7.18 |
| Carbohydrates | 54.59 |
| Gross energy (Kcal/100 g DM) (GE) | 489.72 |
| Protein/energy (P/E) ratio (mg CP/Kcal GE) | 51.34 |

**GE (Kcal/100 g DM) = CP x 5.64 + EE x 9.44 + Carbohydrates x 4.11 (Macdonald *et al.* (1973).**

**P/E ratio (mg protein/Kcal gross energy) = CP/GE x 1000**

**2- Growth performance and organs indices of the fish:**

Table (3) illustrates that there were no significant ($P > 0.05$) differences among treatments in the initial body weight, survival rate (SR) or corrected mortality rate (CMR) of the fish. Yet, there were marketable

decreasing and increasing effects on SR and CMR, respectively due to the dietary inclusion of $AFB_1$ ($T_2$). Moreover, $AFB_1$ significantly ($P < 0.05$) lowered final weight (FW), average weight gain (AWG), average daily gain (ADG) and specific growth rate (SGR) of the fish in $T_2$ groups. Yet, the supplementation of clay (T5) followed by egg shell ($T_3$) significantly ($P < 0.05$) alleviated the toxic effects of $AFB_1$ on FW, AWG, ADG and SGR. However, betafin inclusion synergistically reacted with $AFB_1$, so that $T_4$ was the worst treatment followed by silica addition ($T_6$). In this context, Abdelhamid *et al.* (2002 b&c) reported similar negative effects of $AFB_1$ on tilapia performance including SGR, SR and indices of internal organs. Moreover, Abdelhamid *et al.* (2002b) found that dietary Biogen® supplementation was not useful in $AFB_1$ detoxification. Abdelhamid *et al.* (2002d) came to the same result that dietary inclusion of tafla and aluminosilicate were not sufficient means for removing $AFB_1$ and its toxic effects. Also, Abdelhamid *et al.* (2002a) found that adsorbents, e.g. Antitox plus, Fix-a-tox and Tafla did not significantly reduce $AFB_1$. However, egg shell (including egg shell membrane which contains 10% collagen) can be used as an adsorbent (Gittins and Drakley, 2002). Its fibers show the source of the unique and highly valued type of collagen present in the membrane (Healy *et al.*, 2003a). Moreover, prawn and egg shell wastes could be utilized as adsorbents (Healy *et al.*, 2003b).

**Table (3): Effect of afitoxin $B_1$ ($AFB_1$) on growth performance of *Oreochromis niloticus* (means ± standard errors).**

| Treat. | Body weight (g/fish) | | | ADG | SGR | SR | CMR |
|---|---|---|---|---|---|---|---|
| | Initial weight | Final weight | AWG | | | | |
| $T_1$ | 13.09a ± 0.009 | 25.37a ± 0.154 | 12.28a ± 0.145 | 219.34a ± 2.595 | 1.18a ± 0.008 | 76.66a ± 3.333 | |
| $T_2$ | 13.10a ± 0.003 | 19.15e ± 0.234 | 6.04e ± 0.237 | 107.91e ± 4.243 | 0.68e ± 0.023 | 53.33a ± 8 819 | 30.95a ± 3.353 |
| $T_3$ | 13.10a ± 0.009 | 23.82c ± 0.207 | 10.72c ± 0.197 | 191.42c ± 3.512 | 1.07c ± 0.015 | 70.00a ± 5 774 | 12.50a ± 1.217 |
| $T_4$ | 13.10a ± 0.009 | 18.35f ± 0.111 | 5.25f ± 0.117 | 97.02e ± 4.749 | 0.60f ± 0.011 | 56.66a ±12.018 | 26.19a ±13.482 |
| $T_5$ | 13.11a ± 0.000 | 24.63b ± 0.185 | 11.52b ± 0.185 | 205.71b ± 3.297 | 1.13b ± 0.014 | 73.33a ± 8.819 | 8 33a ± 0.832 |
| $T_6$ | 13.10a ± 0.009 | 22.46d ± 0.335 | 9.36d ± 0.330 | 167.14d ± 5.895 | 0.96d ± 0.024 | 53.33a ± 3.33 | 3.717 |

$T_1$ = Control group
$T_2$ = $AFB_1$ 100 ppb
$T_3$ = $AFB_1$ 100 ppb + egg shell 1%
$T_4$ = $AFB_1$ 100 ppb + betafin 1%
$T_5$ = $AFB_1$ 100 ppb + clay 1%
$T_6$ = $AFB_1$ 100 ppb + silica 1%
AWG = Average weight gain (g/fish)
ADG = Average daily gain (mg/fish/day)
SGR = Specific growth rate (%/d)
SR = Survival rate (%)
CMR = Corrected mortality rate (%)
a-f: Means in the same column having different small letters differ significantly ($P < 0.05$).

The aflatoxic diet ($T_2$) elevated significantly ($P < 0.05$) all organs indices calculated and presented in Table (4) comparing with the control diet ($T_1$). This mean that $AFB_1$ 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 of volume or elevating their water and/or blood contents (Glaister, 1986). Anyhow, $T_3$ and $T_5$ ameliorated the negative effects of $AFB_1$ ($T_2$) on

all tested indices through the presence of 1% of either egg shell or clay, respectively in the aflatoxic diet contained 100 ppb $AFB_1$. However, betafin ($T_4$) and silica ($T_6$) seem to be without positive effect on the aflatoxic diet, particularly on HSI and GSI.

**Table (4): Effect of aflatoxin $B_1$ ($AFB_1$) on indices of *O. niloticus* (means ± standard errors)**

| Treat. Items | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
|---|---|---|---|---|---|---|
| HSI % | 2.86b ± 0.137 | 3.95a ± 0.046 | 3.11b ± 0.055 | 3.06a ± 0.094 | 3.06b ± 0.043 | 3.66a ± 0.149 |
| KSI % | 0.22b ± 0.017 | 0.28a ± 0.009 | 0.26ab ± 0.012 | 0.26ab ± 0.014 | 0.23b ± 0.020 | 0.22b ± 0.008 |
| SSI % | 0.20c ± 0.008 | 0.28a ± 0.006 | 0.22bc ± 0.008 | 0.24b ± 0.009 | 0.24b ± 0.014 | 0.23bc ± 0.015 |
| GSI % | 0.95b ± 0.034 | 1.50a ± 0.087 | 1.07b ± 0.053 | 1.34a ± 0.038 | 1.05b ± 0.038 | 1.33a ±0.046 |

$T_1$ = Control group
$T_2$ = $AFB_1$ 100 ppb
$T_3$ = $AFB_1$ 100 ppb + egg shell 1%
$T_4$ = $AFB_1$ 100 ppb + betafin 1%
$T_5$ = $AFB_1$ 100 ppb + clay 1%
$T_6$ = $AFB_1$ 100 ppb + silica 1%
HSI % = Hepato somatic index.
KSI % = Kidney somatic index.
SSI % = Spleeno somatic index.
GSI % = Gonado somatic index.
a-c: Means in the some row not followed by the same letter differ significantly ($P < 0.05$).

In this concern, Hussein *et al.* (2000) and Abdelhamid *et al.* (2002c) reported that $AFB_1$ negatively affected fish body weight and their internal organs indices. Castro *et al.* (1998) mentioned that betafin showed lower mortality among salmon. Additionally, Magouz (2002) registered positive effects of betafin on growth parameters of tilapia. Although, Vieira *et al.* (2001) found that betaine did not substitute choline effectively in tilapia diets.

**3- Feed and nutrients utilization:**

Table (5) shows that $AFB_1$ ($T_2$) elevated ($P < 0.05$) FCR and lowered ($P < 0.05$) each of PER, PPV and EU comparing with the control ($T_1$). Yet, clay and egg schell addition ($T_5$ and $T_3$, respectively) in the afltoxic diets improved these parameters of feed and nutrients utilization. This negative effect of $AFB_1$ may be attributed to its causative pathological alterations in the gastro-intestinal tract (Murjani, 2003). Similar results were obtained by Abdelhamid *et al.* (2002 b &d). Moreover, Kasper *et al.* (2002) found that betaine did not significantly change feed efficiency. Although, it was said that betafin improves absorption of the nutrients (Anon., 2002) and reduces epithelial damage of the villous (Ombabi, 2002). The better results of clay and egg shell using may be due to their adsorbative characteristics as mentioned before, so prevent or reduce absorption of $AFB_1$ and hence hide its negative effects.

**4- Chemical analyses of fish and blood:**

Aflatoxin $B_1$ significantly reduced DM and CP contents of the fish carcass and blood Hb and uric acid. It significantly increased EE and ash contents of the fish and ALP, GOT and GPT activities of their blood (Tables 6 and 7).

Table (5): Effect of aflatoxin $B_1$ ($AFB_1$) on feed and nutrients utilization of *O. niloticus* (means + standard errors).

| Treat. | FI g/fish | FCR | Protein utilization | | EU % |
|---|---|---|---|---|---|
| | | | PER | PPV % | |
| $T_1$ | 21.42a ± 0.611 | 1.74c ± 0.045 | 2.28a ± 0 054 | 47.03a ± 1.196 | 19.65a ± 0.494 |
| $T_2$ | 20.51ab ± 0.628 | 3.41a ± 0.215 | 1.18c ± 0.081 | 22.84d ± 1.333 | 10.41d ± 0.576 |
| $T_3$ | 20.21ab ± 0.316 | 1.89c ± 0.062 | 2 11a ± 0.069 | 41.56b ± 0.702 | 17.61b ± 0.538 |
| $T_4$ | 19 56b ± 0.219 | 3.73a ± 0.114 | 1.07c ± 0.035 | 22.64d ± 0.522 | 9.94d ± 0.241 |
| $T_5$ | 20.75ab ± 0.349 | 1.80c ± 0.060 | 2.21a ± 0.072 | 45.67a ± 0.134 | 19.11a ± 0.569 |
| $T_6$ | 20.85ab ± 0.662 | 2.23b ± 0.027 | 1.79b ± 0.020 | 33.34c ± 0.306 | 14.71c ± 0.147 |

$T_1$ = Control group
$T_2$ = $AFB_1$ 100 ppb
$T_3$ = $AFB_1$ 100 ppb + egg shell 1%
$T_4$ = $AFB_1$ 100 ppb + betafin 1%
$T_5$ = $AFB_1$ 100 ppb + clay 1%
$T_6$ = $AFB_1$ 100 ppb + silica 1%
FI = Feed intake (g/fish)
FCR = Feed conversion ratio
PER = Protein efficiency ratio
EU = Energy utilization (%)
PPV = Protein productive value (%)
a-d: Means in the same column having different small letters differ significantly ($P < 0.05$).

Table (6): Effect of aflatoxin $B_1$ ($AFB_1$) on carcass composition of *O. niloticus* (means + standard errors).

| Treat. | DM% | % On Dry matter basis | | | |
|---|---|---|---|---|---|
| | | CP % | EE % | Ash % | EC (Kcal/100 g) |
| At the start of the experiment | | | | | |
| | 20.93 | 60.24 | 19.30 | 20.46 | 521.95 |
| At the end of the experiment | | | | | |
| $T_1$ | 25.33a ± 0.355 | 65.02a ± 0.989 | 18.67c ± 0.330 | 16.32b ± 0.025 | 542.96a ± 2.469 |
| $T_2$ | 23.93c ± 0.390 | 61.69b ± 0.429 | 20.34a ± 0.204 | 17.99a ± 0.665 | 539.89a ± 0.490 |
| $T_3$ | 24.90ab ± 0.295 | 62.91b ± 0.369 | 19.09bc ± 0.109 | 18.00a ± 0.265 | 535.03a ± 3.125 |
| $T_4$ | 24.27bc ± 0.295 | 61.95b ± 0.204 | 19.51b ± 0.034 | 18.54a ± 0.104 | 533.56a ± 0.825 |
| $T_5$ | 25.20ab ± 0.219 | 64.95a ± 0.707 | 18.69c ± 0.089 | 16.36b ± 0.104 | 542.76a ± 4.855 |
| $T_6$ | 24.38abc ± 0.054 | 62.01b ± 0.140 | 19.70b ± 0.034 | 18.29a ± 0.075 | 535.66a ± 1.120 |

$T_1$ = Control group
$T_2$ = $AFB_1$ 100 ppb
$T_3$ = $AFB_1$ 100 ppb + egg shell 1%
$T_4$ = $AFB_1$ 100 ppb + betafin 1%
$T_5$ = $AFB_1$ 100 ppb + clay 1%
$T_6$ = $AFB_1$ 100 ppb + silica 1%
DM = Dry matter (%)
CP = Crude protein (%)
EE = Ether extract (%)
EC = Energy content (Kcal/100 g)
a-c: Means in the same column having different small letters are significantly different ($P < 0.05$).

Meanwhile, dietary addition of clay or shell egg to the $AFB_1$ including diets improved this picture.However $AFB_1$ residues were not detectable in the fish carcass. Similar results were recorded by Hussein *et al.* (2000) and Abdelhamid *et al.* (2002 b&d) concerning fish and blood analyses.Anyhow, Abdelhamid *et al.* (2002 c) confirmed that adsorbents still neither obstacle nor sufficient mean for removing $AFB_1$ and its toxic effects. However, Magouz (2002) came to the conclusion that betafin did not influence fish composition.

**Table (7): Effect of aflatoxin $B_1$ ($AFB_1$) on some blood parameters of *O. niloticus* (means ± standard errors).**

| Treat. | Hb g/dl | Uric acid mg/dl | ALP U/L | GOT U/L | GPT U/L |
|---|---|---|---|---|---|
| $T_1$ | 15.38ab ± 1.889 | 3.48a ± 0.278 | 15.46c ± 3.277 | 10.00c ± 1.732 | 38.33b ± 6.983 |
| $T_2$ | 7.10d ± 1.282 | 1.57b ± 0.170 | 50.90a ± 17.412 | 20.67a ± 3.283 | 76.00a ± 9.451 |
| $T_3$ | 12.94abc ± 1.221 | 2.29b ± 0.539 | 20.91c ± 3.633 | 15.00abc ± 1.999 | 48.33ab ± 14.835 |
| $T_4$ | 11.33bcd ± 0.804 | 1.90b ± 0.312 | 35.45abc ± 5.456 | 15.00abc ± 2.645 | 49.33ab ± 10.268 |
| $T_5$ | 16.17a ± 0.480 | 2.62ab ± 0.315 | 15.45c ± 2.404 | 12.00bc ± 1.999 | 40.00ab ± 6.658 |
| $T_6$ | 8.93cd ± 1.999 | 2.36b ± 0.115 | 44.91ab ± 2.363 | 19.33ab ± 2.027 | 66.00ab ± 14.00 |

**$T_1$ = Control group**
**$T_2$ = $AFB_1$ 100 ppb**
**$T_3$ = $AFB_1$ 100 ppb + egg shell 1%**
**$T_4$ = $AFB_1$ 100 ppb + betafin 1%**
**$T_5$ = $AFB_1$ 100 ppb + clay 1%**
**$T_6$ = $AFB_1$ 100 ppb + silica 1%**
**Hb = Hemoglobin (g/dl)**
**ALP = Alkaline phosphatase (U/L)**
**GOT = Glutamic oxalocetic transaminase (U/L)**
**GPT = Glutamic pyruvic transaminase (U/L) or**
**GOT = AST → Aspartate aminotransferase**
**GPT = ALT → Alanine aminotransferase**
**a – d: Mean in the same columns having different small letters are significantly different ($P < 0.05$).**

## CONCLUSION

From the foregoing results and their discussion it could be emphasized that aflatoxin contamination of fish diets is very dangerous from the view point of fish production and public health. Attempting to alleviate aflatoxicosis by fish could be achieved by dietary supplementation of adsorbents, particularly chicken hatchery by-products (egg shell) and clay at an appropriate level to the level of $AFB_1$ in the contaminated diet.

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## إزالة سمية عليقة أسماك البلطى الملوثة بالأفلاتوكسين باستخدام إضافات غذائية من قشر البيض أو البيتافين أو التربة أو السليكا ٠

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تم تغذية إصبعيات أسماك بلطى نيلى على عليقة طافية بها ٢٥% بروتين بدون أفلاتوكسين، أو مع ١٠٠ جزء/بليون أفلاتوكسين $B_1$ بدون إضافات أو مع ١% من أى من قشر البيض أو البيتافين أو التربة أو السليكا، وذلك لمدة ٨ أسابيع بمعدل ٣% من وزن الجسم يوميا لمدة ٦ أيام/أسبوع فى أحواض بلاستيكية سعة ٤٠ لترا فى ٣ مكررات/معاملة٠

خفضت العليقة الملوثة بالأفلاتوكسين من وزن السمك النهائى والزيادة فى الوزن الكلية واليومية ومعدل النمو النوعى والحياتية وكفاءة التحويل الغذائى والمغذيات وبروتين الجسم والهيموجلوبين وحمض اليوريك فى الدم، ورفعت من نسبة نفوق السمك والأوزان النسبية (دلائل) للأعضاء الداخلية ودهن الجسم ورماده والأنشطة الإنزيمية فى الدم٠ وكانت أفضل الإضافات تخفيفا لحدة التلوث الأفلاتوكسينى على هذه القياسات كلها هى قشر البيض أو التربة٠