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AN ATTEPMT TO ALLEVIATE AFLATOXICOSIS ON NILE TILAPIA FISH BY DIETARY SUPPLEMENTATIONS WITH CHICKEN-HATCHERY BY-PRODUCTS(EGG SHELLS)AND SHRIMP PROCESSING WASTES (SHRIMP SHELLS) ON: 2: CLINICAL, BLOOD AND HISTOLOGICAL PARAMETERS.

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ABSTRACT

This experiment was conducted to study the drastic effects of graded levels of aflatoxin-B_1 on clinical symptoms, residual effects, some blood constituents and histopathological changes in 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-B_1 contamination of fingerlings diet fed for 8 weeks.

The effects of aflatoxin-B_1 (AFB_1) were severe clinical lesions in the external organs and postmortem symptoms in the internal organs of the aflatoxicated fish. Significant increase in organs indices was calculated. But decrease in dray matter and increase in ether extract of the fish liver were estimated. 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 were determined. Residues of AFB_1 were found in the whole body of the aflatoxicated fish directly at the end of the experiment and tended to decrease after freezing periods. Severe histological alterations were recorded in livers, kidneys, intestines and gills of the aflatoxicated fish.

These alterations in all tested organs were increased by increasing level of aflatoxin (200 ppb AFB_1).The effects of either adsorbents at levels of 1 and 2%, respectively were useful in reducing the toxic effects of AFB_1 on fish, where it increased significantly the hemoglobin concentration, red blood cells count and uric acid in fish blood. Yet, it decreased significantly white blood cells count, alkaline phosphatase and transaminases activity and most of the organs indices.

However, these adsorbents (at levels of 1% ES and 2% SW) alleviated the toxic effects of AFB_1 on liver composition (DM and EE) of the fish.. Moreover, using these adsorbents at these levels (1% ES and 2% SW) alleviated the adverse effects of AFB_1 on the histopathological changes in different internal organs (livers, kidneys, intestines, and gills) of the aflatoxicated fish.

Keywords: Nile tilapia, Aflatoxin, Egg shells, Shrimp shells, Blood, Residues, Histology.

INTRODUCTION

Aflatoxin B_1 with its global occurrence is considered to be a major risk factor. Aflatoxin B_1 (AFB_1) is a wide spreading 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. 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 (Abdelhamid *et al.,* 1998 and 1999).

Tilapia fishes, particularly *O. niloticus* are sensitive to aflatoxin (Abdelhamid *et al.,* 1998 and Hemeda,1999).

The most applied method for protecting animals against mycotoxicoses is the utilization of adsorbents, which are mixed with a contaminated feed 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 and 2004 b and Hussein *et al.,* 2000).

Therefore, the aim of this study was to give light on the drastic effects of aflatoxin-B_1 on *O. niloticus* fingerlings from the clinical and histological points of view as well as the inhibiting effects of two adsorbent agents, namely egg shell and shrimp waste against aflatoxin-B_1 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), which can detoxify aflatoxin-B_1 contamination of *O. niloticus* fingerlings diet fed for 8 weeks. All materials used herein are the same mentioned in the 1st part of this series (Abdelhamid *et al.,2004 c).*

The chemical analyses of the fish liver at the 4th and 8th week of the experiment was carried out according to the AOAC (2000). Aflatoxin B_1 determination in the fish carcass was determined as described by Abdelhamid (1996).

At the end of the experiments , for all fish, the liver, spleen, kidneys and gonads were removed and weighted 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). Blood samples from each fish of the different groups were collected from the caudal peduncle. Whole blood was used for the determination of hemoglobin (Hb) by using commercial kits (Diamond Diagnostic, Egypt). Also, total erythrocytes count (RBCs) and total leucocytes count (WBCs) were estimated by Haemocytometer. Plasma samples were used for biochemical analysis. Uric acid concentration and activity of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using commercial kits supplied by Diamond, Diagnostic, Egypt. For histopathological examination, representative samples from liver, kidneys, intestine and gills

were proceeded (Bancroft *et al.*, 1990).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-The clinical lesions and postmortem examination of aflatoxicated fish:

External clinical symptoms (protrusive eyes, abdominal distension, hardening of the body, discarding viscera, fins erosion, discarded scales, hemorrhage, discoloration of skin, abdominal shrinkage, operculum erosion and cataract) and postmortem signs (enlarged gall bladder and stomach, distended yellowish liver, viscera covered by a thick layer of mucus and uncharacterized liver and viscera) of aflatoxicated fish were recorded from the 1st week and continued throughout the experiment. However, at the end of the 4th week of the experiment, all groups fed on the diets containing 200 ppb AFB_1 with and without additives died.

These findings are in agreement with those mentioned by Hussein *et al.*(2000) and Abdelhamid *et al.* (2002 b&c).

Moreover, Soliman *et al.*(2000) reported that the earliest symptoms of disease were seen 48 hours after oral administration of 1/10, 1/20 and 1/30 of the AFB_1 - LC_{50}.

2- Organs indices:

Indices of organs of fish fed on different levels of AFB_1 with and without additives were presented in Table 1.

Table (1):Effect of aflatoxin B_1 (AFB_1) and adsorbents on indices(%) of the fish at the end of the experiment.

Treat.	HIS	KSI	SSI	GSI
1	2.68 c	0.18 c	0.14 c	0.97 b
2	2.66 c	0.21 c	0.20 c	0.96b
3	2.63 c	0.19 c	0.14 c	0.97a
4	3.97a	0.30a	0.30a	1.63b
5	3.22 b	0.25b	0.25b	1.06b
6	3.16 b	0.25b	0.25b	1.08 b
7	--------	--------	--------	--------
8	--------	--------	--------	--------
9	--------	--------	--------	--------
10	2.62 c	0.19c	0.14 c	0.44 b
11	2.89bc	0.14c	0.14 c	0.97 b
12	3.38b	0.26b	0.27b	1.01 b
13	3.22b	0.24b	0.27b	1.00 b
14	--------	--------	--------	--------
15	--------	--------	--------	--------

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

The results showed that there were significant increases in indices of aflatoxicated fish (100 ppb AFB_1) with and without additives comparing with the control fish group (zero ppb AFB_1) at the end of the experiment. However, the addition of 1% ES (T_5) and 2% SW (T_{13}) led to significant decreases in HSI, KSI and SSI and insignificant ($P \geq 0.05$) decreases in GSI comparing with the aflatoxicated fish without additives (T_4). Data in Table 2 show the main effects on indices (%) at the end of the experiment . The results showed that there were no significant ($P \geq 0.05$) differences in indices in all fish groups concerning with the type of additives (ES and SW).

However, increasing the concentration of the used additives led to significant decreases in different indices of fish at the end of the experiment. Yet, significant increases were recorded in indices of aflatoxic fish (100ppb AFB_1) comparing with the untreated fish (zero ppb AFB_1). However, in the present study, the positive effects of ES and SW may be due to their adsorptive characteristics, so prevent or reduce absorption of AFB_1 and hence hide its negative effects on indices of fish. Similarly, negative effects of AFB_1 on indices of fish were recorded by Hussein *et al.*(2000) and Abdelhamid *et al.*(2002c and 2004a). Anyhow, AFB_1 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. However, Abdelhamid *et al.* (2002a) found that adsorbents, e.g. Antitox plus, Fix-a-tox and tafla did not significantly reduce aflatoxicosis. Recently, 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.

Table (2): Main effects on indices (%) of the fish at the end of the experiment .

Items	HSI	KSI	SSI	GSI
E.S	3.06	0.23	0.23	1.11
S.W	3.13	0.23	0.23	1.09
AF_0 ppb	2.69 b	0.19 b	0.19 b	0.97 b
AF_{100} ppb	3.49 a	0.27 a	0.27 a	1.24 a
AF_{200} ppb	---------	---------	---------	---------
0 %Add.	3.33 a	0.24 a	0.24 a	1.30 a
1 %Add.	2.97 b	0.23 ab	0.23 b	1.00 b
2 %Add.	2.98 b	0.22 b	0.22 b	1.00 b

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

3- Chemical analysis of the liver:

The results in Table 3 illustrate the toxic effects of AFB_1 on liver composition (DM and EE) of *O. niloticus*. These results showed that AFB_1 caused decreases of DM and increases of EE in the livers of the aflatoxic fish comparing with the control group (zero ppb AFB_1) at the end of the experiment. Yet, increasing AFB_1 level (200 ppb) increased the adverse effects of AFB_1 on DM and EE contents of the fish livers. However, addition of 1% egg shell (T_5) and 2% shrimp waste (T_{13}) alleviated these toxic effects

of AFB_1 on the liver composition of the fish comparing with the aflatoxicated fish without additives (T_4 and T_7). So that, T_7 (200 ppb AFB_1) was the worst treatment.The negative effects of AFB_1 on liver composition may be concerned with the toxic effects of AFB_1 on the hepato-somatic index. This may be due to that liver is the target organ for AFB_1, since it was proved that AFB_1 is a strong hepatic mycotoxin (Hussein *et al.*,2000; Soliman *et al.*,2000 and Nguyen *et al.*,2002). Also, aflatoxins are suspected to cause both acute hepatocellular disease and hepatocarcinoma (Abdelhamid *et al.*,1990, 1996 & 2002e). However, the effects of ES and SW may be due to their adsorptive characteristics, so prevent or reduce absorption of AFB_1 and hence hide its negative effects on liver composition of the fish.

Table (3): Chemical analysis of liver of the fish fed on different levels of dietary AFB_1 and adsorbents at the end of the experiment.

Treat.	DM	EE
1	62.67	28.09
2	61.93	27.68
3	62.34	28.24
4	59.47	30.82
5	60.84	29.30
6	60.71	29.48
7	56.43	32.64
8	57.76	31.75
9	57.49	31.84
10	61.97	27.56
11	62.57	28.12
12	60.68	29.89
13	60.94	29.75
14	57.63	31.87
15	57.31	31.69

DM = Dry matter (%).
EE=Ether extract (%).

4- Blood parameters:

Results in Table 4 show that there were significant decreases in concentration of hemoglobin (Hb), red blood cells count (RBS) and uric acid level and increases in white blood cells count(WBC) and in activity of ALP, AST and ALT of aflatoxicated fish comparing with the control groups (zero ppb AFB_1) with and without additives. Yet, the addition of 1% ES (T_5) and 2% SW (T_{13}) to the aflatoxicated fish diet led to significant increases in Hb, RBCs and uric acid and decreases in WBCs, ALP, AST and ALT comparing with the aflatoxicated fish without additives (T_4). So that, T_4 was the worst treatment among all fish groups. However, the positive effects of ES and SW may be due to their adsorptive characteristics as mentioned before, so prevent or reduce absorption of AFB_1 and hence hide its negative effects on blood parameters. The negative effects of AFB_1 on blood hematological and biochemical parameters in the present study are in full agreement with the findings reported in other studies (Hussein *et al.*,2000 Soliman *et al.*,2000;

Abdelhamid *et al.*,2002 c, d &e and Shehata *et al.*, 2003). Moreover, the present findings confirm those reported by Abdelhamid *et al.* (2002b) who mentioned that the aflatoxin contaminated diets reduced ($P < 0.01$) blood values of PCV, Hb and total protein. However, increased WBCs count is an indicator for the late phase of carcinogenicity of AFB_1 as mentioned by Harvey *et al.* (1995). In the present results, increasing GOT and GPT activity by AFB_1 treatments indicated damage of the liver. Moreover, evidence of acute aflatoxin B_1 nephrotoxicity was provided by distended gall bladder indicating disrupted osmoregulation (i.e. water retention) as reported by (Carpenter *et al.*, 1995).

Table (4): Effect of aflatoxin B_1 (AFB_1) and adsorbents on hematological and blood biochemical parameters of the fish at the end of the experiment.

Treat.	Hb (g/dl)	WBCs $10^3/mm^3$	RBCs $10^6/mm^3$	Uric acid (mg/dl)	ALP (U/L)	AST (U/L)	ALT (U/L)
1	18.46ab	12.72b	2.87 a	4.11 a	18.05c	11.67bc	36.67e
2	18.40ab	12.50b	2.90 a	3.95 a	17.84c	10.33 c	35.00e
3	18.14ab	13.26b	2.86 a	4.06 a	18.63c	10.33 c	37.33e
4	9.43 d	14.45a	2.02 d	1.80 c	61.63a	21.33 a	77.33a
5	15.99bc	12.84b	2.58 b	3.18 b	21.50c	13.00bc	56.33bcd
6	15.14 c	12.53b	2.41 c	3.11 b	20.47c	13.00bc	58.33 bc
7	-------	-------	-------	-------	-------	-------	-------
8	-------	-------	-------	-------	-------	-------	-------
9	-------	-------	-------	------	-------	-------	-------
10	18.55ab	12.88b	2.72ab	3.93 a	18.30c	10.67 c	42.33 de
11	18.90 a	13.29b	2.79 a	3.96 a	18.60c	11.33 c	43.33cde
12	14.93 c	12.91b	2.77 a	2.83 b	37.91b	17.33ab	71.33 ab
13	16.40abc	14.04b	2.77 a	3.47 ab	20.69c	14.33bc	60.33 b
14	-------	-------	-------	-------	-------	-------	-------
15	-------	-------	-------	-------	-------	-------	-------

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

Table 5 shows that there were no significant ($P \geq 0.05$) differences in hematological and blood biochemical parameters (Hb, WBCs, RBCs, uric acid, ALP, AST and ALT) concerning with the type of additives (ES and SW) at the end of the experiment.

Table (5):Main effects on hematological and blood biochemical parameters of the fish fed on different levels of dietary AFB_1 and adsorbents at the end of the experiment.

Items	Hb	WBCs	RBCs	Uric acid	ALP	AST	ALT
E.S	15.93	13.05	2.61	3.37	26.35	13.28	50.17
S.W	16.11	13.21	2.66	3.35	29.20	14.44	55.22
AF_0 ppb	18.49a	12.89 b	2.83a	4.02a	18.25b	11.00b	38.56b
AF_{100} ppb	13.55b	13.37 a	2.43b	2.70b	37.31a	16.72 a	66.83a
AF_{200} ppb	-----	------	-----	------	-----	------	------
0 %Add.	13.95b	13.58 a	2.45b	2.95 b	39.84a	16.50a	57.00
1 %Add.	16.97a	12.78b	2.74 a	3.47 a	23.89b	12.83b	51.25
2 %Add.	17.14a	13.03b	2.71 a	3.65 a	19.60c	12.25b	49.83

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

Yet, increasing levels of these additives led to significant increases in Hb, RBCs and uric acid and decreases in WBCs, ALP and GOT. Meanwhile, there were no significant ($P \geq 0.05$) differences in GPT by increasing levels of additives comparing with the control group (zero% additives).On the other side, AFB_1 caused significant decreases in Hb, RBCs and uric acid and increases in WBCs, ALP, GOT and GPT comparing with the untreated fish (zero ppb AFB_1).

5- Residues of aflatoxin-B_1 (ppb):

Data in Table 6 illustrate the residue (ppb) of AFB_1 in the whole body of the fish fed on different levels of dietary AFB_1 when it was analyzed simultaneously at the end of the experiment and after different freezing periods (1^{st},2^{nd},4^{th},8^{th} and 12^{th} week). The results in this Table show that the AFB_1 residues (ppb) in the aflatoxicated fish were found directly at the end of the experiment only in groups T_4 (100 ppb AFB_1), T_7 (200 ppb AFB_1) and T_{12} (100 ppb AFB_1+1% shrimp waste). Aflatoxin-B_1 level was reduced by going on the freezing time of the fish samples in all of these groups of the aflatoxicated fish. So, it could be concluded that AFB_1 residues decreased by increasing freezing period of the aflatoxicated fish. On the other side, there were no residual effects in the aflatoxicated fish body in all treatments. Yet, the addition of ES at levels of 1% (T_8) and 2% (T_9) and SW at levels of 1% (T_{14}) and 2% (T_{15}) to the aflatoxicated fish (200 ppb) and also the supplementation with ES at levels of 1% (T_5) and 2% (T_6) and SW at level of 2% (T_{13}) to the aflatoxicated fish (100 ppb) led to adsorptive effects of the dietary aflatoxin. Meanwhile, there was no adsorptive effect of the supplementation with 1% SW(T_{12}) to the aflatoxicated fish (100 ppb). In this context, Soliman *et al.* (1998) found that the presence of Fix-A-tox in the contaminated diet led to a significant decreases in aflatoxin residue in the fish. However, in the present results the effects of ES and SW may be due to their adsorptive characteristics, so prevent or reduce absorption of AFB_1 and hence there were no AFB_1 residues in the fish body and muscles. In this respect El-Banna *et al.* (1992) reported that AFB_1 residues in the fish flesh showed a cumulative effect related to level of AFB_1 and feeding period. Also, Hussain *et al.* (1993) suggested that aflatoxin B_1, G_1 and G_2 were detected in muscles of treated groups of fish. In the same trend, Soliman *et al.* (1998) mentioned that the significant increases of aflatoxin residue was observed in fish flesh after 6 months. Also, Abdelhamid *et al.* (1998) recorded high level of aflatoxin-B_1 residues (246-303 ppb). On the other hand, Abdelhamid *et al.* (2002 b&c) reported that there were no AF- residues in fish body(may be for a long storage period before analysis). They added that the absence of AFB_1 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. Since, Omar *et al.* (1996) reported that gray mullet is highly sensitive to AF followed by common carp, red tilapia and Nile tilapia. Moreover, Soliman *et al.* (2000) proved that common carp is more susceptible to AFB_1 than *O. niloticus*. These variations may be also attributed to time and method of storage before analysis for the residues.

Table (6): Aflatoxin-B_1 (AFB_1) residues (ppb) in the whole fish body at different weeks of frozen storage after the end of the experiment.

Treat.	Direct at the end of the experiment	Freezing weeks				
		1	2	4	8	12
4	10.41	6.18	4.26	2.52	1.92	1.64
5	0.00	---	---	---	---	---
6	0.00	---	---	---	---	---
7	16.35	9.60	5.24	3.08	2.38	1.96
8	0.00	---	---	---	---	---
9	0.00	---	---	---	---	---
12	8.18	5.85	3.37	2.34	1.86	1.56
13	0.00	---	---	---	---	---
14	0.00	---	---	---	---	---
15	0.00	---	---	---	---	---

6- Histological effects of aflatoxin-B_1:

6.1- Liver:

The histological examination revealed that liver of fish fed on the control diet without AFB_1 or any dietary additives (T_1) showed intact hepatic architecture, as illustrated by normal hepatic lobules with regular arrangement of the hepatocytes within each lobule (Fig. 1). Feeding fish on 100 ppb AFB_1 contaminated diets without any additives (T_4) showed some pathological effects on the liver in term of collapsed areas resulting from compensatory enhlysema were irregularly spread in the hepatic lobular plates. Also, pleomorphic nuclei and slight vacuolization of the cytoplasm were seen in hepatocytes and lipofuscin was common in the hepatic lobules. In addition, infiltration of fibroblast cells and monocytes around the hyperplasic bile ducts. Furthermore, mild congestion of the blood vessels with microscopic foci of hemorrhage in the portal lobules were noted (Fig. 2). In some fishes of T_4, the liver showed intact hepatic lobular architecture, but high infiltration of monocytes and fibroblast and basophilic granular structures were seen within the portal area. Also, there was pronounced thickening of the lobular endings with basophilic cells (Fig. 3). In other individuals of fish in T_4, large widening of the preportal area with lower infiltration of fibroblast cells occurred in the liver. Also, atrophy of some hepatocytes and vascuolation of others, in particular around the blood sinusoids, with irregular lobular plates were observed. Increasing the level of AFB1 to 200 ppb without any additives (T_7) resulting in severe lesions in the liver of fish. In most fishes, the normal hepatic lobular architecture was absence. High congestion with large areas of hemorrhage and areas of basophilic necrotic hepatocytes adjacent the portal area were observed. Also, great dilatation of the blood sinusoid occurred within the hepatic lobules. In others, severe congestion with large area of hemorrhage, necrotic hepatocytes and infiltration of monocytes and spindle cells were seen in the portal tract.

The observed pathological effects of AFB_1 were nearly similar to those reported by Abdelhamid *et al.* (2002 c) on big or small *O. niloticus* fish fed diets contaminated with 1500 and 1000 ppb AFB_1, respectively. Also, Nguyen *et al.* (2002) found similar lesions in liver of Nile tilapia fish fed 100 mg AFB_1/kg diet for 8 weeks. Hussein *et al.* (2000) and Soliman *et al.* (2000) reported similar pathological effects on Nile tilapia fish fed dietary AFB_1. Moreover, in rabbits fed on a diet containing 50 ppb AFB_1, (Abdelhamid *et al.*, 2002 d) found similar effects of this level on the liver.

The present histopathological findings of AFB_1 with levels of 100 and 200 ppb, being more severe with 200 than 100 ppb were associated with marked reduction in growth performance and significant changes in blood parameters of fishes fed AFB_1 diets as compared with the control group. In general, there were individual variations in level of severity of these lesions, which may indicate individual variation in tolerance of fishes to toxicity of AFB_1. This tolerance may be attributed to (live body weight) LBW and the physiological status of the fishes (Abdelhamid *et al.*, 2002 c). It is of interest to note that most lesions were found in hepatocytes and with lower extent in the portal region. Abdelhamid *et al.*(2002 d) explained that hepatocytes are the first targets to the aflatoxins, as they were affected by the toxin when the blood passes firstly through them.

In this study attempts to eliminate or reduce the toxic effects of AFB_1 were carried out by adding 1 or 2% egg shell and 1 or 2% shrimp waste to diets of fish. When 1% egg shell (ES) was added to the control diet (T_2), fishes showed normal hepatic lobular architecture. Adding 1% ES to 100 ppb AFB_1 diet (T_5) resulted in some improvement or reducing the severity of the hepatic lesions in terms of reducing the degree of congestion, hemorrhage and necrosis of the hepatocytes (Fig. 4). Small widening and slight congestion of the portal area as well as minimal piece meal necrosis in some individuals. Enlargement, dilatation and mild congestion of the portal vessels in the others (Fig. 4).On the other hand, in liver of fish fed 1% ES and 200 ppb AFB_1 diet (T_8) showed abnormal hepatocytes arrangement within the hepatic lobules, but some irregularity in the hepatocytes with mild necrosis were observed. Widening of the blood sinusoids and thick plates of hepatocytes with cellular pleomo were also noted.

On the basis of these finding, interestingly to note that addition of 1% ES was more beneficial on the reducing the pathological effects of 100 than 200 ppb aflatoxin. It is worthy to note that the pathological effects on the liver of diet of T_8 was nearly similar to that of T_4. Also, Diets of T_5 showed mild lesions on the liver as compared to T_4, which indicated the impact of adding 1% ES to diets contaminated with 100 ppb AFB_1 on reducing the toxic effect of aflatoxin. By increasing level of ES to 2% (T3) in the control diet, small dilatation with mild congestion were seen in the central vein. Also, low vacuolization and necrosis were observed in the hepatocytes.

In fish fed diets contaminated with 100 ppb AFB_1 and 2% ES (T_6), the liver showed intact hepatic lobular architecture with mild congestion of the portal vessels and blood sinusoids. There were monocytes aggregation around the portal vessels and bile ducts within the portal area. In fishes of T_9 fed 200 ppb AFB_1 and 2% ES, the liver showed normal hepatic lobules with normal

hepatocyte arrangement around the central vein, which showed mild infiltration of monocytes. In other fishes, the liver showed severe congestion in the bile duct and blood vessels with fibroblast and monocytes infiltration. Small spaces in the blood sinusoids adjacent the central vein were seen. Also, individualization and dissociation of the hepatocytes were noted within the hepatic lobules. In some cases, basophilic focus within the hepatic lobules and severe congestion of the bile ducts and widening the adjacent blood sinusoids were seen (Fig. 5). Based on the pathological effects on the liver of fish fed 100 or 200 ppb AFB_1 with adding 1 or 2% ES, the histological examination revealed reducing these effects by adding 1% ES, regardless level of AFB_1. It is worthy noting that the effect of adding 1% ES on the liver of aflatoxicated fish was nearly similar to addition of 0.5 % silica in diets of rabbits fed 25 or 50 ppb AFB_1 (Abdelhamid *et al.*, 2002 d).

Generally, adding 1% ES to aflatoxic diets showed higher improvement than adding 2% ES on the histological structure of the liver in fish. In the other treatment groups, (T_{10}-T_{15}) addition of shrimp waste (SW) was studied with 1 or 2% level. In fish fed on the control diet and 1% SW, the liver showed intact hepatic lobular architecture. Adding 1% SW to diets contaminated with 100 ppb AFB_1 showed similar lesions to that occurred in fish fed on a diet containing 200 ppb AFB_1 and 2% ES (T_9) and those fed 100 ppb AFB_1 without additives (T4). However, adding 1% SW to 200 ppb AFB_1 diet (T_{14}) resulted in similar effect to feeding fishes on 200 $AFB_1$and 2% ES (T_9) as illustrated in Fig. 34. These findings cleared that adding 1% SW could reduced the pathological effects of AFB_1 as level of AFB_1 decrease from 200 to 100 ppb AFB_1. By increasing level of shrimp waste to 2% in the control diets (T_{11}), normal histological structure of the liver was obtained.

In fish fed on 100 ppb AFB_1 and 2% SW diet (T_{13}) the liver showed enlargement of the lymphatic vessels and bile duct with infiltration in the bile duct by monocytes. Also, the hepatocytes showed regularity within the hepatic lobules and some fibroblast cells were spread within blood sinusoids (Fig. 6). In the other cases, area of degenerated hepatocytes and slight congestion were found in the central vein. However, in fishes fed on 200 ppb AFB_1 and 2% SW showed mild congestion and enlargement of the portal vein with monocytes and fibroblast infiltration. Also, disappearance of some nuclei of the hepatocytes was observed.

It is interest to note that increasing the level of shrimp waste to 2% to reduce the aflatoxic lesions of both levels 100 and 200 ppb AFB_1 on the fish liver showed marked positive effect compared to 1% SW. According the obtained findings with different levels of AFB_1 and, it was observed that adding 2% SW was more beneficial on reducing the pathological effects of AFB_1 on the liver of fish. Based on the forgoing findings on the liver as the target organ of the AFB_1, it could concluded that adding 1% egg shell or 2% shrimp waste showed improvement on the histological structure of the liver and in turn on liver function of aflatoxic fish. This conclusion was almost in accordance with growth performance and blood parameters in fish of different treatment groups studied.

Fig.(1): Section in liver of the control *O. niloticus* (T_1, zero ppb AFB_1) showing normal hepatic lobules with regular arrangement of the hepatocytes within each lobule. (x100, H&E stain)

Fig.(2): Section in liver of aflatoxicated *O. niloticus* with 100 ppb AFB_1 (T_4) showing mild congestion of the blood vessels with microscopic focci of hemorrhage in the portal lobules. (x 100, H&E stain)

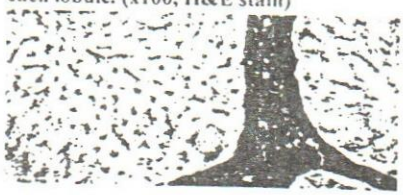

Fig.(3): Section in liver of aflatoxicated *O. niloticus* with 100 ppb AFB_1 (T_4) showing thickening of the lobular endings with basophilic cells. (x 100, H&E stain)

Fig.(4): Section in liver of aflatoxicated *O. niloticus* with 100 ppb AFB_1+1% egg shell (T_5) showing enlargement, dilatation and mild congestion of the portal vessels. (x 100, H&E stain)

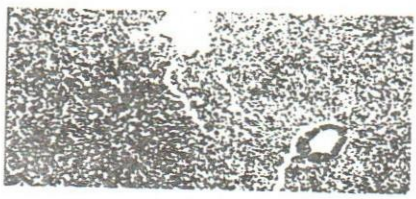

Fig.(5): Section in liver of *O. niloticus* fed on diet contaminated with 200 ppb AFB_1+2% egg shell (T_9) showing severe congestion of the bile ducts and widening the adjacent blood sinusoids. (x 100, H&E stain)

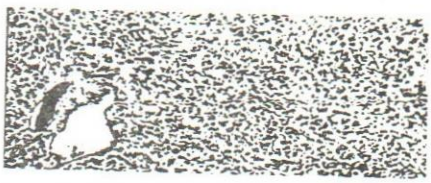

Fig.(6): Section in liver of *O. niloticus* fed on diet contaminated with 100 ppb AFB_1+2% shrimp waste (T_{13}) showing regularity within the hepatic lobules and some fibroblast cells were spread within abnormal blood sinusoids. (x 100, H&E stain)

6.2- Kidney:

The histological examination of the kidney of fish fed on the control diet without any dietary additives (T_1) revealed intact renal cortex and medulla architecture. The glomeruli and renal tubules were apparently normal (Fig. 7). Addition of 1% ES to the control diet (T_2) did not show any pathological effects on the kidney tissue only slight congestion of the glomeruli with normal proximal and distal renal tubules were seen within the renal cortex. Increasing level of ES to 2% in the control diet (T_3) showed intact renal cortex and medulla with normal tubules of Henel's lope. However, the lining epithelium was basophilic with dark stain nuclei.These results indicated that adding 1or 2% ES to the control diet of fish did not adversely effect the normal histogenesis of the kidney.

When fish were fed on aflatoxicated diets with 100 ppb AFB_1 (T_4) kidney tissue of fish showed neoplastic signs and periglomerular and peritubular cell infiltration (Fig. 8). Within the renal cortex the glomeruli showed mild congestion, and the renal tubules had degenerated epithelial cells and interstitial hemorrhage (Fig. 9). Adding 1% ES to fish diets aflatoxicated with 100 ppb (T_5) resulted in elimination of the toxic effect of aflatoxins. Kidney of fish showed intact glomeruli within the renal cortex with slight degeneration of the epithelial cells lining the renal tubules (Fig. 10).Increasing level of ES to 2% in the aflatoxicated diet with 100 ppb (T_6), failed to reduce the toxic effect of aflatoxins. In kidney of fish in T_6 moderate congestion of the glomerular capillaries was seen within the renal cortex. Also, the adjacent renal tubules were degenerated with interstitial hemorrhage of blood vessels. In the renal medulla, the tubules were nearly normal with mild interstitial hemorrhage.

Based on these findings, adding 1% ES reduced the toxic effect of aflatoxin further than adding 2% ES on kidney tissue of fish. The beneficial effect of adding 1% was more than 2% ES on the histological structure of the kidney of fish> It may be attributed to increasing level of calcium in diet of fish. By increasing level of aflatoxin in the control diet to 200 ppb (T_7), kidney of fish showed severe lesions in the renal cortex and medulla with abnormal architecture of the renal tissue.The pronounced effect of 200 ppb level (T_7) was observed in the renal cortex in terms of severe congestion of the glomeruli, interstitial hemorrhage and chronic nephritis (Fig. 11).

As observed for adding 1% Es to aflatoxicated diets with 100 ppb, also adding 1% ES to 200 ppb diet (T_8) was beneficial on the renal tissue, being more marked on the medulla than cortex. In renal cortex, congestion of the glomerular capillaries and mild interstitial hemorrhage, however the adjacent renal convoluted tubules were apparently normal (Fig. 12). On the other hand, the renal medulla showed nearly normal tubules with slight interstitial infiltration of blood cells.

It is of interest to note that adding 2% ES showed adverse effect compared to adding 1% ES. In fish fed 200 ppb AFB_1 and 2% ES (T_9), kidneys showed intact glomeruli and the adjacent renal convoluted tubules had marked congestion and mild interstitial hemorrhage. However, the renal tubules within medulla showed degenerated epithelial cells with areas of bysis and necrosis.

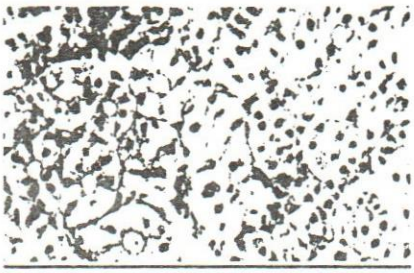

Fig. (7): Section in kidney of the control *O. niloticus* (T_1) showed normal kidney tissue. Tubules of the renal cortex are appearently normal. (x 400, H&E stain)

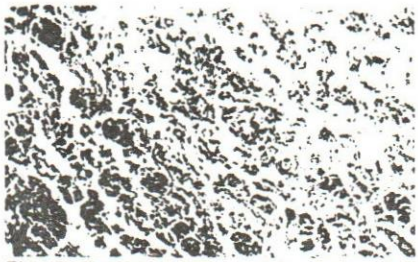

Fig. (8): Section in kidney of *O. niloticus* fed 100 ppb AFB_1 diet (T_4) showing neoplastic signs and periglomerular and peritubular cell infiltration. (x 100, H&E stain)

Fig. (9): Section in kidney of *O. niloticus* fed 100 ppb AFB_1 diet (T_4) showing renal cortex with mild congested glomeruli. The epithelium of renal tubules is markedly degenerated and interstitial hemorrhage. (x 400, H&E stain)

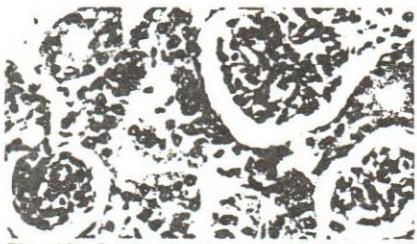

Fig. (10): Section in kidney of *O. niloticus* fed 100 ppb AFB_1 and 1% ES diet (T_5) showing renal cortex with intact glomeruli and slight degeneration of the epithelium lining the convoluted renal tubules. (x 400, H&E stain)

Fig. (11): Section in kidney of *O. niloticus* fed 200 ppb AFB_1 diet (T_7) showing severe congestion of the glomeruli, interstitial hemorrhage and chronic nephritis. (x 400, H&E stain)

Fig. (12): Section in kidney of *O. niloticus* fed 200 ppb AFB_1 and 1% ES (T_8) showing congestion of the glomerular capillaries and mild interstitial hemorrhage. The adjacent renal convoluted tubules are appearently normal. (x 400, H&E stain)

These findings indicated beneficial effect of adding 1% ES on reducing the toxic effect of aflatoxins on both renal cortex and medulla. However, adding 2% ES restored the lesions in medulla of fish 100 ppb AFB_1 (T_6) and in cortex of fish fed 200 ppb AFB_1 (T_9).Adding 1 or 2% SW to the control diet resulted in intact architecture of the kidney in fish of T_{10} and T_{11}, respectively. The kidney showed normal glomerular capillaries and adjacent renal tubules.

In fish fed 100 ppb AFB_1 diet with 1% SW (T_{12}) showed lower lesions in the renal cortex than those obtained in T_4 . The glomeruli showed slight congestion and interstitial hemorrhage of the blood vessels within the renal cortex. On the other hand, adding 2% SW to 100 ppb (T_{13}) diet resulted in marked improvement of the kidney tissue histogenesis. The renal cortex showed nearly intact glomerular capillaries and the adjacent renal convoluted tubules were within normal. It is worthy noting that adding 2% SW to 200 ppb AFB_1 diet (T_{15}) showed marked improvement on the renal cortex than adding 1% SW to 200 ppb AFB_1 diet (T_{14}).In fish of T_{14}, the kidney showed renal cortex containing nearly intact glomerular capillaries with mild congestion and marked degeneration of the epithelial cells lining the adjacent renal tubules. However in T_{15} glomeruli were within normal and the adjacent tubules had some degeneration of the epithelial cells.

Similar findings to those in the present pathological signs of 100 and 200 ppb AFB_1 diets were observed by Abdelhamid *et al.* (2002 b&c) on fish fed 500-2000 ppb AFB_1.Also, Jantrarotai *et al.* (1991); El-Bouhy *et al.* (1993); Soliman *et al.* (2000) found similar findings. Interestingly to note that the changes in histological structure of the kidney were associated with those occurred in the liver of fish in all treated groups, but some histological changes in the kidney were attributed to the dietary additives especially to level of ES, which contained high level of calcium. So, the histological examination may conclude the use of 2% SW rather than 1% ES.

6.3- Intestine:

The histological examination of fish revealed marked pathological effects of aflatoxicated diets on the intestine. In fish fed the control diet (T_1) or the control diet with 1 (T_2) or 2% (T_3) ES showed intact intestinal tissue (Fig. 13). The mucosa and musculosa layers are within normal structure and the villous and musculosa were intact (Fig. 14). It is of interest to note that adding ES resulted in some histometric changes in villi of fish. The villi showed higher density in fish fed the control diet with 1% ES (T_2) and were shorter in fish fed the control diet with 2% ES (T_3) than those fed control diet alone (T_1). Unexpected findings were observed for the effect of feeding fish on 100 (T_4) and 200 (T_7) ppb AFB_1 diets. The intestine showed severity of the aflatoxic effect. Generally, the intestinal architecture was absent and the mucosa structure was destroyed and the musculosa layer was very thin.

When diets aflatoxicated with 100 or 200 ppb were supplemented with 1 or 2% Es (T_5,T_6,T_8 and T_9), respectively, fish showed intestine with abnormal architecture and thick musculosa layer (Fig. 15 for 100 ppb diet with 1or 2% ES and Fig. 16 for 200 ppb diet with 1or 2% ES).These findings indicated slight improvement in the intestinal structure of fish fed aflatoxicated diets with 1 or 2% ES.

Fig. (13): Cross- section in intestine of control *O. niloticus* (T_1) showing intact intestinal tissue. (x 100, H&E stain)

Fig. (14): Cross- section in intestine of *O. niloticus* fed the control diet with 2% ES (T_3) showing intact villous architecture and musculosa is within normal. (x 100, H&E stain)

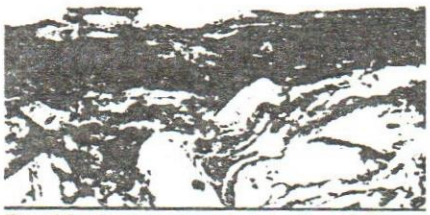

Fig. (15): Cross- section in intestine of *O. niloticus* fed 100 ppb AFB_1 with 1% ES (T_5) or 2% ES (T_6) showing abnormal intestinal architecture of mucosa and thickening musculosa layer. (x 100, H&E stain)

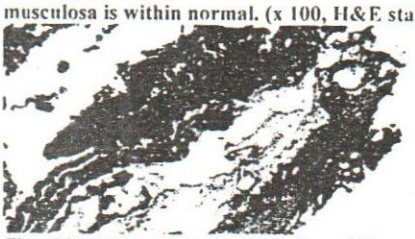

Fig. (16): Cross- section in intestine of *O. niloticus* fed 200 ppb AFB_1 with 1% ES (T_8) or 2% ES (T_9) showing abnormal intestinal architecture of mucosa and thickening musculosa layer. (x 100, H&E stain)

Fig. (17): Cross- section in intestine of *O. niloticus* fed 100 ppb AFB_1 with 1% SW (T_{12}) showing intact musculosa and absence of mucosa layer. (x 100, H&E stain)

Fig. (18): Cross- section in intestine of *O. niloticus* fed 100 ppb AFB_1 with 2% SW (T_{13}) showing intact mucosa and musculosa architecture, but the villi are wider and shorter and musculosa is thicker. (x 100, H&E stain)

Adding 1 or 2% SW to the control diet fed to fish in T_{10} and T_{11}, respectively resulted in normal intestinal architecture with intact villi and musculosa layer. However, this addition led to longer villi in T_{10} and T_{11} than those of fish fed the control diet alone (T_1). On the other hand, adding 1% SW to diet aflatoxicated with 100 ppb resulted in marked improvement in the musculosa layer, but the musculosa architecture was absent (Fig. 17).It is worthy noting that, fish fed 100 ppb aflatoxicated diet with 2% SW (T_{13}) showed normal intestinal tissue with intact architecture of the mucosa and musculosa, but the villi showed shorter, wide and lower density than the control fish (Fig. 18).

From the considerable point of view, it was observed that adding SW both levels did not eliminate the pathological effects of AFB_1.In fish fed 200 ppb AFB_1 diet with 1 (T_{14}) or 2% (T_{15}) SW showed damaged and destructive intestinal structure. The examined lesions in intestine of fish as affected by AFB_1 are nearly similar to that obtained by Kandial *et al.* (1991) on broiler chicks fed diet aflatoxicated with 100 ppb. However, Abdelhamid *et al.* (2002 b) found an increase of number of goblet cells and marked inflammatory cellular infiltration with edema in intestine of Nile tilapia fish fed 500-2000 ppb AFB_1.The author found that similar lesions without edema in fish fed aflatoxicated diets with 2 or 4% Biogen®. Based on these findings, it was observed that adding 1 or 2% ES slightly improved the pathological effects of aflatoxicated diets with 100 or 200 ppb. However, adding 2% SW to the aflatoxicated diets was beneficial only with the lower level of toxicity (100 ppb AFB_1) to improve the intestinal architecture of fish.

6.4- Gills:

The histological examination of fish fed the control diet showed intact lamellar architecture of the gills (Fig. 19). Feeding fish on the control diet with 1% ES (T_2) did not show any changes in structure of gills, being within normal with distribution of goblet cells and epithelial layer lining the lamellae. Also, adding 2% ES did not affect the peripheral boarders of the lamellae, which were lining with normal epithelial cells in fish of T_3. In fish fed diets aflatoxicated with 100 ppb AFB_1 (T_4) marked congestion and hemorrhage with hyperplasia were seen in the epithelial layer lining the lamellae (Fig. 20), beside severe congestion and degeneration of the epithelial layer lining the end of the lamellae. Increasing level of AFB_1 to 200 ppb in the control diet (T_7) resulted in severe lesions in the lamellae architecture of gills. Marked degeneration of the epithelial layer lining the secondary lamellae and filament interstitum were observed (Fig. 21).

The attempts to eliminate the toxic effect of AFB_1 by adding 1% ES (T_5) to diets aflatoxicated with 100 ppb failed to restore the pathological effects of the toxin on gills. Mild congestion of the lining epithelium and inflammatory cells mainly lymphocytes within filament intersitium were noted (Fig. 22). Moreover, adding 2% ES to diet aflatoxicated with 100 ppb AFB_1 (T_6) more severe lesions on the histological structure of gills than those observed in fish of T_5. Severe congestion and hemorrhage of the main lamellae and mild hyperplasia of the secondary lamellae were seen in fish of T_6. On the other hand, elimination of the toxic effect of 200 ppb with the same levels of ES showed similar trend to that occurred with 100 ppb AFB_1.

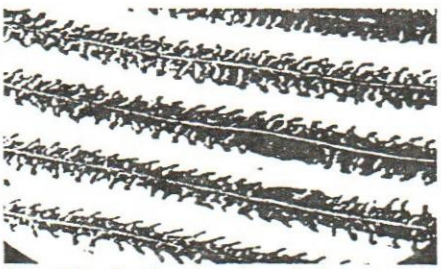

Fig. (19): Section in gills of the control *O. niloticus* (T_1) showing intact architecture of the lamella. (x 100, H&E stain)

Fig. (20): Section in gills of *O. niloticus* fed aflatoxicated diet with 100 ppb (T_4) showing congested lamellae and hyperplasia of the lining epithelial layer of the secondary lamellae. (x 100, H&E stain)

Fig. (21): Section in gills of *O. niloticus* fed aflatoxicated diet with 200 ppb (T_7) showing severe lesions in term of pronounced degeneration of the secondary lamellae and abliteration of normal lamellar architecture. (x 100, H&E stain)

Fig. (22): Section in gills of *O. niloticus* fed aflatoxicated diet with 100 ppb and 1% ES (T5) showing mild congestion and marked lesions in the epithelial layer lining the lamellae. (x 100, H&E stain)

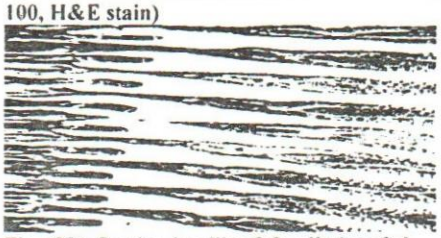

Fig. (23): Section in gills of *O. niloticus* fed aflatoxicated diet with 200 ppb and 2% ES (T_9) showing that lamellae are nearly within normal with slight inflammentation within filament interstitium. (x 100, H&E stain)

Fig. (24): Section in gills of *O. niloticus* fed aflatoxicated diet with 200 ppb and 2% SW (T_{15}) showing intact lamellae architecture with slight congestion hemorrhage of the epithelial layer. (x 100, H&E stain)

In fish fed 200 ppb aflatoxicated diet with 1% ES (T8), gills showed mild congestion and hemorrhagic hyperplasic of the epithelial layer lining the lamellae. Meanwhile, increasing level of ES to 2% showed low lesions in gills. The lamellar architecture was apparently within normal with slight inflammentation within filament interstitum (Fig. 23).

These findings indicated beneficial effects of adding ES to aflatoxicated diets to eliminate the toxic effects, being better for 2% ES with 200 ppb level of AFB_1. Other attempts were conducted to eliminate the toxic effect of AFB_1 by adding SW. In fish fed the control diet with 1 (T_{10}) or 2% (T_{11}) SW showed intact lamellar architecture of the gills with normal distribution of the epithelial and goblet cells of the secondary lamellae and blood vessels within filament interstitial. In case of adding 1% SW (T_{12}) or 2% (T_{13}) SW to aflatoxicated diets with 100 ppb, no marked signs of improvement were obtained on the histological structure of the gills. In fish of T_{12}, marked destruction and damage of the lamellar architecture, was noted. However, in fish of T_{13}, desquamation of the epithelial layer and congestion of blood vessels were observed.

On the other hand, similar pathological effects were obtained when 1% SW was added to 200 ppb diet of fish in T_{14}. There was hyperplasia of the epithelium of the secondary lamellar and inflammatory cells (lymphocytes) and hemorrhage within filament interstitial. It is worthy noting that adding 2% SW to 200 ppb diet showed marked and beneficial effect on the pathological examination of the gills in T_{15}. Marked improvement was observed for the lamellar architecture, but slight congestion and hemorrhage were seen in the epithelial layer lining the lamellae (Fig. 24).The present pathological effects of 100 or 200 ppb AFB_1 are in agreement with those reported by Abdelhamid *et al.* (2002 b) on Nile tilapia fed aflatoxicated diets with 500-2000 ppb AFB_1. Also, Robert (1978) and Hussein *et al.* (2000) found similar results. Findings of addition of SW are in according with those obtained by adding 2 or 4 g/kg diet from Biogen® to AFB_1 diets Abdelhamid *et al.* (2002 b).

Based on these findings, it is of interest to note that the toxic effects of both level of AFB_1 on gills of fish decreased by adding 2% ES and apparently were eliminated by adding 2% SW to aflatoxicated diets. The effects of aflatoxin on histological changes in all tested organs are directly correlated with the level of AFB_1. The present study clearly showed that when high level of AFB_1 was introduced into the control diet, it led to pronounced pathological signs in all tested organs. However, when the low level of AFB_1 was added to diet of fish, there were wide variation in the tested organs. Generally, addition of ES or SW showed milder lesions on all organs, being better with 1% ES and 2% SW. The mechanism that reduces or eliminates the toxic substance from fish is not clearly understood. However, Boonyaratpalin *et al.* (2001) pointed to an increased level of alkaline phosphatase, when higher concentrations of AFB_1 were given. In spite the several attempts to eliminate the toxicity due to AFB_1-contaminated diets, proper storage of feedstuffs or formulated diets is very important to prevent AFB_1- toxicity.

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محاولة تخفيف آثار التسمم الغذائي الأفلاتوكسينى على الأسماك البلطي النيلى بإضافات غذائية من مخلفات مفاقس بيض الدجاج (قشر البيض) ومخلفات تجهيز الجمبري (قشر الجمبري):

٢ – على الدلائل المرضية وقياسات الدم والفحص النسيجي.

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في الدراسة الحالية تم إجراء تجربة لدراسة التأثيرات السامة للأفلاتوكسين ب١ على دلائل الأعضاء, ومدى تأثيره على مقاييس وخواص الدم المختلفة لإصباعيات أسماك البلطي النيلى. وكذلك اشتملت الدراسة على اختبار تأثير بعض المواد المدمصة للعمل على إزالة التأثيرات السامة لهذا السم الفطري الخطير على الأسماك وأيضاً دراسة مدى تأثير هذا السم الفطري على التركيب النسيجي (الهستولوجى) لبعض الأعضاء الداخلية لهذه الأسماك, وكان ذلك لمدة ٨ أسابيع. استخدم في هذه التجربة عدد ٤٥٠ وحده من إصبعيات البلطي النيلى تزن ١٣,٠٠ جراماً في المتوسط (وزن ابتدائي)، تم توزيع هذه الأسماك عشوائياً وتقسيمها لـ ١٥ معاملة كل معامله ٣ مكررات (كل مكررة بها ١٠ سمكات /حوض من البلاستيك سعة ٤٠ لتر). وكانت الأسماك تغذى على العلائق المختبرة على مرتين يومياً لمدة ٦ أيام في الأسبوع، وكان معدل التغذية اليومي للأسماك ٣% من وزن الجسم الحي. ولقد استخدمت عليقة أسماك طافية تحتوى ٢٥,١٤% بروتين خام. وتم إضافة الأفلاتوكسين ب١ إلى العليقة بتركيزات صفر- ١٠٠- ٢٠٠ جزء في البليون بدون أو مع إضافة قشر البيض أو مخلفات الجمبري بمستويات صفر,١ ،٢ %. التأثيرات لكل من نوعى المادتين المدمصتين المستخدمتين (قشر بيض و مخلفات الجمبري) بتركيزات ١, ٢% على التوالي أوضحت أهمية استخداميهما لتقليل التأثيرات السامة للأفلاتوكسين ب١ على أسماك البلطي النيلى حيث تسببتا في حدوث زيادة معنوية في كل من تركيز هيموجلوبين الدم وعدد خلايا الدم الحمراء وتركيز حمض اليوريك في الدم، بينما هاتين المادتين المدمصتين تسببتا في إحداث انخفاض معنوي لعدد خلايا الدم البيضاء والأنشطة الإنزيمية المختلفة (ALP,AST,ALT) في دم الأسماك. وكذا حسنتا من دلائل الأعضاء (الكبد- الكلى- الطحال) كما تسببتا في حدوث انخفاض غير معنوي في دليل المناسل لأسماك البلطي النيلى، وقد أدتا هاتان المادتان المدمصتان (١% قشر بيض ، ٢% مخلفات الجمبري) إلى خفض التأثيرات السامة للأفلاتوكسين ب١ على تركيب كبد الأسماك. الأكثر من ذلك أن استخدام هاتين المادتين بنفس المستويين السابقين (١% قشر بيض، ٢% مخلفات جمبري) قد خففتا من التأثيرات الهستوباثولوجية السيئة للأفلاتوكسين على مختلف الأعضاء الداخلية للأسماك (كبد- كلى- أمعاء- خياشيم) للأسماك الملوثة به. ولقد ثبت أن لقشر البيض ضعف قدرة مخلفات الجمبري في تخفيف آثار وسمية الأفلاتوكسين، لذلك كان تركيز ١% قشر بيض مساويا لتركيز ٢% مخلفات الجمبري في تخفيف هذه الآثار. وعليه فإن الأفلاتوكسين ب١ الملوث للعلائق يمثل خطورة شديدة على صحة وإنتاجية الأسماك والصحة العامة للإنسان. ويوصى باستخدام قشر البيض (١%) أو مخلفات الجمبري (٢%) كإضافات علفية للعمل على التخفيف من التأثيرات السامة للأفلاتوكسين ب١ في علائق الأسماك الملوثة به.