Effect of Aflatoxicosis And Different Antimycotoxins On Health And Growth Of Oreochromis Niloticus

El-Bouhy Z , El-Nobi, G and El-Murr A
Department of Fish Diseases and Management ,
Faculty of Vet. Medicine, Zagazig University

ABSTRACT

A total number of 400 *Oreochromis niloticus* With an average body weight 18 ± 1.0g Were obtained from Abbassa Fish Hatchery ,The fish were used to determine the effect of mild and moderate aflatoxicosis with and without antimycotoxins (chemical as Bentonite and biological as dried *bacillus subtlis* fermentation extract) in diet on health and growth of *O. niloticus.*

The results revealed that, the addition of 0 .5 mg Aflatoxin B1(AFB1)in 1kg diet had no effect on health and growth of the fish but the histology revealed coagulative necrosis varied from scattered foci in low to large areas in liver and kidney which recovered with addition of either Bentonite or dried *bacillus subtlis* fermentation extract. Fish fed on diet with 2.0 mg AFB1 show mortality , reduced growth performance, the fish did not respond to tested reflexes , reduce feed conversion ratio ,liver was dark red friable, gills and kidney were congested. The addition of chemical antimycotoxin (with 1%in diet) and biological antimycotoxin (0.05% in 1kg diet) improve the growth performance and ameliorate the adverse effect of AFB1on health of tested *O. niloticus.*

INTRODUCTION

Aflatoxin contamination occurs over large geographic regions and in many potential feedstuffs, such as cottonseed, peanut, corn, rice, dried fish, shrimp, and meat meals. Aflatoxin B (AFB) is one of the most toxic of all naturally occurring carcinogens. A major epidemic one of liver tumors (hepatomas) which struck US trout hatcheries in the early 1960s, and was traced to contaminated cottonseed meal in the feeds *(1).*

The aflatoxin B1 (AFB1) is a polycyclic aromatic hydrocarbon, produced by some strains of the ubiquitous fungi *Aspergillus avus*, *A. parasiticus* and *A. nomius,* found to be a potent immune modulator in endotherms*(2).*

The most prevalent and famous type is Aflatoxin B1 (AFB1) which is very hepatotoxic, carcinogenic and immuno suppressive. AFB1 doesn't affect only fish but other animals and human. *(3).*

Many methods are used for antidoting aflatoxin like clay or 2% sodium bentonite which had a significant decrease in the absorption of AFB1 (20 ppm) in trout fish *(4).*

The inclusion of non-nutritive sorptive clays in contaminated feeds has promised a practical and cost-effective method for reducing toxic effects of AF in contaminated feedstuffs *(5).*

It was reported that some clays can reduced the uptake of AF from the gastrointestinal tract and improve productive performance, immune function and finally animal health. Among the clays, hydrated sodium calcium aluminosilicate (HSCAS) is the most extensively studied *(6).*

The present study was planned to determine the effect of mild and moderate aflatoxicosis and to antimycotoxins (chemical, Bentonite and biological, dried *Bacillus subtlis* fermentation extract) in diet on health and growth of *O. niloticus.*

MATERIAL AND METHODS

A total number of four hundreds *Oreochromis niloticus* fish with average body weight 18 ± 1.0 g., were obtained from Abbassa Fish Hatchery, Sharkia province, Egypt. in polyethylene bags containing dechlorinated water. Fish were apparently healthy and free from any external lesion and parasites. Fish were kept in a cement ponds provided with aerator for

15 day for acclimatization before start of experiment.

The fish were divided into 8 groups with two replicate (25 fish replicate)$^{-1}$ each replicate was stocked in large cage made from plastic meshed material of 1mm holes with 80 cm length × 80 cm weight × 80 cm depth .The cages were located above the bottom of the pond. Each pond was divided into 4 equal cages

The experimental fish were fed on basal diet contain 34% crude protein and 3000 kcal/kg metaboilzable energy. The diet composed of fish meal, poultry by product soybean, fish oil, flour, corn, minerals and vitamin mixture. Fish were fed with tested diet at rate of 3%body weight daily and fish were fed 4-5 times per day.

Aflatoxin B1 (AFB1)

Aflatoxin B1 (AFB1) (Sigma, USA) was dissolved in chloroform and added to the diet in required dose.

ATOX

Is a commercial product made in TOLSA,S.A company , Spain and imported by Pro Vet Care (PVC) . Each 1 kg. contain 600 gm Bentonite .

NETRTOX

Is a biological Commercial product made in Agrarian marketing corporation company (USA),imported by IFT company, contains (dried *bacillus subtlis* fermentation extract 370 g in 1kg) .

Effect of aflatoxicosis and different antimycotoxins on Health and Growth of Oreochromis niloticus fingerlings

Groups of fish, diets and doses of AFB1 are shown in Table 1. The experiment was conducted for 12 weeks.

Clinical signs and post mortem lesion was recorded. Evaluation of general health condition of fish, clinical signs, post mortem lesions and mortality were recorded ***(7,8)***. Growth performance, body gain ***(9)***, body gain % ***(10)*** and food conversion ratio (FCR) ***(9)*** were determined . Blood sample were taken from fish at end of the experiment to determine some hematological and biochemical parameters. The blood sample was collected in plain centrifuge tubes and centrfugated at 3000 r.p.m for 15 minutes for serum separation.

Histopathological examination of liver, intestine ,spleen and kidney was carried out to tested *O.niloticus* ***(11)***.

The obtained data was statistically analysed using analysis of variance procedure ***(12)***.

Table 1. Different dietary treatments of *Oreochromis niloticus*

Group (n=25)	Dietary treatment
1	Control
2	ATOX (Bentonite) 1%
3	(0.5mg AFB1/kg)
4	(0.5mg AFB1/ kg) ATOX (Bentonite) 1%
5	(0.5mg AFB1/ kg) Netrotox (dried *Bacillus subtlis* fermentation extract) 0.05%
6	(2.0mgAFB1/kg)
7	(2.0mgAFB1/kg) ATOX (Bentonite) 1%
8	(2.0mgAFB1/kg) Netrotox (dried *Bacillus subtlis* fermentation extract) 0.05%

RESULTS AND DISCUSSION

Effect of aflatoxicosis and different antimycotoxins on health and survivability

The results demonstrated in Table 2 revealed that, fish exposed to 0.5mg AFB1/kg and control fish groups showed similar survivability rate (96%). While fish exposed to 2 mg AFB1/kg showed 82% survivability. Addition of bentonite or dried *Bacillus subtlis* fermentation extract revealed 96% survivability. Nearly similar results were previously obtained ***(13)***. These deaths may be attributed to impaired immunity, renal damage and anemia produced by aflatoxin ***(1, 14)***.

Fish exposed to 2.0 mg AFB1/kg diet were observed to expel feed after ingestion and showed black coloration at tail region ***(18)***. These lesions recovered with addition of bentonite or dried *Bacillus subtlis* fermentation.

Table 2. Effect of aflatoxicosis and different antimycotoxins on survivability of *Oreochromis niloticus*

Group / Mortality in weeks	1 Control	2 Bentonite	3 (0.5mg AFB1)	4 - (0.5mg AFB1) Bentonite	5 -0.5mg AFB -dried *Bacillus subtlis* fermentation extract)	6 2.0mgAFB1	7 2.0mgAFB1 Bentonite	8 2.0mg AFB -dried *Bacillus subtlis* fermentation extract)
0-3	0	1	0	1	0	2	0	0
3-6	0	0	1	0	0	0	0	0
6-9	1	0	0	0	1	1	1	0
9-12	0	0	0	0	0	1	0	0
Total mortality no	1	1	1	1	1	4	1	0
mortality %	4	4	4	4	4	18	4	0
Survivability %	96	96	96	96	96	82	96	100

Effect of aflatoxicosis and different antimycotoxins on growth

The results demonstrated in Table 3 revealed fish groups fed on diet contains 0.5 or 2.0 mg AFB1/kg in diet displayed significant lower body gain and body gain %.

Similar results were previously displayed *(13,18)* . This may be attributed to that AFB cause impaired liver function, reduced feed efficiency, body weight loss, increased susceptibility to secondary infectious diseases and cellular necrosis *(19)*.

The addition of chemical antimycotoxin (Bentonite) as feed additive to control diet didn't affect the growth .

Addition of Bentonite or dried *Bacillus subtlis* fermentation extract to fish exposed to AFB1 revealed significantly improvement in the body gain and body gain % ,but the biological one gave better results in both parameters *(17)*. This may be due to that Bentonite a can reduced the uptake of AFB1 from the gastrointestinal tract and improve productive performance *(6)*. Fish fed on 2.0 mg AFB1/kg diet showed significant decrease in feed intake. This may be attributed to that fish expelled feed after ingestion as it was mentioned in previous research *(18)*.

Effect of aflatoxicosis and different antimycotoxins on some biochemical parameters

Table 4 showed that fish fed on diets containing AFB1 g showed creatinin levels similar to fish of control. Fish exposed to 0.5mg or 2mg AFB1/kg diet resulted in significant increase in ALT levels .When antimycotoxins are added to the diet the ALT level returned to its normal level. This increase in AlT may be due to the hepatotoxic effect of aflatoxin *(20)*.

Fish group fed on diet contains 2.0 mg AFB1/kg showed higher cortisol levels than group fed on 0.5mg AFB1 in diet, but both groups had higher values than control. The addition of antimycotoxins decreases the levels of cortisol. Similar results were previously obtained *(15-17)*. AFB is considered strong chemical stressors which normally increase cortisol *(15)*.

Effect of aflatoxicosis and different antimycotoxins on Histopathological Findings

The histological alterations were recognized in the organs of the fish received feed contain different concentrations of aflatoxin were similar but the severity was dose related.

The liver showed coagulative necrosis, varied from scattered foci from low to large areas with numerous leukocytic and melanomacrophages infiltrations at the margins (Fig 1). These areas represented by karyolysis and pyknosis in the nuclei of hepatocytes. Acute cellular swelling and ballooning degeneration were seen besides diffuse fatty infiltration . This may be attributed to the hepatotoxic effect of AFB1 *(20)*.

Table 3. Effect of aflatoxicosis and different antimycotoxins on some growth parameters of *Oreochromis niloticus*

Group / Parameter	1 Control	2 Bentonite)	3 (0.5mg AFB1)	4 - (0.5mg AFB1) -Bentonite	5 -0.5mg AFB -dried *Bacillus subtlis* fermentation extract)	6 2.0mgAFB1	7 2.0mgAFB1 Bentonite	8 2.0mg AFB -dried *Bacillus subtlis* fermentation extract)
Initial weight	18.35^{a} ±0.08	18.15^{a} ±0.31	17.85^{ab} ±0.14	18.35^{a} ±0.02	17.5^{b} ±0.17	16.95^{c} ±0.14	18.3^{a} 5 ±0.14	16.45^{d} ±0.14
Final weight	27.6^{ab} ±0.34	27.65^{ab} ±0.37	26.25^{d} ±0.37	28^{a} ±0.11	26.75^{cd} ±0.14	20.9^{e} ±0.34	27.7^{ab} ±0.11	27.1^{bc} ±0.05
Body gain	9.3^{c} ±0.23	9.5^{bc} ±0.05	8.4^{d} ±0.23	9.95^{b} ±0.25	9.5^{bc} ±0.17	3.95^{e} ±0.20	9.35^{bc} ±0.02	10.65^{a} ±0.20
Body gain %	50.65^{b} ±1.0	53.95^{b} ±0.31	46.7^{c} ±0.75	54.2^{b} ±1.3	54.25^{b} ±1.5	23.25^{d} ±1.0	50.95^{b} ±0.54	64.75^{a} ±1.8
Feed intake	22.25^{b} ±0.37	22.05^{b} ±0.02	24.4^{b} ±1.6	24.2^{b} ±0.11	22.2^{b} ±0.34	17.7^{c} ±0.8	24.55^{b} ±0.54	29.5^{a} ±1.4
Feed conversion ratio	2.35^{d} ±0.02	2.3^{d} ±0.0	2.85^{b} ±0.08	2.4^{d} ±0.05	2.3^{d} ±0.05	4.45^{a} ±0.02	2.6^{c} ±0.05	2.75^{bc} ±0.08

Means within the same raw carrying different superscripts are significant ($P \leq 0.05$)

Table 4. Effect of aflatoxicosis and different antimycotoxins on some biochemical parameters of *Oreochromis niloticus*

Group / Parameter	1 Control	2 Bentonite)	3 (0.5mg AFB1)	4 - (0.5mg AFB1) -Bentonite	5 -0.5mg AFB -dried *Bacillus subtlis* fermentation extract)	6 2.0mgAFB1	7 2.0mgAFB1 Bentonite	8 2.0mg AFB -dried *Bacillus subtlis* fermentation extract)
ALT (µ/L)	36.6^{c} ±0.49	35.6^{c} ±0.46	56.6^{a} ±0.55	55.2a ±0.93	44.9^{b} ±0.68	57.06^{a} ±0.43	44.5^{b} ±0.6	44.1^{b} ±0.97
Creatinin mg/dl	0.63^{a} ±0.11	0.67^{a} ±0.11	0.66^{a} ±0.02	0.65^{a} ±0.03	0.62^{a} ±0.02	0.71^{a} ±0.11	0.65^{a} ±0.02	0.6^{a} 2 ±0.01
Cortisol µ g/dl	7.2^{d} ±0.34	6.9^{d} ±0.29	10.0^{c} ±0.3	7.2^{d} ±0.15	7.1^{d} ±0.24	18.3^{a} ±0.41	11.0^{b} ±0.21	7.9^{d} ±0.43

Means within the same raw carrying different superscripts are significant ($P \leq 0.05$)

The intestine showed mucinous degeneration, desquamation and sloughing of the lining epithelium besides leukocytic infiltration in the submucosa . Nearly similar lesions were previously recorded ***(14)***

The kidneys revealed cloudy swelling, congestion and hemorrhages (Fig 2). Hyaline droplet degeneration was also detected in the cytoplasm of some renal tubules. The glomeruli were proliferated and showed hypercellular and the Bowman's capsule and basement membrane of the glomerular capillaries were thickened. *Oncorhynchus mykiss* fish fed on diet containing AFB1showed similar renal toxic lesions ***(20)***.

A-Effect of bentonite Treatment

The histopathological lesions of these groups were moderately ameliorated in comparison with the aflatoxin-contaminated groups.

The liver showed portal areas with necrotic pancreatic acini and lymphocytes, and melanomacrophages infiltration (Fig 3). Hemorrhages and brown pigments inside the cytoplasm of hepatic cells were seen scattered throughout the hepatic parenchyma. Hydropic

degenerations and vacuolation of hepatocytes were observed. Numerous eosinophilic Mallory bodies were also detected in the cytoplasm Small necrotic areas were seen particularly adjacent the necrotic pancreatic acini . Mild congestion and fatty infiltration were also visualized.

The kidney showed congestion, haemorrhage, cloudy swelling and focal hyaline droplet degeneration. The anterior kidney revealed moderate necrosis in the hemopoietic tissue and melanomacrophages centres .

The intestine revealed focal necrotic mucosa and lymphocytes infiltrating the submucosa. Mucinous degeneration and desquamation of the lining epithelium were recorded. This may be attribute to that Bentonite can reduced the uptake of AF from the gastrointestinal tract and improve productive performance *(6)* .This was confirmed that adding bentonite decrease the toxicity of AFB1 by adsorption mechanism and AFB1 adsorbed to Bentonite particles and so decrease the bioavaibilty of AFB1. *(17)*

B-effect of biological-Treatment

The lesions in these groups were completely alleviated toward the normal particularly with low concentration of aflatoxins and showed mild degenerative changes with an attempt to regeneration and congestion in the examined organs (Figs 4). Similar results were previously found *(21)*. This improvement in histopathology may be due to the that these bacteria were useful to aquatic animals not only as food but also as biological controllers of fish disease and activators of nutrient regeneration. *(22)*. Also may be due (dried *Bacillus subtlis* L-Form) act by biotransformation enzymatic cleavage of epioxide ring of mycotoxin which will be changed into non toxic substance. *(23)*.

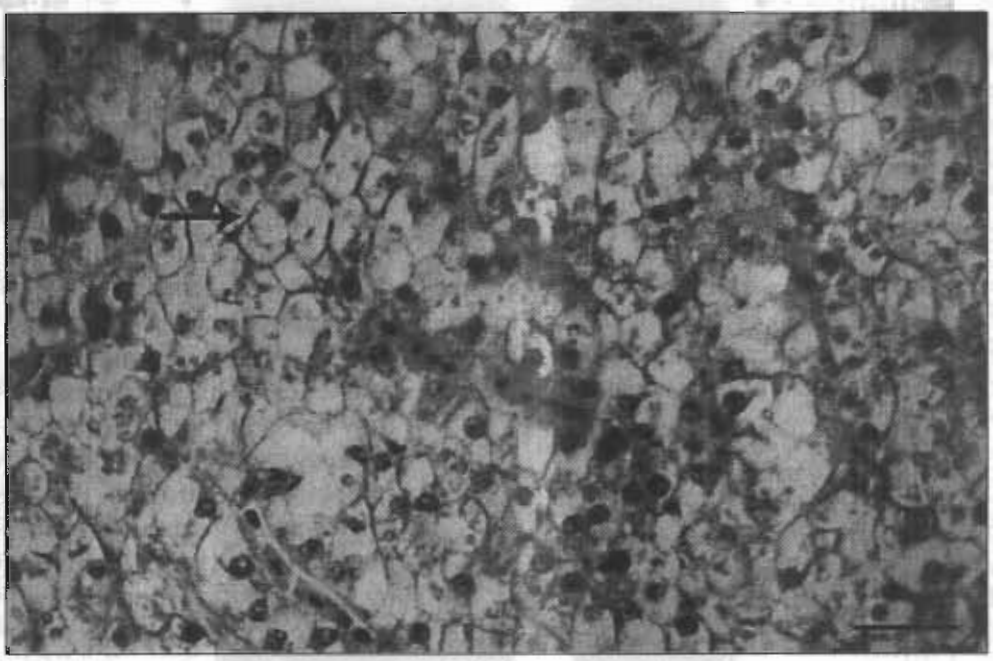

Fig 1. Section in Liver of fish received 2.0 mg AFB1 showing coagulative necrosis (arrow), HE (bar 100 µm).

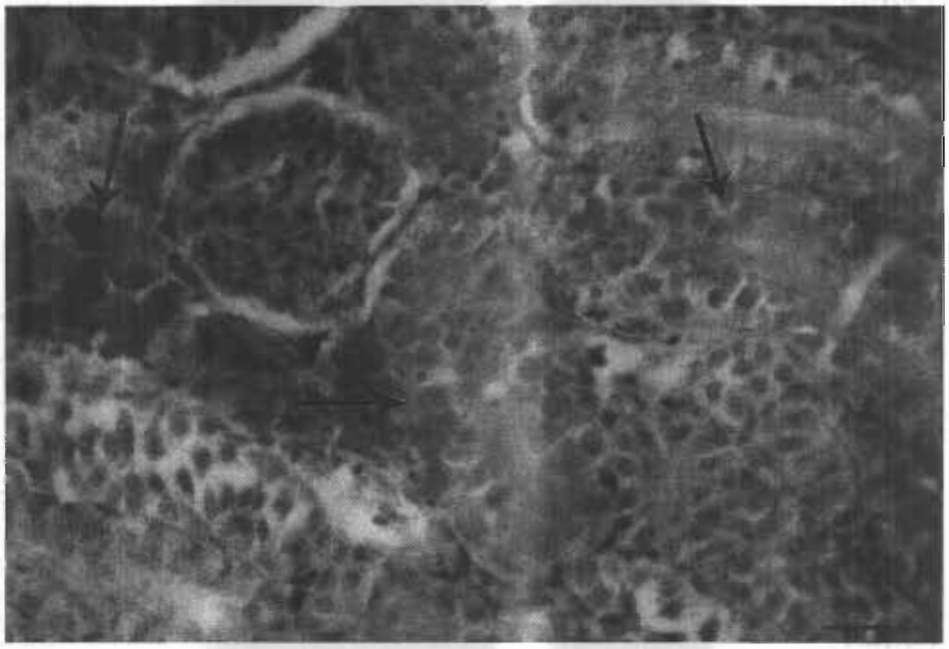

Fig 2. Section in kidney of fish received 0.5 mg AFB1 showing cloudy swelling and hemorrhages (arrow), HE (bar 100 µm).

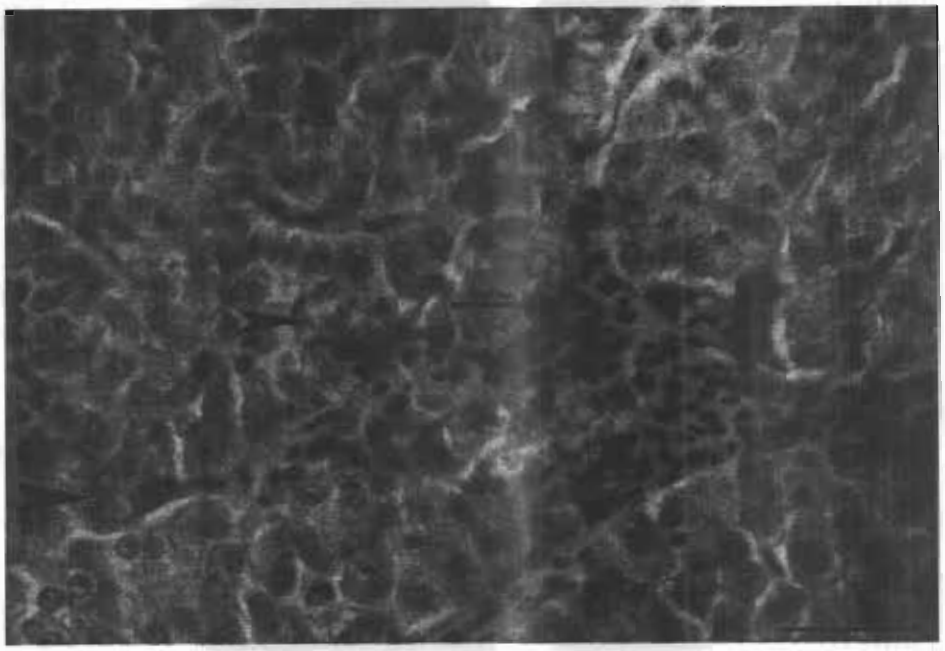

Fig 3. Section in liver of fish fed on diet with(2.0 mg AFB1 and bentonite)showing necrotic pancreatic acini infiltrated with lymphocytes (arrow), and melanomacrophages (arrow head), HE (bar 100 µm).

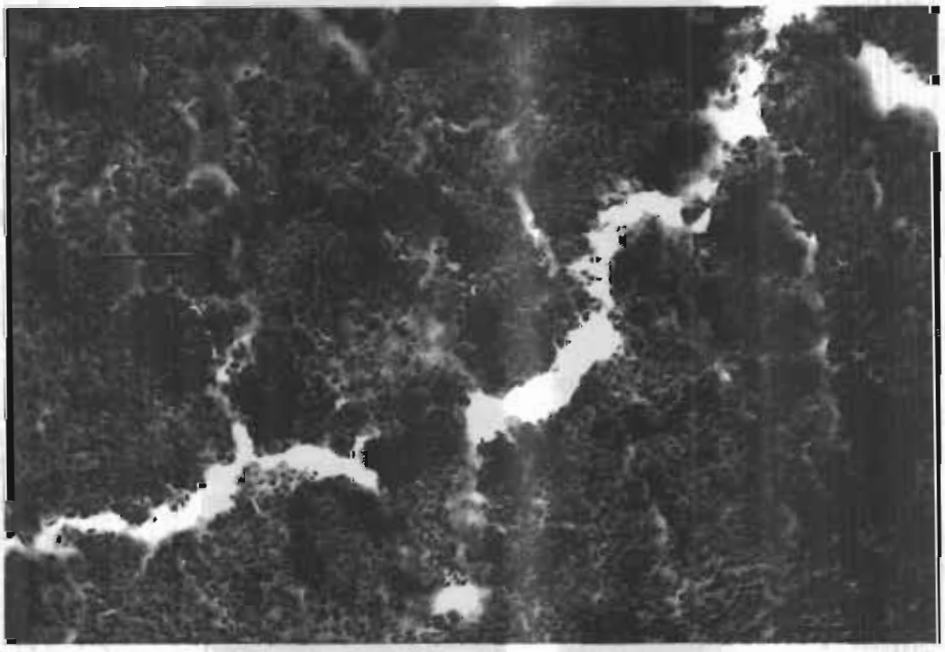

Fig 4. Section in kidney of fish received 2.0mg AFB1and -dried *bacillus subtlis* fermentation extract)showing regenerated renal tubules (arrow), HE (bar 100 µm).

REFERENCES

1. *Wolf H and Jackson EW (1963).* Heptomas in rainbow trout: descriptive and experimental epidemiology Science 142, 676–678.

2. ***Sahoo P K and Mukherjee S C (2001):*** Immunosuppressive effects of aflatoxin B1 in Indian major carp (*Labeo rohita*). Comp. Immunol., Microbiol. Inf. Dis. 24, 143–149

3. ***Williams J H , Phillips T D , Jolly P E , Stiles J K , Jolly C M , Aggarwal D (2004):*** Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences and interventions. Am. J. Clin. Nutr. 80, 1106–1122.

4. ***Ellis RW, Clements M, Tibbetts A and Winfree R (2000):*** Reduction of the bioavailability of 20 mg/kg aflatoxin in trout feed containing clay Aquaculture 183_2000.179–188.

5. ***Ramos A J , Johana F G and Hernandez, (1996):*** Prevention of Toxic Effects of Mycotoxins by Means of Nonnutritive Adsorbent Compounds. Journal of Food Protection, 59, 631–641

6. *Ramos AJ and Hernandez E (1997):* Prevention of aflatoxicosis in farm animals by means of hydrated sodium calcium aluminosilicate addition to feedstuffs: a review. Animal Feed Science and Technology, 65, 197–206

7. *Luky Z (1977):* Methods for the diagnosis of fish diseases. Amerind publishing Co. New Delhi, India.

8. *Noga, E J (1996):* Fish diseases: Diagnosis and treatment Mosby- year book, Inc., St. Louis, Missouri

9. *Siddiqui A , Howloder M and Adam A (1988):* Effect of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile Tilapia (*Oreochromis niloticus*). Aquaculture 70: 63-73..

10. ***Janucy R and Ross B (1982):*** A guide to Tilapia feeds and feeding. University of Sterling. Institute of Agriculture. Sterling, Scotland.

11. ***Robert R (1989):*** Fish pathology, 2nd ed. Bailliere Tindall London, Philadelphia, Sydney, Tokyo, Toronto

12. ***SAS Institute Inc (1996):*** Statistical analysis system for windows 6.12. Ed. Cary, NC. USA.

13. ***El-Banna R , Teleb H M , Hadi M M , Fakhry F M (1992):*** Performance and tissue residue of tilapias fed dietary aflatoxin. Vet. Med. J. 40, 17– 23

14. ***Hamilton P (1990):*** Problems with mycotoxins persist, but can be lived with. Feedstuffs 62, 22–23.

15. ***Sharma R P (1993):*** Immunotoxicity of Mycotoxins. Journal of Dairy Science, 76, 892–897.

16. ***Marin D E, Taranu I, Bunaciu R P, Pascale F, Tudor D S , Avram N , Sarca M, Cureu I , Criste R D , Suta V and Oswald I P (2002):*** Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. Journal of Animal Science, 80, 1250–1257.

17. ***Huff W E , Kubena L F , Harvey R B and Phillips T D (1992):*** Efficacy of hydrated sodium calcium aluminosilicate to reduce the individual and combined toxicity of aflatoxin and ochratoxin A1. Poult. Sci.71, 64–69.

18. ***Nguyen Anh Tuan , John M Grizzle, Richard T, Lovell Bruce B Manning A George E Rottinghaus (2002):*** Growth and hepatic lesions of Nile tilapia (*Oreochromis niloticus*) fed diets containing aflatoxin B1 Aquaculture 212 (2002) 311 –319.

19. ***Pier AC, Richard JL and Cysewski SJ (1980):*** Implications of mycotoxins in animal disease. J Am Vet Med Assoc 176:719–724.

20. ***Williams D E , Orner G , Willard K D, Tilton S, Hendricks J D, Pereira C, Benninghoff A D and Bailey G S (2009)***: Rainbow trout (*Oncorhynchus mykiss)* and ultra-low dose cancer studies. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 149 (2), 175–181

21. ***Miles, R D , Arafa A S and Harms R H (1981)***: Effect of a living on freeze-dried *lactobacillus acidopbilus* culture on performance, egg quality, and gut microflora in commercial layers. Poult. Sci. 60:993–1004.

22. ***Yasuda K and Taga N A (1980):*** Mass culture method for *Artemia salina* using bacteria as food. Mer 1980;18(53):62.

23. ***Doerr J A (2008):*** Cell-wall deficient lactobacilli: an emerging option for the control of mixed challenges in commercial poultry and livestock operations. . Proc. 5th World Mycotoxin Forum, the Netherlands. P.135.

الملخص العربى

تأثير سمية الافلاتوكسن ومضادات السموم الفطرية على صحة ونمو البلطى النيلى

زينب مصطفي عبد السلام ، جمال النوبى أحمد ، عبدالحكيم المر

قسم أمراض ورعاية الأسماك، كلية الطب البيطرى – جامعة الزقازيق

أستخدم عدد 400 من أصبعيات البلطى النيلى بمتوسط وزن 18 ±1.0 جرام لتحديد تأثير الافلا توكسن واثنان من أنواع مضادات السموم الفطرية على صحة ونمو البلطى النيلى.

أوضحت نتائج الدراسة مايلى:

1- أن إضافة الافلاتوكسن B1بنسبة 0.5 مج لكل كيلو جرام من للعليقة المقدمة الي إصبعيات البلطي النيلي لم تسفر عن اختلاف معنوي في معدلات النمو الطبيعية لسمك البلطي أما إضافة 2مج ج لكل كيلو جرام من العليقة نتج عنها نقص معنوي في معدلات الأوزان والنمو وزيادة في معدلات نفوق الاسماك الي جانب ظهور بعض العلامات الظاهرية مثل اسمرار الزعنفة الذ يليه

2- أظهرت الأسماك المعرضة لتركيزات مختلفة من الافلاتوكسن تغيرات إثناء الفحص الهستو باثولوجي للأسماك و تزيد هذه التغيرات بزيادة تركيز الافلاتوكسن

3- مع إضافة كل من مضاد سموم كيميائي يحتوي علي بنتونيت بنسبة 1% و اضافة مضاد سموم بيولوجي يحتوي علي بكتريا الا كتو باسلس ستلس0.05 % حدث تحسن معنوي في معدلات النمو والفحص الهستو باثولوجي و قد اظهر مضاد السموم البيولوجي نتائج أفضل نسبيا من مضاد السموم الكيميائي