Trials To Detoxificate Aflatoxin Contaminated Fish Diet And Its Use Safely (Residual And Histopathological Study)

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ABSTRACT

This experiment aimed to measure the influence of dietary probiotics and prebiotics on aflatoxin contaminated fish diet. A total of 80 Nile tilapia(*Oreochromis niloticus*) fingerlings with an average initial body weight of about 14 g was randomly allocated to 8 aquaria Each treatment was performed in two replicates at a rate of 10 fish for each replicate. Nile tilapia fingerlings supplemented with four diets for 70 days. First group was fed on basal diet without aflatoxin or feed additives(control), 2nd gp was fed on basal diet but contaminated with 150 ppb aflatoxin, 3rd gp was fed contaminated diet plus 0.1% Rotamin and 4th group was fed contaminated diet plus 0.2% Power top. Histopathological results showed severe degenerative lesions in the liver of fish exposed to aflatoxin B₁without feed additives, while the groups exposed to aflatoxin B₁with 0.1% Rotamin or 0.2% Power top showed mostly normal liver and reduction in the aflatoxin B₁ residues in fish body.

INTRODUCTION

Aflatoxins (AFs) are produced by genus Aspergillus, mainly Aspergillus flavus Aspergillus. parasiticus and Aspergillus nomius, that grow on a variety of raw material growth, harvest, storage transportation for example, the cereals used in the preparation of food and feed commodities (1,2). Aflatoxins (AFs), a group of potent mycotoxins with mutagnic, carcinogenic, teratogenic, hepatotoxic and immunosupresive properties, are of particular importance because of their major occurrence and adverse effects on animal and human health, generalized as "aflatoxicosis" (3-5). Liver is the primary target organ of metabolic action of aflatoxin, and most reports are based on the compositional changes in liver tissue (6). Aflatoxin is a potent liver toxin and carcinogenic, with aflatoxin B1 being the most toxic compound (7). The liver enzymes are changed with observation of malignant tumors (8,9). The hepatic damage represented by severe vacuolar degeneration and focal necrosis of the hepatocytes. (10,11). Most of chemicals and antibiotics are

ineffective in cleaning an infective cultivation system and also their uses are major expenses that significantly reduce the profitability for fish production so, prevention is better than cure (12,13). Therefore, several alternative strategies to use of antimicrobials have been proposed such as immunotherapy like probiotics as live yeast Saccharomyces cerevisae and another immunostimulants prebiotics such as alginic acid, mannon oligosaccharides and B- glucan which that may serve as dietary supplements to improve fish performance and immune responses (14). Several studies were performed on the yeast Saccharomyces cerevisiae as Immune stimulants and concluded that addition of Saccharomyces. cerevisiae to the common fish diet activates phagocytic activity and phagocytic index (15,16). In recent years, yeasts have also been reported to have high adsorption ability against mycotoxins in aqueous solution; Saccharomyces cerevisiae (Sc) had the potential to bind AFB1 (17,18). The yeast Sc was reported to be the most efficient microorganism for aflatoxin B1 quenching (19). A prebiotic is defined as no

digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or more of the gut-beneficial microbe groups Clinoptilolite (prebiotic) possibly supplies a significant amount of minerals to the diets of animals, providing that some minerals are present in this material in a form that can be assimilated by the body (21). Clinoptilolite prevent laboratory animals from toxic and teratogenic effects of the diet originated mycotoxins (22) It is suggested clinoptilolite be preferred in order to prevent domestic animals and their products from residual carcinogenic side effects (23).

This work was performed to determine the histopathological changes in liver and aflatoxin B_1 residues in musculature of fish as biological and adsorbant indices, respectively for evaluation the detoxification effect of the used additives on aflatoxin.

MATERIAL AND METHODS

Experimental fish

A total of 80 apparently healthy Nile tilapia fish (monosex male) fingerlings were obtained from a private fish farm at Tolombat 7 (Kafrelsheikh) Fish were transported to the wet lab of Sakha aquaculture research unit, Kafrelsheikh governorate, Egypt. Fingerlings

with an average initial body weight of about 14 g. were randomly divided into 4 equal groups two replicates each of 10 fishes were distributed through a total of 8 fully prepared glass aquaria measuring 60x35x40 cm. Fish were maintained in the aquaria for two weeks before the beginning of the experiment for acclimation. These aquaria supplied with chlorine free tap water according to (24). The aquaria were continuously aerated by electric pump and held at 25 ± 2 °C and half of the water was changed daily.

Experimental diets and feeding design

The present feeding trial was started from mid of augst and lasted 10 weeks and the diets were formulated according to previous reports (25) as shown in table (1). The basal diet was considered as a control diet. After homogenous mixing was obtained, forty ml water per hundred g diet was slowly added to the mixture for pelleting the diet according to (26) Nile tilapia fingerlings were fed on four diets. First group was fed on basal diet without aflatoxin or feed additives, second group was fed basal diet contaminated with aflatoxin(150 ppb), third group was fed on aflatoxin contaminated diet but supplemented with 0.1% Rotamin fourth group was fed on aflatoxin contaminated diet and supplemented with 0.2% Power top. The aflatoxin in dose of 150 ppb was added according to (27). The diet was daily provided at 3% of body weight for twice daily for a period of 70 days (10 weeks) as described by (28).

Table 1. Basal diet formulation throughout the experimental period

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Physical composition Yellow corn	%	Chemical composition	01
Soy bean meal44% Corn glutine 62% Fish meal (60%) Wheat bran Sunflower oil Dicalcium phosphate Common salt Vit. & min. mix ** The used premix (Multivite Car)	33 37 15 6 2 4.5 2 0.2 0.3	ME Kcal/kg* Crude protein Calcium Available phosphorus Lysine Methionine+ cystine	3118 32.3 .9 5 1.5

The used premix (Multivita Co.) composed of vitamin A 12000000 IU, vitamin D_3 2200000 IU, vitamin E 10000 mg, vitamin K_3 2000 mg, vitamin B1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B12 10 mg, Niacin 30000 mg, Biotin 50 mg, Folic acid 1000 mg, Pantothenic acid 10000 mg, Iron 30000 mg, Manganese 60000 mg, Copper 4000 mg, Zinc 50000 mg, Iodine 1000 mg, Cobalt 100 mg, Selenium 100 mg, calcium carbonate (CaCO₃) carrier to 3000g.

Antimycotoxins

Rotamin: A commercial product containing 88% Clinoptilolite, 5% Foldspars, 2% Montmorillonit, 2% Cristobalite and 3% Muscovite (National Activities Group Company).

Power Top: A commercial product consisting of 80% active yeast; Saccharomyces cerevisae, 10% Mannan Oligo Saccharides and 10% Beta Glucan (Media Vet. Company).

Aflatoxin B1

It was kindly provided by Microbiology DEPT, Iowa Uni., USA using Aspergillus parasiticus NRRL 2999. Concentration of the produced AFB1 was calculated and incorporated into the experimental diets at a rate of 150 ppb according to the method described previously (27).

Histopathology

Three fishes from each experimental group were taken immediately at the end of the experiment for histopathological examination. Tissue specimens from the liver, were collected, fixed in 10% neutral buffered formalin solution, dehydrated in serial grades of ethyl alcohol, cleared by xylol, embedded in paraffin wax, sectioned at 3-5 microns, stained Haematoxyline and Eosin (H&E) according to (29) and then examined microscopically for recording the hisopathological alterations

Residues of AFB1 in the whole fish body

Samples from three fishes from each of the experimental group were taken immediately at the end of the experiment to determine the residues of AFB1 in the whole fish body (30).

RESULTS

The histopathological examination demonstrated that no detectable pathological changes were observed in the liver of fish group fed on basal diet without aflatoxin or feed additives (Fig.1). However, fish exposed to aflatoxin either alone or with some additives showed pathological changes varied from group to another as follow, The group which fed on basal diet contaminated by 150 ppb aflatoxin without feed additives showed marked congestion in liver (Fig.2), severe fatty change of the hepatocytes (Fig.3), marked vacuolation together with focal necrosis of the (Fig.4) hepatocytes (Fig.5). The group which was fed basal diet contaminated by 150 ppb aflatoxin and supplemented with 0.1% Rotamin showed mostly normal liver except mild congestion, focal fatty change and hydropic degeneration of hepatocytes mostly together with rare affaction of the cytoplasm (Fig.6). However, the group fed basal diet contaminated by 150 ppb aflatoxin and supplemented with 0.2% Power top showed normal liver except very mild congestion (Fig.7).

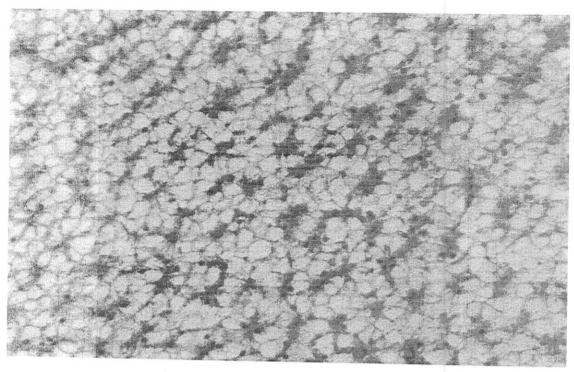


Fig.1. Liver of the group fed basal diet without aflatoxin B_1 or feed additives showing no detectable pathological changes (H α E X400).

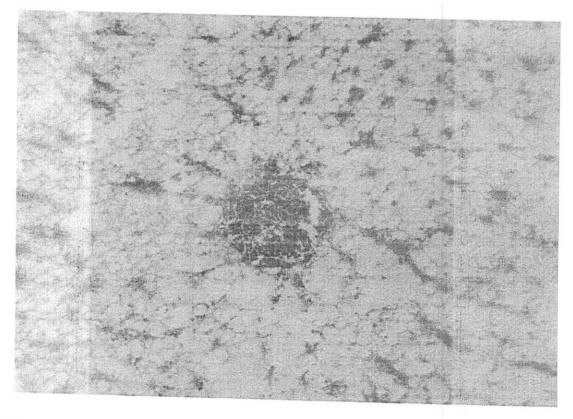


Fig.2. Liver of the group fed basal diet with aflatoxin B_1 without feed additives showing marked congestion and fatty changes (H α E X400).

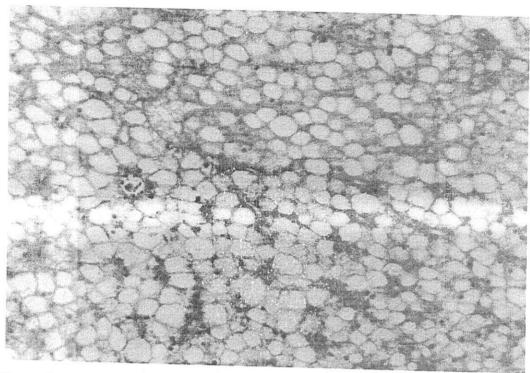


Fig.3. Liver of the group fed basal diet with aflatoxin B_1 and without feed additives showing severe fatty changes (H α E X400).

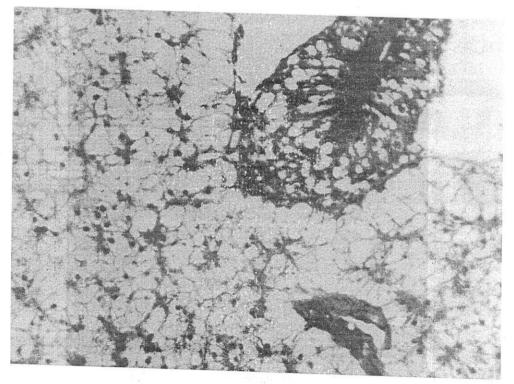
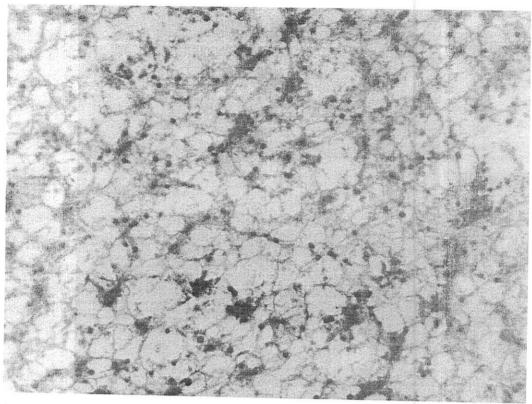


Fig.4. Liver of the group fed basal diet with aflatoxin B_1 without feed additives showing marked vaculation in the pancreatic acini (H α E X400).



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Fig.5. Liver of the group fed basal diet with aflatoxin B_1 without feed additives showing focal necrosis (H α E X400).

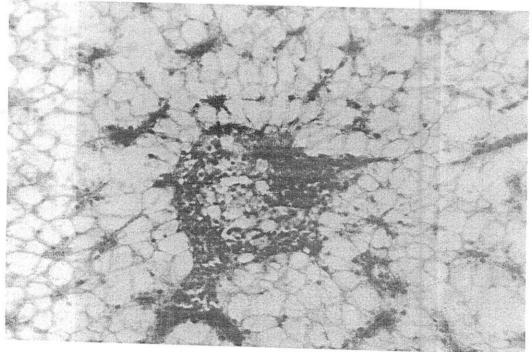


Fig.6. Liver of the group fed basal diet with aflatoxin B_1 with Rotamin showing mild congestion and focal fatty change and hydropic degeneration of hepatocytes (H α E X400).

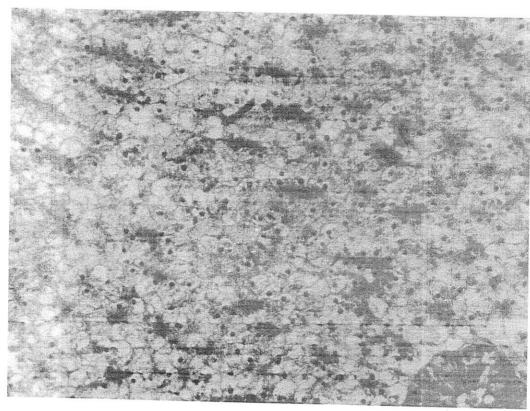


Fig.7. Liver of the group fed basal diet with aflatoxin B_1 and power top showing very mild congestion (H α E X400).

The achieved data concerning with aflatoxin B₁ residues in the whole fish body musculature are shown in Table (2) illustrated that the highest level aflatoxin B₁ residues in group fed basal diet contaminated by 150 ppb aflatoxin without feed additives being 8.3 ppb aflatoxin B₁, followed by the group fed basal diet contaminated by aflatoxin and

supplemented with 0.1% Rotamin (2.25 ppb), and the group fed basal diet contaminated by aflatoxin ppb and supplemented with 0.2% Power top (2.12 ppb) respectively While aflatoxin B_1 residues was non detected in the group fed basal diet free from aflatoxin B_1 or feed additives.

Table 2. Residues of aflatoxin B₁ in the tilapia fish musculature

Groups 1 -control	AFB ₁ residues in fish body (ppb)
2- fish fed aflatoxin contaminated diet.	non detected
3- fish fed aflatoxin contaminated diet with 0.1% Rotamin	8.30
4- fish fed aflatoxin contaminated diet with 0.2% Power top.	2.25
Power top.	2.12

DISCUSSION

Dietary aflatoxins are absorbed from the alimentary canal and pass to different organs and the liver is the primary target organ of metabolic action of aflatoxin (6) . In present study the histopathological lesions observed in fish group exposed to aflatoxin B_1 without feed additives agree with those obtained by (7)

Aflatoxin is a potent liver toxin and carcinogen, with aflatoxin B1 being the most toxic compound Such lesions agree also with those obtained by (31) who reported focal necrosis in the liver of Oreochromis niloticus. While the reported lesions in the groups exposed to aflatoxin B₁with feed additives were supported by pervious reports (32). Several biological, chemical and environmental detoxifying agent affect the biosynthesis and degree of hazard action of aflatoxins. The biological factors include - strain variability and competing microflora. The chemical factors include - the type of substrate, type of nutrients and antifungal agents. The environmental factors include - temperature, water activity, atmosphere gases, light intensity and pH. Our results are in accordance with recent study (33) who reported that both of chemical and biological detoxifying effect on fish diet contaminated with 200 ppb aflatoxin was effective with some superiority to the biological detoxification.

Aflatoxin B₁ residues findings concerning fish group exposed to aflatoxin $\widetilde{B_1}$ without feed additives are similar to those obtained by (34), AFB1 residues in the O. niloticus flesh showed a cumulative effect related to the levels of ration contaminated with AFB1 and feeding period Moreover, the feed additives ameliorate the results of aflatoxin B₁, residues findings concerning the groups exposed to aflatoxin \widetilde{B}_1 These findings are in agreement with those of other authors (35,36). The probiotic in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species; and enhancement of nutrition of host species through the production of supplemental digestive enzymes of the used probiotics These results agree with pervious findings (37). Immuno stimulants enhance the macrophage immune force of the fish to eliminate unwanted pathogens in their blood stream. The use of immuno stimulants for prevention of diseases in fish is considered an important attractive and promising field. Our results supported by (17,18) who reported that

in recent years, yeasts have also been reported to have high adsorption ability against mycotoxins in aqueous solution; Saccharomyces cerevisiae (Sc) has the potential to bind aflatoxin B₁.

CONCLUSION

It could be concluded that fish diets contaminated with aflatoxin B_1 induced severe hepatic damage and the use 0.1% Rotamin or 0.2% Power top could reduce the harmful histopathological lesions and aflatoxin B_1 residues.

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Authors wish to express their gratitude to Dr. Nagwan El-Habashi, Lecturer of pathology, College of Veterinary Medicine, Kafrelsheikh University, for her help in histopathology.

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الملخص العربي

محاولات لإزالة سمية الأفلاتوكسين من علائق الأسماك واستعمالها بأمان

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**أستاذ تغذية الحيوان والدواجن وأمراض سوء التغذية ونائب رئيس جامعة كفر الشيخ

الأفلاتوكسين ب١من أهم وأخطر الملوثات الغذائية والتي ينجم عنها أضرار صحية واقتصاديه في أسماك البلطي ولقد أجريت هذه التجربة لدراسة هذه الآثار السامة للأفلاتوكسين ب١ على اصبعيات البلطي النيلي ,وكذلك كمحاولة لإزالة الآثار السيئة لهذه السموم عن طريق إضافة بعض البروبيوتك, والبريبيوتك ولقد الجريت هذه التجربة على عدد ٨٠ سمكه تم تقسيمها إلى أربع مجموعات وقسمت كل مجموعه إلى مكررين بكل مكرر عدد ١٠ من الا سماك، حيث تم تغذية المجموعة الأولى على عليقه أساسيه وأعتبرت مجموعة ضابطة (بدون إضافة الأفلاتوكسين ب١ أوأ إضافات غذائية) و المجموعة الثانية على العليقه الأساسيه ملوثة بالأفلاتوكسين ب١ (١٥٠ جزء في البليون افلاتوكسين ب١). و المجموعة الثالثة تناولت العليقه الأساسيه ملوثة والمجموعة الرابعة على العليقه الأساسيه ملوثة بالأفلاتوكسين ب١) مضاف اليها روتامين (بريبيوتك) ١٠٠% والمجموعة الرابعة على العليقه الأساسيه ملوثة بالأفلاتوكسين ب١ (١٥٠ جزء في البليون افلاتوكسين ب١) مضاف اليها روتامين (السابيع وفي. نهاية مضاف إليها ٢٠٠% بور توب (بروبيوتك) وتم تغذية الأسماك على هذة العلائق لمدة ١٠ اسابيع وفي. نهاية التجربه تم اخذ عينات من الكبد للفحص الهستوباثولوجي وكذلك لقياس متبقيات الأفلاتوكسين في العضلات.

وقد اظهرت النتائج أن العلائق الملوثة بالأفلاتوكسين أدت الى تأثيرات سيئة على الكبد مثل أحتقان وتضخم ونخر فى الكبد focal necrosisوكذلك سهولة تفتت الكبد، كما رسبت متبقيات السم في لحوم تلك الأسماك ولقد أظهرت النتائج أيضا أن إضافة مضادات السموم الى العليقة المحتوية على الأفلاتوكسين ب١ أدت الى تخفيف الأثار السميه والباثولجية للافلاتوكسين على الأسماك

الخلاصة: إضافة كل من روتامين (بريبيوتك) وبور توب (بروبيوتك). يمكنها الحد من الآثار السميه للخلاصة : إضافة كل من روتامين (بريبيوتك) وبور توب (بروبيوتك). للافلاتوكسين ب١ على الأنسحة وكذلك يقلل من وجود متبقيات للافلاتوكسين ب١ في الأنسجة.