Effects of contaminated feed with *Aspergillus flavus* on some hematological and biochemical parameters on *Cyprinus carpio* L. 1758

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Summary

In this investigation the common carp, *Cyprinus carpio* L. 1758 was exposed to contaminated feed with *Aspergillus flavus* under laboratory aquarium conditions (glass ponds) for 90 days. Results revealed that the contaminated feed with (*A. flavus*) has significantly affect in body weight change, blood and biochemical parameters of groups fed on contaminated feed with *A. flavus*. Body weight changes were decreased in group feeding on contaminated feed with *A. flavus* in comparison with control group. The results showed decreases in RBCs, PCV% and Hb concentration but WBCs was increased in treatment fish in comparison with the control group. In addition, serum Glutamic pyruvic trans-aminase, Glutamic oxaloacetic trans-aminase and total cholesterol as compared to control showed an increase ($P<0.05$) in these parameters in contaminated fish but total protein was decreased in same groups. In conclusion, *A. flavus* is produced toxic compounds that represent a serious source of contamination in foods; this confirms the infection of aquacultures' fishes with *A. flavus*. So, one should not store foods for long periods or under poor conditions, fish health problems may arise.

Keywords: *Cyprinus carpio*, *Aspergillus flavus*, Body weight, Blood and biochemical parameters.

Introduction

Fishes are considered as an important source of human dietary protein worldwide, especially in poor countries (1). Most cases of inflammation of the enteritis in fishes are due to incorrect feeding which contains some types of fungi (2). The true fungi, member of the Phycomyctesare are those which cause the more important mycotic diseases of fishes (3). Fish diseases are the major cause of limited fish production in fish farms. Mycosis diseases are divided into two types integumentary and systemic mycosis, Aspergillosis is from the second types of mycosis diseases (4). A wide variety of phycomycetes and fungi imperfect have been associated with disease in fishes (5). *Aspergillus flavus* are more fungus widespread in nature, where they are present in the air and soil and can grow in fruits, vegetables and grains during storage and marketing causing a decrease in the nutritional and material value (6). Aflatoxin is a toxic compound produced by (*A. flavus*). The molds can grow in improperly stored feeds and feeds with lesser quality of components. These toxins have been incriminated as the case of high mortality in cattle and in some case of death in human beings (7). The carcinogenic effect of aflatoxin has been studied in fishes such as Salmonid, Rainbow trout, Catfish, Tilapia and Indain major carps (7-9). Metabolic activity within the liver is controlled by enzymes. Liver plays an important role in detoxification of poisonous and toxic substances (10). Highest value of transaminase enzymes are seen in case of hepatocyte necrosis occurring in case of fungal poisoning and viral hepatitis (11). Therefore, the present study aims to measure the effect of contaminated feed with *A. flavus* on the body weight changes, blood and biochemical parameters of common carp *Cyprinus carpio.*

Materials and Methods

Fish groups (25 fish samples) were divided as followed: control group, and another group feeding on contaminated feed with (*A. flavus*). Commercial feed was used with tap water and contaminated with 1×10^9 spores of culture (12)

Aspergillus flavus acquired from the Agriculture Research Directors.

A total of 25 fish samples (Common carp) were collected from Al-Mahawel region in Babylon province, during the period from March till May 2014. These samples were transferred a fresh to the laboratory in Fish and Animal Resource Center/ Agriculture Research Director by plastic containers and acclimatized to laboratory conditions (24.5 °C) for two weeks. Fishes were feed with commercial feed twice a day, at a feeding rate of 3% of the body weight, 23% proteins as shown in (Table, 1). Feeding period of 90 days was worked. Samples were divided into three aquariums (60 × 30 × 30 cm) with 40 liters of water after it was cleaned and disinfected by sodium chloride Nacl 3%, also fishes were disinfected with Nacl 3% to remove external parasites. Total and standard lengths were taken and fishes were weighted by balance type Mettler PE 3600 gm. The range and (mean) of weight was 38.6-63.9 gm (51.25 gm). Fish samples were divided into two groups as follows: Control group (C), fish feed throughout the experimental period on the feed is non-contaminated with *A. flavus*, and group (T) feeding on contaminated feed with *A. flavus*.

Table, 1: The components of experimental diet according to (13).

Contents	Percentage %
Animal protein	10
Soybean	25
Yellow corn	17
Local barley	22
Nakhala	25
Vitamins + Salts	1
Total	100

Blood samples were obtained from the caudal vein of fishes by using a 23-gauge needle and 3 ml syringe. The blood samples from each fishe was divided into two parts, the first part was used heparinized tubes for the evaluation of the hematological parameters including the red blood cell (RBCs), white blood cell (WBCs), packed cell volume (PCV%), and hemoglobin concentration (Hb), these parameters were determined as described by (14). The second part of the blood samples were used non-heparinized tubes for serum biochemical analysis, centrifuged at 3000 rpm for 10 min. and the obtained serum were aspirated into sterile vials and kept in deep freezer (-20°C) for the later analysis of the serum biochemical parameters including:

Glutamic oxaloacetic trans-aminase (GOT) was measured by a Randox kit following the method of (15) on a spectrophotometer at 546 nm wave length.

Glutamic pyruvic trans-aminase (GPT) was performed in blood serum with the help of a Randox kit according to the method of (15), using a spectrophotometer at 546 nm wave length. Total protein (16) and cholesterol (17) were measured using kits from ASSEL. These analyses were estimated using the VEGASYS Chemical Analyzer Device (AMS Co., Italy).

Data on weight, blood and biochemical parameters for the control and treatment fishes were analyzed using analysis of paired sample (T-Test). Comparison between means was done using stander error mean (SEM), by the (SPSS) was used.

Results and Discussion

This study was determine the effect of contaminated feed with *A. flavus* in fish culture farms (Common carp), *A. flavus* is produce a toxic compounds, it's a severe source of contamination in foods and feeds in many parts of the world (18). The results of this study, (Table, 2) observed decreases weight of *C. carpio* which were different in all of the treatment periods, weight ranged between 47.00 gm at the first month but at the end of treatment period 31.36 gm, these changes of total weight were due to the effect of aflatoxin in appetite, growth and not completely consumed (19).

Table, 2: Body weight (gm) changes of *Cyprinus carpio* exposed to contaminated feed with *A. flavus*.

Experimental groups	Exposure period (days)		
	30 days	60 days	90 days
C	51.72 ± 4.37 A	54.36 ± 4.58 A	80.38 ± 7.01 A
T	47.00 ± 3.73	44.40 ± 3.20 A	31.36 ± 3.51* B

Values are Mean±SEM (n=25) *Different capital letters denote significant results (P<0.05) between different groups. (C) control group: Fish feed throughout the experimental period on the feed is non-contaminated with *A. flavus*. (T) group: Fish feed throughout the experimental period on the feed is contaminated with *A.flavus*.

Table (3), the blood picture counts of *C. carpio* daily feed on contaminated feed with *A. flavus*. It observed a decrease in RBCs count, (1.56×10^6 cell/mm^3) at the first month and (1.26×10^6 cell/mm^3) at the end of treatment period. Also, there was a decrease in PCV, 25.14% at the first month and 19.98% at the third months and a decrease in hemoglobin concentration (5.80 gm/ 100 ml) at the first month and (4.94 gm/ 100ml) at the end of feeding with contaminated feed. These changes in blood picture count that belong to destruction and hemolysis of red blood cells RBCs are due to the toxic effect of aflation of *A. flavus*, these results agree with (10, 20 and 21). Also (Table, 3) showed an increase in white blood cells count, a minimum (29.52 $\times 10^3$ cell/ mm^3) at the first month and a maximum (31.46×10^3 cell/ mm^3) at the end of feeding period. In addition, these results are due to the toxic compound which causes disorder in immune system response (22-24).

Table, 3: Changes in blood parameters of *C. carpio* exposed to contaminated feed with *A. flavus*.

Parameter	Treatment	Exposure periods (months) 1	2	3
RBCs× 10^6 (cell/ mm^3)	C	1.48 ± 0.05	1.61 ± 0.02	1.85 ± o.o3
	T	1.56 ± 0.02	1.47 ± 0.02	1.26 ± 0.02*
WBCs×10^3 (cell/ mm^3)	C	29.06 ± 0.31	29.68 ± 0.38	28.88 ± 0.32
	T	29.52 ± 0.20	30.12 ± 0.14	31.46 ±0.61*
PCV (%)	C	28.72 ± 0.17	29.36 ± 0.16	31.70 ± 0.30
	T	25.14 ± 0.15	21.92 ± 0.29	19.98 ± 0.39*
Hb (gm/100 ml)	C	5.86 ± 0.35	6.04 ± 0.29	6.58 ± 0.21
	T	5.80 ± 0.20	5.54 ± 0.23	4.94 ± 0.26*

Values are Mean±SEM (n=5) *Different capital letters denote significant results (P<0.05) between different groups. (C) control group: Fish feed throughout the experimental period on the feed is non-contaminated with *A. flavus*. (T) group: Fish feed throughout the experimental period on the feed is contaminated with *A. flavus*.

Aflatoxicosis causes loss of appetite and disorder in digestion, absorption and metabolite process in fishes and other animals due to stress and disorder which affect all body organs especially in the liver and kidney (24), stress factors such as aflatoxin exposure cause show the changes in the biochemical properties, in (Table, 4) showed an increase of serum GOT and GPT to the various exposure periods, showing a minimum of GPT (49.23 U/L) at the first month and a maximum (52.22 U/L) at the third months. Also, it shows a minimum of GOT (196.99 U/L) at the first month and a maximum (250.58 U/L) at the end of treatment period. Total cholesterol level in serum increased a minimum (198.13 mg/dl) at the first month and a maximum (244.25 mg/dl) at the end of treatment.

Table, 4: Changes in biochemical parameters of *C. carpio* exposed to contaminated feed with *A. flavus*.

Parameters	Treatment	Exposure periods 1	2	3
GPT(U/L)	C	48.64 ±0.21	48.59 ± 0.25	48.47 ± 0.36
	T	49.23 ±0.08	50.18 ± 0.09	52.22 ± 0.10*
GOT(U/L)	C	195.40 ± 0.68	199.65 ± 0.44	199.82 ± 0.24
	T	196.99 ± 1.04	220.60 ± 0.19	250.58 ± 9.8*
Cholesterol (mg/dl)	C	190.31 ± 0.21	203.21 ± 5.72	202.68 ± 3.60
	T	198.13 ± 1.44	215.21 ± 6.20	244.25 ±14.24*
Total protein (gm/dl)	C	3.19 ± 0.22	3.04 ± 0.22	4.69 ± 1.32
	T	3.19 ± 0.07	2.99 ± 0.10	2.99 ± 0.08*

Values are Mean±SEM (n=5) *Different capital letters denote significant results (P<0.05) between different groups. (C) control group: Fish feed throughout the experimental period on the feed is non-contaminated with *A. flavus*. (T) treated group: Fish feed throughout the experimental period on the feed is contaminated with *A. flavus*.

Total protein level in serum of common carp exposed to aflatoxin was found to decrease in the treatment fish rather than the control, total protein (3.19 gm/dl) at the first month and (2.99 gm/dl) at the end of treatment period. These changes are due to effect of aflatoxin on liver functions due to pathological effects of all toxins on liver. Also, this toxin cause fatty changes of liver, necrosis of hepatocytes and disorder in metabolic processes of lipids (24). Contaminated of fish feed with *A. flavus* lead to accumulation of

these toxins in fish tissues. The risk for feed contamination may occurre as a result of using the contaminated fish tissues, especially in great quantities. The products of these fungi have an ability to accumulate in the living organisms (25).

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تأثير تلوث الغذاء بفطر الرشاشيات الصفراء على بعض المعايير الدموية والبايوكيميائية لسمكة الكارب الاعتيادي *Cyprinus carpio* L. 1758

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الخلاصة

عرضت أسماك الكارب الاعتيادي *Cyprinus carpio* L. 1758 للغذاء الملوث بفطر الرشاشيات الصفراء مع التغذية وتحت ظروف البيئة المائية المختبرية (بيئة الأحواض الزجاجية) لمدة 90 يوما. أظهرت النتائج تأثير تلوث الغذاء بفطر الرشاشيات الصفراء على وزن الجسم، الصورة الدموية والفحوصات البايوكيمياوية للمجاميع التي غذيت بالغذاء الملوث بفطر الرشاشيات الصفراء. وزن الجسم انخفض في المجاميع التي غذيت بالعلف الملوث بفطر الرشاشيات الصفراء بالمقارنه مع مجموعة السيطرة. كذلك فُحِصَت الصورة الدموية لأسماك مجموعتي السيطرة والمغذية بالغذاء الملوث بفطرالرشاشيات الصفراء لوحظ بالنتائج نقصان بأعداد كريات الدم الحُمر، ونسبة حجم الخلايا المرصوصة وتركيز خضاب الدم، لكن أعداد كريات الدم البيضاء زادت في مجموعة أسماك المغذية بالعلف الملوث بالفطر بالمقارنة مع مجموعة السيطرة.فضلاً عن ذلك قيس كلوتامك بايروفك ترانز امينيز وكلوتامك أوكزالو اسيتيك ترانز امينيز والكولستول الكلي والبروتين الكلي في الأسماك المغذية بالعلف الملوث مع مجموعة السيطرة، لوحظ بالنتائج زيادة هذه القيم في مجموعة المغذية بالعلف الملوث بالمقارنة مع مجموعة السيطرة لكن البروتين الكلي قلت قيمته في نفس المجموعة ($P<0.05$). يستنتج من هذا أن الرشاشيات الصفراء تنتج مركبات سامة تمثل مصدر خطير لتلوث الغذاء وهذا تأكيد على إصابة أسماك التربية بفطر الرشاشيات الصفراء. لذا يجب عدم خزن الطعام لفترة طويلة أو تحت الظروف السيئة لما تسببه من مشاكل في صحة الأسماك.

الكلمات المفتاحية: سمكة الكارب الاعتيادي، الرشاشيات الصفراء، وزن الجسم، الفحوصات الدموية والبايوكيماوية.