ECONOMIC LOSSES DUE TO OCHRATOXICOSIS IN CULTURED FISH FARMS AND THE ECONOMIC IMPORTANCE OF BIOGEN IN ITS PREVENTION

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SUMMARY

This study was carried-out on 320 Oreochromis niloticus fish collected from different private fish farms at Behera, Kafr-El-Sheikh and Alexandria governorates. This fish were transported alive to the laboratory and divided into 4 groups each group contain 80 fish. The first group take Biogen treated diet and the second group fed on regular diet without Biogen and this two groups considered as a control groups, while the 3rd group take a diet containing Biogen and Ochratoxin-A (OTA) and the 4th group take regular diet treated with (OTA) and the period of the experiment lasted 8-weeks. The clinical signs that appeared on the fish treated with OTA take the form of erratic swimming with respiratory and nervous manifestations, abnormal skin pigmentation, increasing the amount of mucous (Slimness) on the skin and gills, severe bilateral exophthalmia, blindness in addition to fin and tail rot.

At the Postmortem lesions, the fish treated with OTA showed that, the liver was friable with pale brownish colouration, congested kidney and congested gall bladder. The posterior portion of the kidney tinged with the blood.

The economic losses resulted from OTA appeared in the form of increasing the mortality rates by about 67.50 % in the group treated with OTA, while the group fed Biogen the mortality percentage in it about 46.25 % and there is a clear decrease in the body weight, weight gain and economic returns. The addition of Biogen improve the weight gain in the group treated with Biogen by about 0.35, 0.63, 0.46, 0.16, 0.02, 0.05, 0.29 and 0.6 Kg, and the economic returns improved by about 2.45, 4.41, 3.22, 1.12, 1.14, 0.35, 2.03 and 4.20 LE than the group treated with OTA for the period from 1 to 8 weeks, respectively.

This study concluded that, the toxicity of fish with Ochratoxins causes a high economic losses to cultured fish via its increasing mortality, decreasing body weight, body weight gain and the economic returns and the addition of Biogen to the fish diet can improve body weight, body weight gain and the economic and productive efficiency of fish production farms.

INTRODUCTION

Fish and fish products play an important and increasing role in solving the human nutritional problems in Egypt. The Egyptian population suffering from deficiency of animal protein, as the daily consumption of animal protein in Egypt about 16 g (AOAD, 1995) and the per capita daily consumption of fish meat about, 7.2 kg/year (FAO, 1994). Meanwhile, the consumption of fish in developed countries about 22.2 kg/year and in developing countries about 10.1 kg/year (FAO, 1994).

Fish supplies about 30 percent of total animal protein in diet of Asian populations, 20% in Africa and 10% in Latin America (FAO, 1996).

One of the main factors affecting fish production and efficiency were fish diseases and especially that resulted from mycotoxins and mainly Ochratoxins especially Ochratoxin A (OTA) which commonly contaminated the human, animal and fish feed (Scudmore et al., 1999; Creepy et al., 2004; Bayder et al., 2005; Bejaoui et al. 2006; Tangani and Pussemier, 2006 and Timperio et al., 2006).

In human the mycotoxins Ochratoxins constitute a major hazards on newly weaned children (Jonsyn et al. 1995 ; Oylami et al., 1996; Scudmore et al., 1999 and Bejaoui et al., 2006). Also there is a common relationship with Balkan Endemic Nephropathy and OTA (Stefanovic et al., 2006), also between OTA and Procine Nephropathy (Radic et al., 1997 and Sauvant et al., 2005) and there is a report called the Ochratoxins is a mycotoxins endemic in Egypt (El-Kady et al., 1995; El-Shaboury 1998 and Wafa et al., 1998).

The mycotoxins Ochratoxins causes severe economic losses to fish industry (Jonsyn and Lahai, 1992), through decreasing fish weight, feed conversion ratio and causes severe mortality among fish farms (Samira-Rezeaka, 1991 and El-Shaboury, 1998).

The Ochratoxins causes severe losses among different fish species especially Tilapia Nilotica, Mugil and Common Carp and this two species of fishes considered as the most distributed fish species among Egyptian fish farms and lakes (El-Zarka et al., 1993 and El-Shaboury , 1998) . Also, this two species accepted by Egyptian consumers and play an important economic role in fish industry in Egypt.

Ochratoxins are a group of structurally related, toxic metabolites produced by seven species of Aspergillus and six species of Penicillium. Aspergillus Ochraceus from which the toxins acquired their name appears to be the predominant Ochratoxin producer . Furthermore, the toxin production by these fungi is influenced by species and even strain of fungi, time and temperature of incubation, moisture content of substrate and type of substrate. (Scudmore et al., 1999; Bayder et al., 2005 and Tangani et al., 2006).

The most important clinical signs that appeared on the fish suffered from Ochratoxicosis include , nervous and respiratory manifestations as well as skin erosion, congestion of the skin, fin and tail rot, exophthalmia and congestion. While, the most important postmortem changes were enlargement of all internal organs especially liver, kidney, spleen and gall bladder. (Easa, 1997 and Saad , 2002).

The main economic losses resulted from affection of cultured fish with Ochratoxins resulted from decrease of fish body weight, weight gain and hindrance of the food conversion efficiency. (Easa, 1997 and Saad, 2002).

The addition of some immunostimulants as the Biogen can inhibit the effect of Ochratoxins on the cultured fish and increase the economic and productive efficiency of fish production farms via increasing the body weight and weight gain (Easa, 1997 and Saad, 2002).

The aim of this study, is the determination of the effect of Ochratoxins on the efficiency of fish farms and determination of the lesions and symptoms of Ochratoxicosis on infected fish, in addition to determination of the role of the Biogen as feed additives in improving the losses resulted from Ochratoxins in fish farms.

MATERIALS AND METHODS

1 Fish:

A total number of 320 Oreochromis niloticus random fish sample, were collected from different private fish farms at Behera, Kafr-El-Sheikh and Alexandria governorates. The fish were transported alive to the laboratory of Poultry and Fish Diseases Department Faculty of Veterinary Medicine Alexandria University in plastic bags containing water enriched by air (2/3). The average body weight of the fish about 30 ± 5 gm.

2. Aquaria:

Fish were kept in prepared glass aquaria (90 X 50 X 35 Cm). These aquaria were used for holding the experimental fish throughout the period of the present study, supplied with chlorine free tap water. The continuous aeration was maintained in each aquarium using an electric air pumping compressors. Water temperature was kept at 22 ± 1 °C. All fish were acclimatized for at least 2 weeks prior to the experiment. (El-gamal, 2005).

3-Fish diets:-

Fish were fed on commercial fish food containing 23-25% crude protein (obtained from Barseek fish culture factory) the diet was daily provided at 3% of body weight as described by and the daily amount of food was offered on two occasions over the day (Regular diet), in addition to in acute and chronic experiment (El-gamal, 2005).

4. Ochratoxin A:

The mycotoxin Ochratoxin A [(C_{20} H_{18} $ClNO_6$) (7-Carboxy-5-Choloro-8-Hydroxy-3, 4-dihydro-3-R-methyl isocoumarin)] was kindly provided by Sigma Chemical Co. U. S. A. also by Sigma-Aldrich Chemie Gmbh, Germany. The OTA that used its number 0-1877 (1 mg) Lot. No. 76 H 4084.

5-Biogen :-

Is an immunostimulant containing the extract of Ginseng plant that increase the vitality and activity of the fish. Also, it increase and activate the antibodies and immunity of the fish against any stress that the fish exposed to it also contain some useful bacteria that help in destruction of the OTA and hindered its effects, also the Biogen activate the liver to get ride of the OTA and also, it considered as a stomachic to the fish that improve the fish appetite. Moreover, the Biogen improve the water quality and decrease the ammonia level with improvement the excretion of heavy metal from the fish body.

6-Toxicity of Ochratoxin-A.

The fish (30±5 gm each) in this experiment divided into 4 groups, two groups of them was injected with 1/10 LD_{50} (10000 ng) (Shehata et al., 1985), the first group take toxin with Biogen ® and the second group take toxin with regular diet.

The other two groups act as a control and one of them take regular diet only and the other take regular diet with Biogen . All the experimental groups were kept under daily observation for 8 weeks.

The clinical signs, mortality and postmortem lesions were recorded throughout the 8-week experimental period.

7-Fish weight:-

During the experiment the fish was weighted weekly and the body weight gain was calculated for every week according to the following equation (Shewita, 2004):

Weight gain = Body weight 2 – Body weight 1.

8-Economic returns:

The return from fish sale per LE according to the following equation (Shewita, 2004 and El-Tahawy, 2004):

Returns = Wight of fish X price of fish (LE).

9-Statistical analysis:

The data of body weight, body weight gain and return losses of exposed fish to OTA were statistically analyzed using ANOVA test according to (SAS, 1987 and Macarthur et al., 2006) to examine the significant effect of the main variables on the studied parameters.

RESULTS AND DISCUSSION

The mortalities due to exposure to OTA in (Table, 1). Showed that the groups fed OTA with regular diet of high mortality percentage (67.50 %) , than the group fed OTA with diet contain Biogen (46.25 %). While the control groups not show any mortalities.

The economic losses for the groups fed OTA with regular diet about 15.8 LE and for the groups fed Biogen with OTA was about 9.8 LE.

Table (2) explain the significant differences ($P < 0.01$) due to the effect of different treatments on body weight of the fish, as the groups fed diet contain OTA show severe decrease in body weight along the period of the experiment , as the body weight of this groups were 2.34, 2.27, 2.04, 2.24, 2.16, 1.74, 1.50 and 1.18 Kg, while the group fed Biogen with OTA showed

improvement in its body weight as its body weight were 2.69, 2.90 , 2.50, 2.40, 2.18, 1.79, 1.79 and 1.78 Kg.

The return results in (Table, 3) indicated that , the OTA causes severe economic losses but by the addition of Biogen there is improvement in the economic returns of this groups. The economic returns of the groups fed OTA were 16.38, 15.89, 14.28, 15.68, 15.12, 12.18, 10.50 and 8.26 LE, but, by the addition of the Biogen the economic returns by reached to 18.3, 20.30, 7.50, 16.8, 5.26, 12.53, 12.53 and 12.46 LE, for the weeks 1, 2, 3, 4, 5, 6, 7 and 8, respectively.

Also, the results in table (4) indicated that, the addition of Biogen to fish diet improves he weight gain and its returns, than the groups without Biogen treatment or OTA feeding diet. The weight gain of the fish due to addition of Biogen were 0.35, 0.63, 0.46, 0.16, 0.02, 0.05, 0.29 and 0.6 Kg and the returns improved by about 2.45, 4.41, 3.22, 1.12, 0.14, 0.35, 2.03 and 4.20 LE for the weeks from 1 to 8, respectively.

This results agreed with those of (Saad, 2002) who reported that the OTA causes decrease of body weight, body weight gain and so reduction of economic and productive efficiency of fish production farms and the addition of Biogen to the fish diet will improve body weight and weight gain with increasing of the fish production farms profits. Saad (2002) and El-gamal (2005) attributed this results to the Biogen act as immunostimulant as it contain the extract of Ginseng plant which increase the vitality and activity of the fish. Also, the Biogen increase and activate the antibodies and immunity of the fish against any stress that the fish exposed to it, also it contain some useful bacteria that help in destruction of the OTA and hindered its effects, moreover the Biogen activate the liver to get ride of the OTA, in addition the Biogen considered as a stomachic to the fish that improve the fish appetite, the Biogen improve the water quality and decrease ammonia level and improve the excretion of heavy metal from the fish body.

The most important behavioural changes appeared on the infected fish with OTA were -erratic swimming with respiratory and nervous manifestations, which appears in the form of swimming vertically and on the lateral sides, swimming in the circle movement, with signs of asphyxia, decreasing of feeding reflex, decrease vitality, losing of body reflex and don't stimulate to external stimuli, at the end of injection the fish appear off-food and death occur suddenly without any clinical appearance.

The most important clinical signs of Ochratoxicosis appeared in Fig. (1 and 2) and it started in the 1st week of administration and continued until the end of the experiment (8th week) and it include, abnormal skin pigmentation, increase the amount of mucous (Slimness) in skin and gills and then decreased gradually, leading to appearance of rusty spots in the external surface of the fish, the eye affected as follow, in the beginning, appearance of eye cataract, which increased gradually leading to severe exophthalmia (bilateral) after that there is destruction of the eye and the fish become blind

especially at the end of the experiment (10-20 %), there is fin and tail rot, which leads to appearance of vertebral column in severe cases (40%), mild ascitis changed to severe ascitis and severe abdominal distension, congested gills with mild signs of gill rot and stunted growth.

At the post-mortem findings (Fig. 3 and 4) we noticed that, the liver is friable with a pale brownish colour, congested kidney especially at the later stages of injection, gas bladder is congested and covered with inflammatory exudates, the abdominal cavity is filled with viscous bloody fluid and sanguineous fluid, at the later stages appearing of gastro-enteritis, blotched area in the kidney especially in the posterior part, while the anterior part was slightly pale in colour, the liver at the beginning of injection was edematous and slightly pale with a focal necrotic area, the spleen is congested.

This results agreed with those of Safinaze (2000) and Saad (2002) where they reported that Ochratoxicosis causes severe behavioral, clinical and post-mortem changes similar to the our results in toxicated fish.

This study concluded that, the toxicity of fish with Ochratoxins causes a high economic losses to cultured fish via its increasing mortality, decreasing body weight, body weight gain and the economic returns and the addition of Biogen to the fish diet can improve body weight, body weight gain and the economic and productive efficiency of fish production farms.

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Table (1): Mortality number and its percentages as well as the economic losses resulted from Ochratoxin-A administration to Oreochromis niloticus fish.

		Biogen treated diet		Regular diet	
Treatment condition	Week number	Mortality number	Return losses (LE)	Mortality number	Return losses (LE)
Control fish	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
	6	-	-	-	-
	7	-	-	-	-
	8	-	-	-	-
	Total mortalities	0/80	-	0/80	-
	Mortality % after 8-weeks	0%	-	0%	-
Treated fish with OTA	1	-	-	-	-
	2	5	1.40	16	4.48
	3	10	2.80	-	-
	4	5	1.40	-	-
	5	5	1.40	6	1.68
	6	10	2.80	12	3.36
	7	-	-	10	2.80
	8	-	-	12	3.36
	Total	37/80	9.8	54/80	15.68
	Mortality % after 8-weeks	46.25%	-	67.50%	-

Table (2): Explain the effect of ochratoxin-A treatment on O. niloticus weight (Kg) with and without Biogene treatment.

Group	Parameter	Fish number	Biogen® treated diet	Fish number	Regular diet
Control fish	Week1	80	3.16±0.03A	80	2.71±0.0B
	Week2	80	3.17±0.03A	80	2.82±0.03AB
	Week3	80	3.19±0.02A	80	2.89±0.02AB
	Week4	80	3.23±0.02A	80	2.99±0.01AB
	Week5	80	3.25±0.02A	80	3.03±0.02A
	Week6	80	3.26±0.02A	80	3.14±0.02A
	Week7	80	3.33±0.03A	80	3.17±0.03A
	Week8	80	3.45±0.04A	80	3.20±0.03A
Treated fish with ochratoxin-A	Week1	80	2.69±0.02B	80	2.34±0.03B
	Week2	75	2.90±0.02AB	64	2.27±0.02B
	Week3	65	2.50±0.02B	64	2.04±0.02B
	Week4	60	2.40±0.04B	64	2.24±0.01B
	Week5	55	2.18±0.01B	58	2.16±0.03B
	Week6	43	1.79±0.01C	48	1.74±0.01C
	Week7	43	1.79±0.02C	38	1.50±0.01C
	Week8	43	1.78±0.02C	26	1.18±0.02C

Means within the same column carrying similar superscripts are significantly different at (P< 0.01)

Table (3): Explain the effect of Ochratoxin-A treatment on O. niloticus returns (LE) with and without Biogene treatment.

Group	Parameter	Fish number	Biogen ® treated diet	Fish number	Regular diet
Control fish	Week1	80	22.12±2.20B	80	18.97±1.98C
	Week2	80	22.19±2.23B	80	19.74±1.74BC
	Week3	80	22.33±3.22B	80	20.23±2.23AB
	Week4	80	22.61±2.66B	80	20.93±2.99AB
	Week5	80	22.75±2.75B	80	21.21±3.22A
	Week6	80	22.82±3.27B	80	21.98±3.21A
	Week7	80	23.31±2.27AB	80	22.19±2.18A
	Week8	80	24.15±2.15A	80	22.40±2.49A
Treated fish with Ochratoxin-A	Week1	80	18.83±2.87C	80	16.38±1.37D
	Week2	75	20.30±2.35B	64	15.89±1.55E
	Week3	65	17.50±2.36CD	64	14.28±1.14F
	Week4	60	16.80±2.37DE	64	15.86±1.61E
	Week5	55	15.26±2.26E	58	15.12±1.12E
	Week6	43	12.53±2.23F	48	12.18±1.13G
	Week7	43	12.53±2.55F	38	10.50±1.14H
	Week8	43	12.46±2.47F	26	8.26±1.12I

Means within the same column carrying similar superscripts are significantly different at (P< 0.01)

Table (4): Explain the effect of ochratoxin-A treatment on O. niloticus (body weight gain and its returns / LE) with and without Biogen treatment.

Group	Parameter	Gain due to addition of Biogen	Returns due to addition of Biogen
		Kg	LE
Control fish	Week1	0.45±0.02B	3.15±0.01A
	Week2	0.35±0.01C	2.45±0.02C
	Week3	0.38±0.02BC	2.66±0.02BC
	Week4	0.24±0.03D	1.68±0.01D
	Week5	0.22±0.02D	1.54±0.01D
	Week6	0.12±0.01F	0.84±0.03F
	Week7	0.16±0.01F	1.12±0.02F
	Week8	0.25±0.02E	1.75±0.01E
Treated fish with Ochratoxin-A	Week1	0.35±0.03BC	2.45±0.02C
	Week2	0.63±0.06A	4.41±0.03A
	Week3	0.46±0.02B	3.22±0.02A
	Week4	0.16±0.01F	1.12±0.01F
	Week5	0.02±0.01H	0.14±0.002H
	Week6	0.05±0.02H	0.35±0.001H
	Week7	0.29±0.01E	2.03±0.02E
	Week8	0.6±0.05G	4.20±0.02G

Means within the same column carrying similar superscripts are significantly different at (P< 0.01)

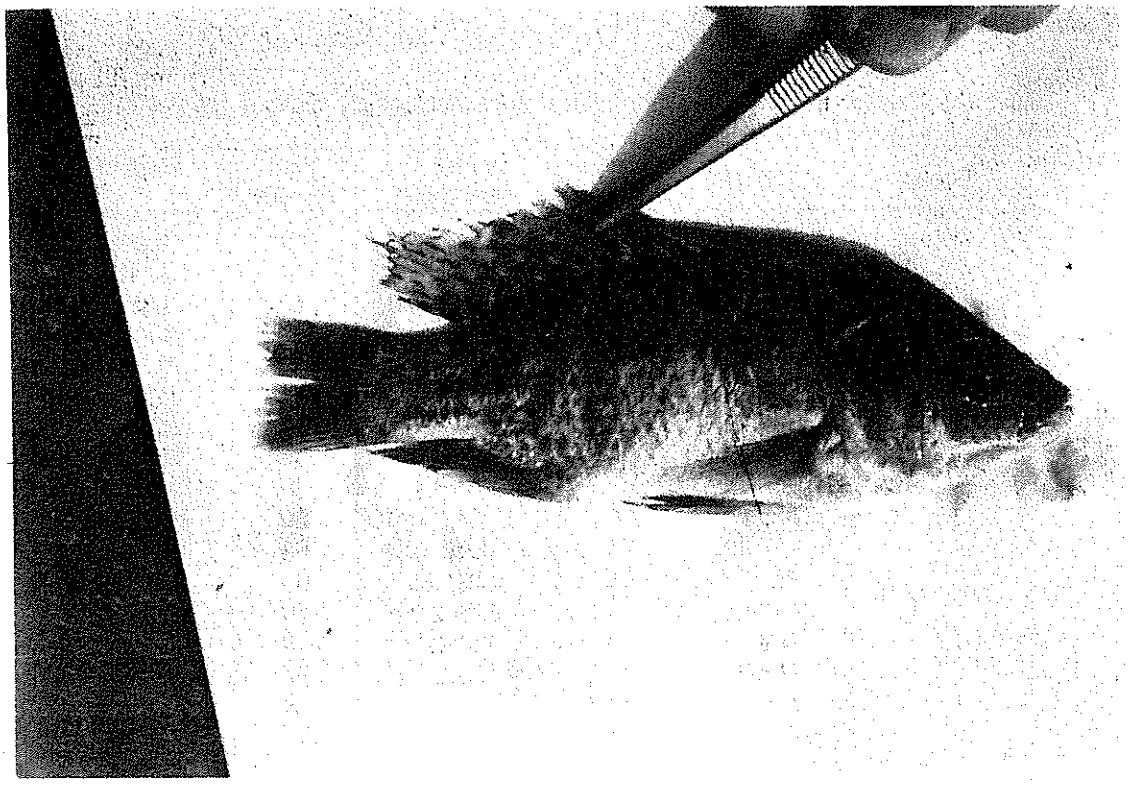

Fig. (1): Darkening of body colour with slight fin rot of Oreochromis niloticus fish suffering from Ochratoxicosis.

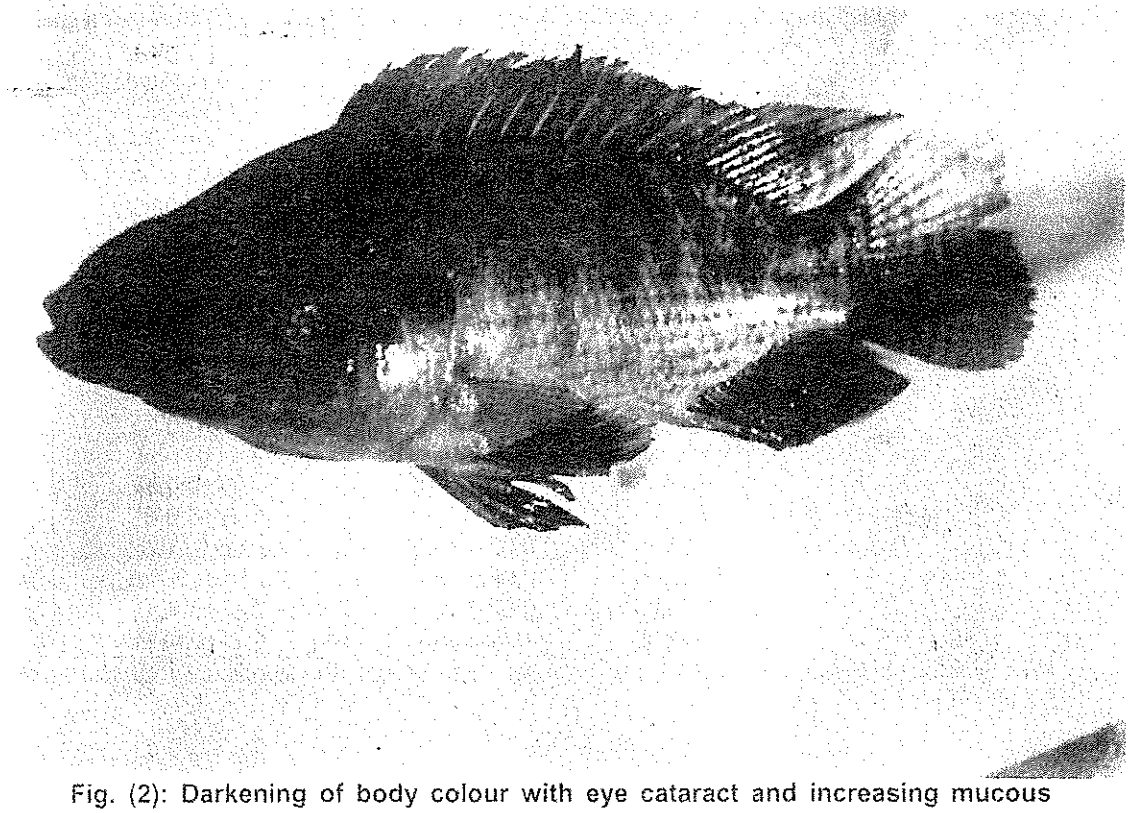

Fig. (2): Darkening of body colour with eye cataract and increasing mucous secretion (Slimness) of Oreochromis niloticus fish suffering from Ochratoxicosis.

Fig. (3): Congestion of all internal organs especially liver and gills of Oreochromis niloticus fish suffering from Ochratoxicosis. (arrows).

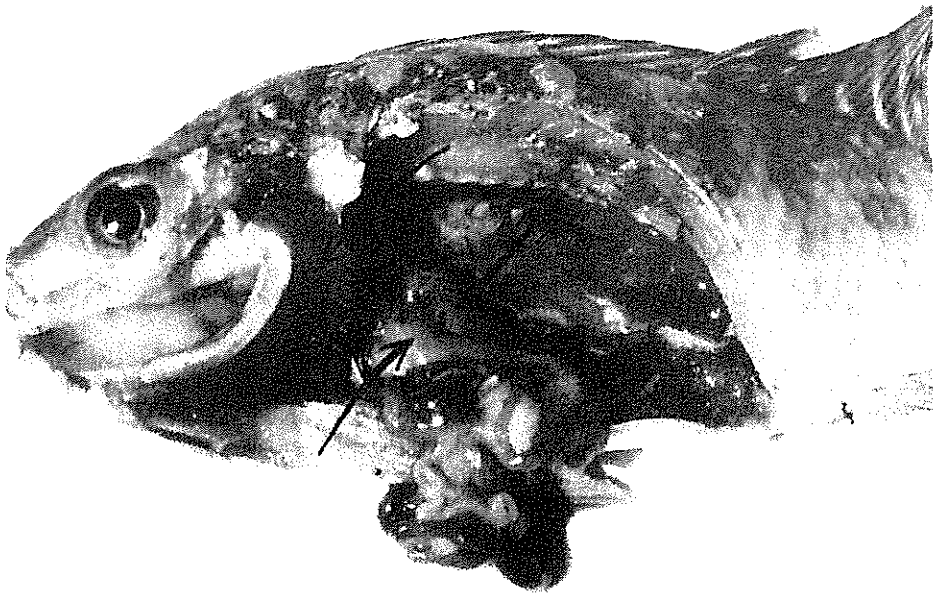

Fig. (4): Congested kidney and gills with slight liver necrosis of Oreochromis niloticus fish suffering from Ochratoxicosis. (arrows)

الملخص العربى

الخسائر الاقتصادية للسموم الفطرية الاوكراتوكسينات فى مزارع انتاج الاسماك ودور البيوجين فى منع التسمم بها

طلعت طلعت سعد و *سند طلعت عطاالله و صافيناز جمعة محمد

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أجريت هذه الدراسة على عينة عشوائية مكونة من 320 سمكة مجمعة من عدد من المزارع الخاصة المتواجدة فى محافظات البحيرة وكفر الشيخ والاسكندرية حيث تم نقل تلك الاسماك حية الى معمل صحة وأمراض الاسماك بكلية الطب البيطرى – جامعة الاسكندرية وتم تقسيمها الى أربعة مجموعات كل مجموعة تحتوى على 80 سمكة بلطى نيلى. حيث تم اعطاء المجموعة الاولى عليقة محتوية على البيوجين والمجموعة الثانية أعطيت عليقة بدون البيوجين وأعتبرت هاتان المجموعتان كمجموعات ضابطة بينما أعطيت المجموعة الثالثة عليقة محتوية على البيوجين والاوكراتوكسين-أ وأعطيت المجموعة الرابعة عليقة منتظمة محتوية على الاوكراتوكسين-أ فقط واستمرت التجربة لمدة 8 أسابيع.

أوضحت التجربة أن أهم الاعراض الاكلينيكية التى ظهرت على الاسماك نتيجة للتسمم بالاوكراتوكسين هى أن الاسماك تعوم بطريقة مضطربة ويظهر عليها علامات واعراض تنفسية وعصبية شديدة ويوجد بقع ذا ت الوان غريبة غامقة على الجلد مع زيادة فى نسبة المخاط على الجلد والخياشيم مع جحوز شديد فى العينين ثم عمى مصحوبا بتآكل فى الزعنفة الزيلية والذيل.

و بالفحص التشريحى للاسماك تبين أن الاسماك التى عوملت بالاوكراتوكسين-أ كان الكبد بها سهل التمزق بالضغط الخفيف عليه وذا لونه بنى باهت و الكلية تميزت باحتقان شديد وأيضا الحوصلة المرارية لوحظ بها احتقان شديد كما تميز الجزء الامامى من الكلية باللون الاحمر المشرب بالدم.

وكانت الخسائر الاقتصادية العالية التى تسبب بها الاوكراتوكسين للاسماك فى صورة زيادة فى نسبة ومعدلات النفوق للاسماك التى عوملت بالاوكراتوكسين عنها فى الاسماك التى

لم تعامل بالاوكراتوكسين حيث بلغت نسبة النفوق 67.5 % فى المجموعة التى عوملت بالاوكراتوكسين ولكن فى المجموعات التى عوملت بالاوكراتوكسين والبيوجين انخفضت نسبة النفوق بها الى 46.25 % كما لوحظ نقص فى وزن الجسم ومعدل الزيادة فى وزن الجسم والعائد الاقتصادى فى المجموعات التى عوملت بالاوكراتوكسين عنها فى المجموعات التى لم تعامل بالاوكراتوكسين أو التى عوملت بالاوكراتوكسين وأضيف لها البيوجين حيث لوحظ أن البيوجين يحسن من معدل الزيادة فى الوزن بمعدل 0.35و 0.63و 0.46 و 0.16 و 0.02 و 0.05 و 0.29 و 0.6 كجم للمجموعات التى عوملت بالبيوجين والاوكراتوكسين عن المجموعة التى عوملت بالاوكرتوكسين فقط وكان العائد لهذه المجموعة 2.45 و 4.41 و 3.22 و 1.12 و 1.14 و 0.35 و 2.03 و 4.20 جنيه على التوالى زيادة عن المجموعة التى عوملت بالاوكراتوكسين فقط وذلك للاسابيع من الاول الى الثامن على التوالى.

لذلك يمكن أن نستخلص من تلك الدراسة أن الاوكراتوكسينات تعتبر من أحد اللسموم الفطرية عالية السمية بالنسبة للاسماك ,واكثرها خطورة اقتصادية على مزارع الاسماك حيث يؤدى الى زيادة فى نسبة النفوق ونقص فى الوزن ونقص معدل الزيادة فى وزن الاسماك وبالتالى نقص فى العائد الاقتصادى لمزارع الاسماك , وان اضافة البيوجين يمكن أن يحسن من العائد الاقتصادى لمزارع الاسماك ويقلل من خطورة الاوكراتوكسين على مزارع انتاج الاسماك.

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