

AN ATTEMPT TO ALLEVIATE AFLATOXICOSIS ON NILE TILAPIA FISH BY DIETARY SUPPLEMENTATIONS WITH CHICKEN-HATCHERY BY-PRODUCTS(EGG SHELLS)AND SHRIMP PROCESSING WASTES (SHRIMP SHELLS) ON: 1- FISH PERFORMANCE AND FEED AND NUTRIENTS UTILIZATION.

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ABSTRACT

This experiment was conducted to study the drastic effects of graded levels of aflatoxin-B₁ on growth performance, survival rate, feed and nutrients utilization, and carcass composition of Nile tilapia, *O. niloticus* fingerlings. Also, it was conducted for experimenting the inhibiting effects of graded levels of natural, cheap and available two adsorbent agent, namely egg shells(ES) and shrimp wastes(SW) against the adverse effects of aflatoxin-B₁ contamination of fingerlings diet fed for 8 weeks. The effects of aflatoxin-B₁ (AFB₁) were significant decreases in body weight, average weight gain, average daily gain, relative growth rate, specific growth rate, survival rate and feed intake of the fish. Significant increases in corrected mortality rate and feed conversion ratio were recorded. Also, significant decreases in protein efficiency ratio, protein productive value and energy utilization were calculated. Reductions ($P \leq 0.01$) in dry matter, crude protein and energy content and increases ($P \leq 0.01$) in ether extract and ash of fish carcass were determined. The effects of either adsorbents at levels of 1 and 2%, respectively were useful in reducing the toxic effects of AFB₁ on fish via adsorbing the toxin from the fish diets, where it increased significantly the body weight, average weight gain, average daily gain, relative growth rate, specific growth rate, survival rate, feed intake, protein efficiency ratio, protein productive value and energy utilization. Yet, it decreased ($P \leq 0.01$) corrected mortality rate. However, these adsorbents (at levels of 1% ES and 2% SW) alleviated the toxic effects of AFB₁ on feed conversion ratio and carcass composition of the fish.

Keywords: Nile tilapia, Aflatoxin, Egg shells, Shrimp shells, Performance, Feed utilization.

INTRODUCTION

Mycotoxins are highly toxic secondary metabolic products of various toxigenic moulds, mainly those belonging to the genera *Fusarium*, *Aspergillus* and *Penicillium*. It has been estimated that at least 300 of these fungal metabolites are potentially toxic to animals and humans. However, the most notorious - from the agricultural point of view - and thus extensively investigated mycotoxin is aflatoxin B₁. Its global occurrence is considered to be a major risk factor. It is the silent enemy. It is a major contaminant in aquafeeds and considered as a causative agent for fish mortality, morbidity and low productivity leading to economic losses, human toxicity and affects public health (Abdelhamid *et al.*, 1998 and 1999).

The Nile tilapia is one of the most important warm water fish species on accounts of its recognized advantages as an aquaculture species.

However, *O. niloticus* are sensitive to aflatoxin (Abdelhamid *et al.*, 1998 and Hemed, 1999).

The most applied method for protecting animals against mycotoxicoses is the utilization of adsorbents, which are mixed with a contaminated feed. These materials supposed to bind the mycotoxins efficiently in the gastro-intestinal tract (Alexander *et al.*, 2001). Different agents have been used for detoxification process (Abdelhamid *et al.*, 2002 c, d & e , 2004 a & b and Hussein *et al.*, 2000). Therefore, the aim of this study was to give light on the drastic effects of aflatoxin-B₁ on performance and feed utilization of *O. niloticus* fingerlings and the inhibiting effects of two adsorbent agents, namely egg shell and shrimp waste against aflatoxin-B₁ contamination of the fish diet fed for 8 weeks.

MATERIALS AND METHODS

The present study was carried out in season 2003, for investigating the best source and level of two natural, cheap and available adsorbent materials, namely egg shells (ES) and shrimp wastes (SW) for detoxifying aflatoxin-B₁ contamination of *O. niloticus* fingerlings diet fed for 8 weeks. The experiment was carried out in in-door wet lab.

A total number of 450 healthy fingerlings were purchased from Al-Manzalah Integrated Fish Farm, General Authority for Fisheries Resources Development, with an average initial body weight of about 13 gram. After an adaptation period of one week, the fishes were randomly divided into 15 treatments, each treatment at three replicates (each contained 10 fingerlings in a 40 L cylindrical plastic aquarium).

Each aquarium was supplied with 35 L dechlorinated tap water and an air-stone connected with an electric compressor and covered with a fishing net. The replacement of the aquaria waters 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.

The experimental fishes received the tested diets twice daily at 9.00 a.m. and 3 p.m., six days a week. 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.

A ground basal floating diet was obtained from Joe Trade Company, Cairo.

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 DM 89.5, CP 25.1, EE 13.1, ash 7.18, NFE 54.6, GE 490 kcal/100g DM and P/E ratio 51.3 mg CP/kcal GE. $GE (Kcal/100 g DM) = CP \times 5.64 + EE \times 9.44 + \text{Carbohydrates} \times 4.11$ (calculated according to Macdonald *et al.*, 1973). The diet was supplemented with aflatoxin B₁ (prepared as described in Abdelhamid *et al.*, 2004 b) at concentrations of 0.0, 100 and 200 ppb without or with additives (ES and SW) at rates of 0.0, 1 and 2% of each as shown in Table (1). Egg shells and shrimp wastes were purchased from the local market from Al-Mansourah and Ezbet El-Borg, Domiatta, respectively.

Table (1): The experimental design.

Treatment No.	Aflatoxin- B ₁ level (ppb)	Adsorbent (%)
1	0.00	0.00 ES and 0.0 SW
2	0.00	1.00 ES
3	0.00	2.00 ES
4	100.00	0.00 ES and 0.0 SW
5	100.00	1.00 ES
6	100.00	2.00 ES
7	200.00	0.00 ES and 0.0 SW
8	200.00	1.00 ES
9	200.00	2.00 ES
10	0.00	1.00 SW
11	0.00	2.00 SW
12	100.00	1.00 SW
13	100.00	2.00 SW
14	200.00	1.00 SW
15	200.00	2.00 SW

At the end of the experiment, the remained fish were sampled from each aquarium and kept frozen for chemical analysis.

The chemical analyses of the basal diet and the whole fish body (at the start, 4th and 8th week of the experiment) were carried out according to the AOAC (2000). Aflatoxin B₁ determinations in the media extract and the basil 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 – pH-meter), dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter), and conductivity (using Jenway Ltd., Model 470-portable conductivity meter).

Body weight of individual fish was measured biweekly to point feed quantity and to calculate growth performance and feed utilization (Abdelhamid, 2003) in form of average weight gain (g/fish) $AWG = \text{Average final weight (g)} - \text{Average initial weight (g)}$. Average daily gain, (mg/fish/day) $ADG = [AWG (g)/\text{Experimental period (days)}] \times 1000$. Relative growth rate, (RGR) = Average weight gain (g)/ Average initial weight (g). Specific growth rate (SGR, %/day) = $[\ln \text{ final weight} - \ln \text{ initial weight}] \times 100 / \text{Experimental period (d)}$. 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%) = $[\text{Retained protein (g)}/\text{Protein intake (g)}] \times 100$. Energy utilization (EU%) = $[\text{Retained energy (Kcal)}/\text{Energy intake (Kcal)}] \times 100$. Survival rate (SR%) = $[\text{End number of the alive fish}/\text{The beginning number of the fish}] \times 100$. Corrected mortality rate (CMR% according to Abbot, 1925) = $[\text{Mortality rate in each treatment} - \text{Mortality rate in the control group}] \times 100 / [100 - \text{Mortality rate in the control group}]$.

The obtained data were statistically analyzed 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- Quality parameters of the rearing water:

All tested water quality criteria were suitable for rearing Nile tilapia fingerlings as cited by Abd El-Hakim *et al.* (2002) and Abdelhamid (2003). Since water temperature ranged between 25 and 28°C, pH values 7.6 – 8.7, conductivity 249 – 286 ms/cm and dissolved oxygen 6.9 – 12.7 mg/l. Also, Abdelhamid *et al.* (2002c) measured these water parameters and suggested their suitability for rearing Nile tilapia fish.

2- Growth performance and survival rate:

2.1- Body weight (BW):

Results in Table 2 show no significant ($P \geq 0.05$) differences on BW at the start of the experiment. However, significant decreases were recorded in BW of the aflatoxicated fish without additives (T_4 , 100 ppb AFB₁ and T_7 , 200 ppb AFB₁). These reductions in BW increased significantly by increasing AFB₁ concentration comparing with the control groups (T_1 , 0 ppb AFB₁, T_2 , 0 ppb AFB₁+ 1% ES, T_3 , 0 ppb AFB₁+2% ES, T_{10} , 0 ppb AFB₁ +1% SW and T_{11} , 0 ppb AFB₁ +2% SW) at different experimental intervals. Yet, the addition of ES (T_5 , 100 ppb AFB₁ +1% ES, and T_8 , 200 ppb AFB₁ +1% ES) and addition of SW (T_{13} , 100 ppb AFB₁ +2% SW, and T_{15} , 200 ppb AFB₁ +2% SW) to the aflatoxicated diets recorded significant increases in BW of fish comparing with the aflatoxicated fish without additives (T_4 and T_7) at different intervals of the experiment. T_7 was the worst treatment in BW followed by T_4 at different experimental intervals.

Data presented in Table 3 show that the type of additives recorded no significant ($P \geq 0.05$) effects on BW at different experimental intervals. Moreover, the increasing levels of additives caused significant increases in BW at different experimental intervals.

While, there were no significant ($P \geq 0.05$) differences recorded in BW between the two concentrations of additives (1 and 2%). Yet, addition of aflatoxin to fish diets at concentrations of 100 and 200 ppb AFB₁ led to significant decreases in BW comparing with the untreated fish (zero ppb, AFB₁) at different experimental intervals.

These decreases in BW increased significantly by increasing the dietary level of AFB₁ comparing with the untreated fish at the 4th week (W_4) of the experiment. Similar negative effects of AFB₁ on BW of fish were recorded in other works (Hussein *et al.*, 2000; Abdelhamid *et al.*, 2002 b&c; Nguyen *et al.*, 2002 and Shehata *et al.*, 2003). In this context, AFB₁ treatment led to a clear reduction in growth rate in a

Table (2):Effect of aflatoxin B₁(AFB₁)on body weight(g), body weight gain(g/fish)and average daily body weight gain(mg/fish)of the fish at different intervals of the experiment.

Treat. Weeks	Body weight		Body weight gain			Daily body weight gain	
	W ₀	W ₄	W ₈	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
1	13.89	17.35 ab	22.56 a	3.46 ab	8.67 a	123.51ab	154.82a
2	13.88	17.31 ab	22.66 a	3.43 ab	8.78 a	122.62ab	156.79a
3	13.88	17.49 a	22.65 a	3.61 a	8.77 a	129.17a	156.67a
4	13.87	15.71 gh	17.94 c	1.83 gh	4.06 c	65.59gh	72.56c
5	13.88	16.30 ef	21.02 b	2.42 ef	7.14 b	86.55ef	127.56b
6	13.88	15.97 gf	20.41 b	2.08 fg	6.52 b	74.52fg	116.55b
7	13.88	15.09 l	-----	1.21 l	-----	43.21l	-----
8	13.89	15.76 g	-----	1.88 g	-----	67.14g	-----
9	13.87	15.37 hi	-----	1.48 hi	-----	52.86hi	-----
10	13.87	17.17abc	22.43 a	3.29 abc	8.56 a	117.74abc	152.86a
11	13.88	17.28 ab	22.72 a	3.40 ab	8.84 a	121.55ab	157.86a
12	13.88	16.52 de	14.72 b	2.63 de	5.84 b	94.16de	104.35b
13	13.88	16.84 cd	22.38 b	2.96 cd	6.50 b	105.71cd	116.07b
14	13.87	16.56 ed	-----	2.69 de	-----	96.07de	-----
15	13.88	16.97 bc	-----	3.08 bc	-----	110.24bc	-----

a-i: Means in the same column having different letters differ significantly ($P \leq 0.01$).

direct relation to the dietary aflatoxin level (Marzouk *et al.*,1994). However, Abdelhamid *et al.*(2002a) suggested that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce the aflatoxicity. 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) to prevent the drastic effects of AFB₁ on the fish.

2.2- Average weight gain (AWG) and average daily gain (ADG):

Average weight gain (g/fish) and average daily gain (mg/fish/day) were calculated and illustrated in Table 2. The results indicated significant decreases in AWG and ADG of the aflatoxicated fish without additives (T₄ and T₇). These reductions in AWG and ADG were increased significantly by increasing AFB₁ levels (T₇) comparing with the control groups (T₁, T₂, T₃, T₁₀ and T₁₁) at different experimental intervals. Yet, the addition of ES at a level of 1% (T₅ and T₈) and the addition of 2% SW (T₁₃ and T₁₅) led to significant increases in AWG and ADG comparing with the aflatoxicated fish without additives (T₄ and T₇) at different experimental intervals. Yet, T₇ was the worst treatment in AWG and ADG followed by T₄ at the different experimental intervals. The reduction in body weight gain in the present study may be attributed to the loss of appetite and reduction of feed intake. Table 3 shows that there were no significant ($P \geq 0.05$) differences in AWG and ADG in all fish groups concerned with type of additives throughout W₀₋₈. However, the AWG and ADG were increased significantly by increasing levels of additives comparing with the untreated fish (zero% additives) at different experimental intervals. Yet, average weight gain and average daily gain were decreased

significantly by increasing the levels of aflatoxin-B₁ at all intervals of the experiment.

Many workers recorded the same negative effects of AFB₁ on AWG and ADG (Hussein *et al.*,2000; Abdelhamid *et al.*,2002 b&c; Nguyen *et al.*,2002 and Shehata *et al.*,2003). Soliman *et al.* (1998) indicated that the presence of Fix-A-tox accompanied with aflatoxin in the experimental diets, caused an alleviative effect towards the adverse effect of aflatoxin. However, prawn and egg shell wastes could be utilized as adsorbents (Healy *et al.*, 2003b).

Table (3):Main effects of aflatoxin and adsorbents on body weight(g),body weight gain(g/fish)and average daily body weight gain(mg/fish)of the fish at different intervals of the experiment.

Items Weeks	Body weight			Body weight gain		Daily body weight gain	
	W ₀	W ₄	W ₈	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
E.S	13.88	16.27	21.21	2.38 b	7.33	85.03 b	130.82
S.W	13.88	16.61	20.96	2.73 a	7.08	97.54a	126.42
AF ₀ ppb	13.89	17.33 a	22.60 a	3.45 a	8.72 a	123.04a	155.64a
AF ₁₀₀ ppb	13.88	16.18 b	19.57 b	2.30 b	5.69 b	82.02b	101.61b
AF ₂₀₀ ppb	13.89	15.81 c	-----	1.93 c	-----	68.79c	-----
0 %Add.	13.88	16.05 b	20.25 b	2.17 b	6.37 b	77.46b	113.69b
1 %Add.	13.88	16.61 a	21.46 a	2.73 a	7.58 a	97.38 a	135.39a
2 %Add.	13.89	16.66 a	21.54 a	2.77 a	7.66 a	99.00a	136.79a

a-c: Means in the same column having different letters differ significantly (P ≤0.01).

2.3- Relative growth rate (RGR) and specific growth rate (SGR):

Tables 4 and 5 illustrate the relative growth rate and specific growth rate (%/day).The results indicated significant decreases in RGR and SGR of the aflatoxicated fish without additives (T₄ and T₇).

Table (4):Effect of aflatoxin B₁ (AFB₁) and adsorbents on specific growth rate(%/day)and relative growth rate of the fish at different intervals of the experiment.

Treat. Weeks	Specific growth rates		Relative growth rates	
	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
1	0.79ab	0.87a	0.25ab	0.62a
2	0.79ab	0.87a	0.25ab	0.63a
3	0.83a	0.87a	0.26a	0.63a
4	0.44g	0.46d	0.13gh	0.29c
5	0.57ef	0.74b	0.17ef	0.51b
6	0.50fg	0.69bc	0.15fg	0.47b
7	0.30h	-----	0.08i	-----
8	0.45g	-----	0.14g	-----
9	0.36h	-----	0.11hi	-----
10	0.76abc	0.86a	0.23abc	0.62a
11	0.78ab	0.88a	0.24ab	0.64a
12	0.62de	0.63c	0.19de	0.42b
13	0.69cd	0.69bc	0.21cd	0.47b
14	0.63de	-----	0.19de	-----
15	0.72bc	-----	0.22bc	-----

a-i : Means in the same column having different letters differ significantly (P ≤0.01).

These reductions in RGR and SGR were increased significantly by increasing AFB₁ level (T₇) comparing with the control groups (T₁, T₂, T₃, T₁₀ and T₁₁) at different experimental intervals. Yet, the addition of ES at 1% level (T₅ and T₈) and 2% of SW (T₁₃ and T₁₅) led to significant increases in RGR and SGR comparing with the aflatoxicated fish without additives (T₄ and T₇) at different experimental intervals. Yet, T₇ was the worst treatment in RGR and SGR followed by T₄ at different experimental intervals.

The reduction in growth rates in the present study may be attributed to the loss of appetite and feed intake reduction.

However, the RGR and SGR were increased significantly by increasing levels of the additives comparing with the untreated fish (zero% additives) at different experimental intervals. Yet, the RGR and SGR were decreased significantly by increasing the levels of aflatoxin-B₁ at all intervals of the experiment. Many workers recorded the same negative effects of AFB₁ on growth rates of tilapia fish (Hussein *et al.*, 2000; Abdelhamid *et al.*, 2002 b&c and Shehata *et al.*, 2003). Yet, Abdelhamid *et al.* (2002a) suggested that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce AFB₁. However, egg shell can be used as an adsorbent (Gittins and Drakley, 2002). Moreover, prawn and egg shell wastes could be utilized as adsorbents (Healy *et al.*, 2003b).

Table (5): Main effects of aflatoxin and adsorbents on specific growth rate(%/day) and relative growth rate of the fish at different intervals of the experiment.

Items	Specific growth rates		Relative growth rates	
	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
E.S	0.560 b	0.750	0.171 b	0.53
S.W	0.638 a	0.730	0.196a	0.51
AF ₀ ppb	0.791 a	0.870 a	0.248a	0.63 a
AF ₁₀₀ ppb	0.545 b	0.610 b	0.165b	0.41 b
AF ₂₀₀ ppb	0.460 c	-----	0.138 c	-----
0 %Add.	0.512b	0.660 b	0.156 b	0.46 b
1 %Add.	0.639 a	0.770 a	0.196a	0.55 a
2 %Add.	0.646 a	0.780a	0.199a	0.55 a

a-c: Means in the same column having different letters differ significantly (P ≤0.01).

2.4- Survival rate (SR) and corrected mortality rate (CMR):

Data in Table 6 shows that there were significant decreases in SR and increases in CMR of the fish treated with aflatoxin-B₁ without additives (T₄ and T₇) by increasing AFB₁ levels (T₇) compared with the control groups (T₁, T₂ and T₃) at different experimental intervals.

However, the dietary addition of ES (1%, T₅ and T₈) and SW (2%, T₁₃) to the aflatoxicated fish diets led to significant increases in SR and decreases in CMR compared with T₄ and T₇ at different experimental intervals. So, the T₇ was the worst treatment in SR and CMR followed by T₄ at all experimental intervals. From the results in Table 7 there were no significant (P ≥0.05) differences in SR in all fish treatments concerning with the type and concentrations of the additives (ES and SW) at different experimental intervals.

However, the SR was decreased significantly by increasing levels of AFB₁ comparing with the untreated fish (zero ppb AFB₁) at different experimental intervals. However, data in Table 7 show that there were no significant ($P \geq 0.05$) differences in CMR in all fish treatments concerning with the type of additives (ES and SW) at different experimental intervals. Yet, increasing levels of additives led to significant decreases in CMR of fish comparing with the control group (zero% additives).

On the contrary, AFB₁ caused significant increases in CMR of the aflatoxicated fish (comparing with the control group, zero ppb AFB₁) which were increased by increasing level of AFB₁. Similar reduction in survival rate by AFB₁ was recorded (Hussein *et al.*, 2000; Abdelhamid *et al.*, 2002 b&c and Salem 2002). Shehata *et al.* (2003) suggested also that aflatoxin-B₁ caused significant increases in the mortality rate of *O. niloticus* fish .

They added that using adsorbents significantly reduced the toxic effect of aflatoxin on SR of fish. The increased survival rate by the adsorbents used herein may be due to their ability for adsorption of mycotoxin in the gastrointestinal tract and thereby decrease the toxic effects on animals (Galvano *et al.*, 2001). On the other side, Abdelhamid *et al.*, (2002a) mentioned that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce AFB₁.

Table (6):Effect of aflatoxin B₁ (AFB₁) and adsorbents on corrected mortality rate (CMR%) and survival rate(SR%) of the fish at different intervals of the experiment.

	CMR ₄	CMR ₈	SR ₄	SR ₈
1	3.33 ef	10.00 c	96.66 a	86.66 a
2	3.33 ef	3.33 c	96.66 a	86.66 a
3	0.00 f	0.00 c	96.66 a	86.66 a
4	20.74bcd	65.55a	76.66 bc	60.00 d
5	7.04 def	24.07 bc	90.00 ab	76.66 ab
6	10.00def	23.33 bc	90.00 ab	73.33 bc
7	41.48 a	-----	56.66 d	-----
8	34.07ab	-----	63.33 cd	-----
9	34.07ab	-----	63.33 cd	-----
10	0.00f	3.70 c	93.33 a	83.33 ab
11	0.00f	0.00 c	96.66 a	83.33 ab
12	17.41cde	55.28 ab	76.66 bc	63.33 cd
13	13.33def	47.06 ab	83.33 ab	76.66 ab
14	38.15 a	-----	60.00 d	-----
15	30.74abc	-----	66.00 cd	-----

a-f: Means in the same column having different letters differ significantly ($P \leq 0.01$).

3. Feed intake and feed conversion:

3.1- Feed intake (FI):

Results in Table 8 show significant ($P \leq 0.01$) decreases in FI of the aflatoxicated fish without additives (T₄, 100 ppb AFB₁ and T₇, 200 ppb AFB₁).

These reductions increased significantly by increasing AFB₁ concentration comparing with the control groups (T₁, 0 ppb AFB₁, T₂, 0 ppb AFB₁+ 1% ES, T₃, 0 ppb AFB₁+2% ES, T₁₀, 0 ppb AFB₁ +1% SW and T₁₁, 0 ppb AFB₁ +2% SW) at different experimental intervals. Yet, the addition of ES

to the aflatoxicated diets (T₅, 100 ppb AFB₁ +1% ES, and T₈, 200 ppb AFB₁ +1% ES) and the addition of SW (T₁₃, 100 ppb AFB₁ +2% SW, and T₁₅, 200 ppb AFB₁ +2% SW) recorded significant increases in FI of fish comparing with the aflatoxicated fish without additives (T₄ and T₇) at different intervals of the experiment. From Table 9, the results show that there were no significant (P ≥ 0.05) differences in FI in all fish treated with both types of additives (ES and SW) at different experimental intervals. Yet, increasing the concentration of these additives caused significant increases in FI, but no significant (P ≥ 0.05) differences were recorded between both concentrations of the additives (1 and 2%) at different intervals of the experiment. However, significant decreases in FI were recorded for the aflatoxicated fish, which increased by increasing the level of aflatoxin (200 ppb) comparing with the control group (zero ppb AFB₁) at different experimental intervals.

Table (7): Main effects of aflatoxin and adsorbents on corrected mortality rate (CMR%) and survival rate (SR%) of the fish at different intervals of the experiment.

Items	Corrected mortality rate		Survival rate	
	CMR ₄	CMR ₈	SR ₄	SR ₈
E.S	17.12	35.95	87.22	78.33
S.W	18.35	43.61	83.33	75.56
AF ₀ ppb		2.84b		92.78 a
AF ₁₀₀ ppb	14.88 b	45.31a	82.22 b	68.33 b
AF ₂₀₀ ppb	36.67 a	-----	61.11 c	-----
0 %Add.	21.85a	52.03a	81.67	73.33
1 %Add.	16.66ab	36.87b	85.83	77.50
2 %Add.	14.69b	30.44b	88.33	80.00

a-c: Means in the same column having different letters differ significantly (P ≤ 0.01).

The similar negative effects of AFB₁ on FI were recorded in other researches (Abdelhamid *et al.*, 2002b and Salem, 2002). Also, in the same trend, Nguyen *et al.* (2002) suggested that fish fed diets containing 10 and 100 mg AFB₁/kg expel feed after ingestion.

3.2- Feed conversion ratio (FCR):

Results in Table 8 present that there were significant increases in FCR of the aflatoxicated fish comparing with the control ones (zero ppb AFB₁) at different experimental intervals.

These increases of FCR were increased significantly by increasing the dietary AFB₁ level. This increase in FCR may be attributed to the very low weight gain values which were recorded in aflatoxicosis, where FCR calculation depends on weight gain besides FI. So, T₇ was the worst treatment in FCR at different intervals of the experiment. Results in Table 9 show that there were no significant (P ≥ 0.05) differences in FCR of the fish treated with both types of additives (ES and SW) throughout the experiment (W₀₋₈).

Table (8):Effect of aflatoxin B₁ (AFB₁) and adsorbents on feed conversion and feed intake (g/fish) of the fish at different intervals of the experiment.

Treat.	Feed conversion		Feed intake	
	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
1	2.71 fg	2.43 de	9.36ab	20.97a
2	2.72 fg	2.40 e	9.33abc	20.94a
3	2.58g	2.42 de	9.34abc	20.99a
4	5.06 c	4.85 a	9.24bcd	14.66d
5	3.93 de	2.86 cd	9.38ab	20.38bc
6	4.50cd	3.08 c	9.32abc	20.09c
7	7.60 a	-----	9.09e	-----
8	4.99c	-----	9.23bcd	-----
9	6.20 b	-----	9.17de	-----
10	2.83fg	2.47 de	9.31bcd	20.88a
11	2.74 fg	2.39 e	9.30abcd	20.93a
12	3.54ef	3.51 b	9.20cde	20.42bc
13	3.20efg	3.20 bc	9.43a	20.74ab
14	3.44ef	-----	9.24bcd	-----
15	3.04 fg	-----	9.36ab	-----

a-g :Means in the same column having different letters differ significantly(P ≤0.01).

However, the addition of ES led to significant increases in FCR from the start to 4th week (W₀₋₄) of the experiment comparing with the fish treated with SW. Anyhow, there were no clear effects on FCR concerning with both types of additives in the treated fish groups.

Yet, the levels (1 and 2%) of these additives (ES and SW) caused significant decreases in FCR comparing with the control group (zero % additives), while there were no significant (p ≥0.05) differences in FCR between both concentrations (1 and 2%) of the additives at different experimental intervals.

However, feed conversion ratios were increased significantly by increasing the level of AFB₁ at all intervals of the experiment. Similar negative effects of AFB₁ on FCR of *O. niloticus* fish were recorded in other studies (Abdelhamid et al., 2002b&c and Salem, 2002).

Table (9):Main effects of aflatoxin and adsorbents on feed intake(g/fish) of *O. niloticus* at different intervals of the experiment.

Items	Feed conversion		Feed intake	
	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
E.S	4.48 a	3.00	9.27	20.51
S.W	3.79 b	3.14	9.28	20.60
AF ₀ ppb		2.72 c	2.42 b	9.34a
AF ₁₀₀ ppb	4.21 b	3.72 a	9.30a	20.16b
AF ₂₀₀ ppb	5.48 a	-----	9.20b	-----
0 %Add.	5.12 a	3.64 a	9.23b	20.32b
1 %Add.	3.58 b	2.81 b	9.28a	20.66a
2 %Add.	3.71 b	2.77 b	9.32a	20.69a

a-c: Means in the same column having different letters differ significantly (P ≤0.01).

4- Protein and energy utilization:

4.1- Protein efficiency ratio (PER):

Protein efficiency ratio of *O. niloticus* fish fed on different levels of AFB₁ with and without additives, namely ES and SW at different experimental intervals was calculated (Table 10).

The results indicated that there were significant decreases in PER of the aflatoxicated fish comparing with the control ones (zero ppb AFB₁) at different experimental intervals.

This reduction in PER was increased significantly by increasing the level of AFB₁ (T₇). So, T₇ was the worst group in PER. The addition of 1% ES (T₅) and 2% SW (T₁₃) increased significantly the PER comparing with the aflatoxicated fish groups without additives (T₄ and T₇).

Table (10):Effect of aflatoxin B₁ (AFB₁) on protein efficiency ratio (PER), protein productive value (PPV) and energy utilization(EU) of the fish at different intervals of the experiment .

Treat.	PER		PPV		EU	
	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
1	1.47 ab	1.64 ab	-----	36.56a	-----	14.74a
2	1.46 ab	1.67 a	-----	36.79a	-----	14.83a
3	1.54a	1.66 a	-----	37.03a	-----	14.85a
4	0.79 gh	0.82 d	-----	15.64d	-----	6.78c
5	1.02 f	1.39 bc	-----	29.79b	-----	12.25b
6	0.89fg	1.29 c	-----	28.23bc	-----	11.69b
7	0.53 l	-----	9.63e	-----	4.84d	-----
8	0.81g	-----	14.77c	-----	6.43c	-----
9	0.64 hi	-----	12.57d	-----	5.88c	-----
10	1.40ab	1.63 ab	-----	36.00a	-----	14.55a
11	1.45 ab	1.68 a	-----	37.49a	-----	15.16a
12	1.14de	1.14c	-----	25.32c	-----	10.70b
13	1.25cd	1.25 c	-----	27.62bc	-----	11.57b
14	1.16de	-----	19.22b	-----	8.84b	-----
15	1.31bc	-----	21.67a	-----	10.12a	-----

a-i: Means in the same column having different letters differ significantly (P ≤0.01).

The present results agree with the findings of Salem (2002) who showed that dietary aflatoxin inclusion was responsible for lower (P < 0.05) protein efficiency ratio proportional to the mycotoxin levels. Results in Table 11 showed that there were no significant (P ≥0.05) differences in PER for the fish treated with both types of additives (ES and SW) throughout the entire period of the experiment (W₀₋₈). However, the addition of SW led to significant increases in PER from the start to 4th week (W₀₋₄) of the experiment comparing with the fish treated with ES. Anyhow, there were no clear effects on PER concerning with both types of additives in the treated fish groups. Yet, the levels (1 and 2%) of these additives (ES and SW) caused significant increases in PER comparing with control group (zero % additives).

4.2- Protein productive value (PPV) and energy utilization (EU):

Results in Table 10 reveal the presence of significant decreases in PPV and EU of the aflatoxicated fish compared with the control fish (zero ppb AFB₁) with and without additives throughout the entire period of the

experiment. Yet, at 4th week (W₀₋₄) interval there were significant decreases in PPV and EU of the aflatoxicated fish (200 ppb AFB₁) without additives (T₇) compared with all aflatoxicated fish groups (200 ppb AFB₁) with 1 and 2% egg shell (T₈ and T₉) and shrimp waste (T₁₄ and T₁₅), respectively. Therefore, the addition of 1% egg shell (T₅ and T₈) and 2% shrimp waste (T₁₃ and T₁₅) led to significant increases in PPV and EU of the aflatoxicated fish comparing with the aflatoxicated fish without additives (T₄ and T₇) at 4th week (W₀₋₄) and 8th week (W₀₋₈) of the experiment. However, the decreases in PPV and EU were significantly increased by increasing the level of AFB₁, so that T₇ and T₄ were the worst treatments during both intervals, respectively. Negative effects of AFB₁ on PPV and EU may be attributed to the impairment of digestion and adsorption due to aflatoxin as mentioned by Huff *et al.*(1977). Moreover, aflatoxin toxicity is expressed in the disruption of protein synthesis through conversion to 2,3-epoxide binding to DNA and inhibiting RNA synthesis (Yu, 1981). Also, Takahashi *et al.* (1995) reported that aflatoxin binds to DNA and inhibits protein synthesis by fish. Additionally, Murjani (2003) reported that these negative effects of AFB₁ may be attributed to its causative pathological alterations in the gastrointestinal tract.

Similar, results were obtained by Abdelhamid *et al.*(2002 b&d). Moreover, Abdelhamid *et al.*, (2002a) mentioned that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce AFB₁ toxicity. However, the better results of egg shell and shrimp waste used in present study may be due to their adsorptive characteristics as mentioned before, so prevent or reduce absorption of AFB₁ and hence hide its negative effects on the nutrients utilization. Also, Galvano *et al.* (2001) suggested that the increase in PPV and EU by adsorbents may be due to there ability for adsorption of mycotoxin in the gastrointestinal tract and thereby decreasing toxic effects on animals Results in Table 11 show that the addition of SW led to significant increases in PPV and EU of the fish comparing with the ES at the 4th week interval (W₀₋₄).

Table (11):Main effects of aflatoxin and adsorbents on protein efficiency ratio (PER), protein productive value (PPV) and energy utilization(EU) of the fish at different intervals of the experiment.

Items	PER		PPV		EU	
	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈	W ₀₋₄	W ₀₋₈
E.S	1.02 b	1.41	12.33b	30.67	5.72b	12.52
S.W	1.17 a	1.36	16.84a	29.77	7.93a	12.25
AF ₀ ppb	1.47 a	1.66 a	-----	36.74a	-----	14.81a
AF ₁₀₀ ppb	0.98 b	1.12 b	-----	23.71b	-----	9.96b
AF ₂₀₀ ppb	0.83 c	-----	-----	-----	-----	-----
0 %Add.	0.93 b	1.23 b	9.63b	26.10b	4.84b	10.76b
1 %Add.	1.17 a	1.46 a	17.00a	31.98a	7.64a	13.08a
2 %Add.	1.18 a	1.47 a	17.12a	32.59a	8.00a	13.32a

a-c: Means in the same column having different letters differ significantly (P ≤0.01).

Yet, supplementation of ES led to insignificant (P ≥0.05) increases in PPV and EU of fish at 8th week (W₀₋₈). However, increasing the concentration of these additives caused significant increases in PPV and EU of *O. niloticus*

fish comparing with the control group (zero% additives). But no significant ($P \geq 0.05$) differences were observed between both concentrations of the additives (1 and 2%) in PPV and EU of fish during either experimental intervals. Yet, significant decreases were recorded in PPV and EU of the aflatoxicated fish (100 ppb AFB₁) comparing with the control group (zero ppb AFB₁) at the 8th week of the experiment.

5- Carcass composition of the fish:

Results in Table 12 show the carcass composition of the fish fed on different levels of AFB₁(0, 100 and 200 ppb) with and without additives, namely ES and SW. The results illustrated that aflatoxin-B₁ significantly reduced DM, CP and EC of the fish carcass.

Table (12):Effect of aflatoxin B₁ (AFB₁) and adsorbents on carcass composition of the fish at the 4th week and 8th week of the experiment.

Treat.	DM	% On Dry matter basis			
		CP	EE	Ash	EC
At the start	21.84	58.12	19.92	21.96	515.84
At the end					
1	25.21 ab	64.74 a	18.51 fg	16.76 g	539.83 a
2	25.14 ab	64.76 a	18.46 fg	16.78 g	539.51 a
3	25.28 ab	64.84 a	18.32 g	16.85 g	538.61 a
4	23.21 e	60.85 c	19.97 bc	19.19 c	531.69 b
5	24.93 d	62.77 b	18.83 fg	18.40 d	531.78 b
6	24.91 d	62.68 b	18.95 ef	18.38 de	532.36
7*	22.25 f	59.04 e	20.71 a	20.26 a	528.42 bc
8*	22.31 f	59.85 d	20.04 b	20.11 a	526.75 c
9*	22.30 f	59.76 d	20.64 a	19.60 bc	531.89 b
10	25.12 bc	64.73 a	18.49 fg	16.78 g	539.62 a
11	25.33 a	64.85 a	18.54 fg	16.62 g	540.73 a
12	24.84 d	62.53 b	19.54 cd	17.94 ef	537.08 a
13	24.96 cd	62.87 b	19.38 de	17.76 f	537.49 a
14*	22.34 f	59.62 d	20.42 ab	19.96 ab	529.04 bc
15*	22.42 f	59.74 d	20.64 a	19.63 bc	531.68 b

a-g: Means in the same column having different letters differ significantly ($P \leq 0.01$).

* Carcass composition of *O. niloticus* at the 4th week.

Yet, it significantly increased EE and ash contents of the fish carcass. These adverse effects of aflatoxin-B₁ on fish carcass composition were increased by increasing the level of AFB₁ (200 ppb). However, dietary addition of 1% ES (T₅) and 2% SW (T₁₃) to the AFB₁ including diets alleviated the toxic effects of AFB₁ on carcass composition of the fish at the end of the experiment. However, the positive effects of egg shell and shrimp waste used in the present study may be due to their adsorptive characteristics as mentioned before, so prevent or reduce absorption of AFB₁ and hence hide its negative effects on carcass composition of the fish. The same adverse effects of AFB₁ on carcass composition were recorded by Abdelhamid *et al.* (2002b&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 and ash percentages. 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. In accordance with the present findings, Abdelhamid *et al.* (2002b) reported that the aflatoxic diets significantly reduced the fish flesh crude protein content but increased its fat and ash contents proportional to the dietary levels of the aflatoxin.

Results in Table 13 present no significant ($P \geq 0.05$) differences in DM and CP of the fish carcass concerning with both type of additives (ES and SW). Yet, the addition of SW to the fish diets led to significant increases in EE and EC of the fish carcass.

Meanwhile, dietary supplementation of the ES caused significant increases in ash content of fish carcass at the end of the experiment.

On the other side, increasing levels of the adsorbents used led to significant increases in DM,CP and EC and decreases in EE and ash contents of the fish carcass comparing with the control group (zero% additives).

In addition, AFB₁ recorded significant decreases in DM, CP and EC and increases in EE and ash of fish carcass comparing with the control group (zero ppb AFB₁) at the end of the experiment.

Table (13):Main effects on carcass composition of *O. niloticus* fed on different levels of dietary AFB₁ and adsorbents at the end of the experiment.

Items	DM	CP	EE	Ash	EC
E.S	23.95	62.14	19.38 b	18.48 a	533.42 b
S.W	23.96	62.11	19.57 a	18.32 b	535.06 a
AF ₀ ppb	25.22 a	64.77 a	18.47 c	16.75 c	539.68 a
AF ₁₀₀ ppb	24.34 b	62.09 b	19.44 b	18.47 b	533.67 b
AF ₂₀₀ ppb	22.31 c	59.51c	20.53 a	19.97 a	529.36 c
0 %Add.	23.55 c	61.54 b	19.73 a	18.73 a	533.31 b
1 %Add.	24.12 b	62.37 a	19.30 b	18.33 b	533.46ab
2 %Add.	24.20 a	62.46 a	19.41 b	18.14 c ±0.36	535.46 a

a-c: Means in the same column having different letters differ significantly ($P \leq 0.01$).

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محاولة تخفيف آثار التسمم الغذائي الأفلاتوكسيني على الأسماك البلطي النيلي بإضافات غذائية من مخلفات مفاقس بيض الدجاج (قشر البيض) ومخلفات تجهيز الجمبرى (قشر الجمبرى) على: ١- أداء السمك واستفادته من الغذاء والمغذيات. عبد الحميد محمد عبد الحميد ، عبد الخالق السيد عبد الخالق ، أحمد إسماعيل محرم وفتحى فتوح خليل قسم إنتاج الحيوان - كلية الزراعة - جامعة المنصورة - المنصورة - ج.م.ع.

تم إجراء تجربة لدراسة التأثيرات السامة للأفلاتوكسين ب_١ على معدل النمو، معدل الإعاشة، ومدى الاستفادة من الغذاء والعناصر الغذائية المختلفة لإصباغيات أسماك البلطي النيلي. وكذلك اشتملت الدراسة على اختبار تأثير بعض المواد المدعمة للعمل على إزالة التأثيرات السامة لهذا السم الفطري الخطير على الأسماك، وكان ذلك لمدة ٨ أسابيع. استخدم في هذه التجربة عدد ٤٥٠ وحده من إصبعيات البلطي النيلي والتي تم الحصول عليها من المجمع السمكي المتكامل بالمنزلة- التابع لهيئة تنمية الثروة السمكية- وزارة الزراعة. كانت الأسماك تزن ١٣,٠٠ جراما في المتوسط (وزن ابتدائي)، تم توزيع هذه الأسماك عشوائيا وتقسيمها لـ ١٥ معاملة كل معاملة ٣ مكررات (كل مكررة بها ١٠ سمكات /حوض من البلاستيك سعة ٤٠ لتر). وكانت الأسماك تغذى على العلائق المختبرة على مرتين يوميا لمدة ٦ أيام في الأسبوع، وكان معدل التغذية اليومي للأسماك ٣% من وزن الجسم الحى. ولقد استخدمت عليقة أسماك طافية تحتوى ٢٥,١٤% بروتين خام. وتم إضافة الأفلاتوكسين ب_١ إلى العليقة بتركيزات صفر - ١٠٠ - ٢٠٠ جزء في البليون بدون أو مع إضافة قشر البيض أو مخلفات الجمبرى بمستويات صفر، ١، ٢، ٤%. التأثيرات لكل من نوعى المادتين المدعمتين المستخدمتين (قشر بيض ومخلفات الجمبرى) بتركيزات ١، ٢% على التوالي أوضحت أهمية استخدامهما لتقليل التأثيرات السامة للأفلاتوكسين ب_١ على أسماك البلطي النيلي حيث تسببتا فى حدوث زيادة معنوية فى كل من وزن الجسم ومتوسط الزيادة الكلية واليومية فى الوزن ومعدل النمو النسبى ومعدل النمو النوعى ومعدل الإعاشة وكمية الغذاء المأكول ومعدل الاستفادة من البروتين والقيمة الإنتاجية للبروتين والاستفادة من الطاقة فى جسم الأسماك. وكذا حسنتا من معدل النفوق المصحح، وقد أدت هاتان المادتان المدعمتان (١% قشر بيض، ٢% مخلفات الجمبرى) إلى خفض التأثيرات السامة للأفلاتوكسين ب_١ على كل من معدل التحويل الغذائى وتركيب جسم الأسماك. ولقد ثبت أن لقشر البيض ضعف قدرة مخلفات الجمبرى فى تخفيف آثار وسمية الأفلاتوكسين، لذلك كان تركيز ١% قشر بيض مساويا لتركيز ٢% مخلفات الجمبرى فى تخفيف هذه الآثار. وعليه فإن الأفلاتوكسين ب_١ الملوث للعلائق يمثل خطورة شديدة على إنتاجية الأسماك. ويوصى باستخدام قشر البيض (١%) أو مخلفات الجمبرى (٢%) كإضافات علفية للعمل على التخفيف من التأثيرات السامة للأفلاتوكسين ب_١ فى علائق الأسماك الملوثة به.