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Hazard Effect Of Ochratoxin On Tilapia Fish And Ways For Reduction.

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ABSTRACT

This study was done to show the positive effect of spices on reduction of ochratoxin toxicity in nile tilapia as (Black seed, Basil , Curcuma and dried ginger) are added to ochratoxic (600 µ kg-1) diet for Nile tilapia fingerlings. These diets were offered daily at 3-5% daily of actual biomass in glass aquaria in 3 replicate per treatment in an indoor feeding experiment lasted in 12 weeks. The ochratoxic-A diet gives the worst results in all growth parameters and in blood biochemical tests of experimented fish. Species reduce ochratoxicosis symptoms in fish, since improved all the above tested parameters of ochratoxicated fish. Generally, the obtained results in the present study indicated that Basil was the best detoxifying agent of ochratoxin-A, then Black seed followed by curcuma and finally the dried ginger respectively.

Keywords: Nile tilapia**,** Ochratoxin-A, growth performance, Biochemical tests, , Species.

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INTRODUCTION

Ochratoxin produced by Aspergillus ochraceus , A. niger and Pencillium (Anli and Alkis, 2010).The health hazardous of ochratoxin A (OTA) in human includes hepatotoxic, carcinogenic, teratogenic, genotoxic, nephrotoxic and immunosuppressive. In addition, it may be implicated in human disease Balkan Endemic Nephropathy (BEN) and in the development of urinary tract tumors (Toscani et al., 2007; Anli and Alkis, 2010).

Nile tilapia, O. niloticus (L.) is an important species for freshwater aquaculture. Improving fish performance and disease resistance of cultured organisms are major challenges facing fish culturists. Moreover, bacterial diseases are one of the limiting factors for fish culture including Nile tilapia (Rahman *et al*., 1997, 2001 and Li *et al*., 2006).

Spices are the main sources of natural antioxidants and antimicrobial compounds. A large number of plants have been used in traditional medicine for the treatment and control of several diseases. The present article gives an idea about the use of medicinal plants in aquaculture.

Attempts to use the natural materials such as Species could be widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance Levic *et al*., (2008).

MATERIALS AND METHODS

This study was conducted in the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt to evaluate the ability of natural products to detoxify the drastic effects of OCTA on the Nile tilapia for 12 weeks. The first group was the control (T1), OCTA (T2), Black seed T3), Basil (T4), curcuma (T5) and dried ginger (T6) three replicates have been used for each treatment.

A group of 180 Nile tilapia O. niloticus fingerlings (obtained from Abbassa hatchery), with average initial body weight of 8.0g were used in this study. Fish were maintained in the aquaria for 2 weeks before beginning of experiment for acclimization purpose. The fish in all experiments were distributed into the aquaria at stocking rate of 10 fish per aquarium. The experimental treatments were tested in 3 aquaria for each. The experiment continued for 12 weeks. During the experimental period the fish were fed the experimental diets at rate of 3% for the first six weeks, the feeding rate was 5% of live body weight daily till the end of the experiment. The diets were given twice daily, the amount of the feed was adjusted bi-weekly based on actual body weight changes. At the end of experiment fish were taken for chemical analysis . Body weight of individual fish was measured biweekly to point feed quantity and to calculate growth performance and feed utilization.

Average weight gain (g/fish) AWG= Average final weight -Average initial weight (g).

Average daily gain (g/fish/day) DG= AWG (g) / Experimental period (days).
Feed conversion ratio (FCR)= feed intake (g) / live weight gain (g).
Relative growth rate (RGR) = [(W2 – W1) / W1] x 100.
Where W2 = average final body weight (g), W1 = average initial body weight (g).

A basal diet kg-1 (fish meal (120 g), soybean (430 g), wheat bran (209 g) and yellow corn (180.30 g), corn flour (15 g), corn oil (40 g), fish oil (30.30 g) also vitamins and mineral salt were added (30 g). The chemical composition of the diet was crude protein 30.19%, ether extract 7.18%, crud fiber 5.1% and gross energy 444.97 kcal kg-1. The basal diet was considered as a control (T1). They were milled and ochratoxin-A was added at concentration 600 µ kg-1 to all diets (T2, T3, T4, T5 and T6), except control (T1). Anti-toxin was added for all treatments 10g /kg feed.

Chemical analysis of diets and whole–fish body from each treatment at the beginning and at the end of experiment were analyezed according to the standard methods of AOAC (1990) for moisture, protein, fat and ash. Gross energy was calculated according to NRC (1993).

Ochratoxin extracted from fish feed was added to fish diet .

Blood samples were collected and transferred for centrifugation at 3500 rpm for 15 min to obtain blood plasma for determination of uric acid according to Berham (1972), aspartate aminotranseferase (AST), alanine aminotransferase (ALT) according to Varley (1976).

The obtained data were statistically analyzed by using one-way analysis of variance (ANOVA) procedure. Analysis system were done using SPSS program version SPSS (PASW)18 Statistics ver. 18.0 (SPSS, Richmond,USA) as described by Dytham,(1999). Differences between treatments were tested with Duncan's multiple range test (Duncan 1955). All data were expressed as means ± standard error. All data were expressed as means ± standard error. The significance level was set at the probability level of $P<0.05$.

RESULTS AND DISCUSSION

Growth performance:

Data presented in Table (1) showed there were no significant ($P<0.05$) differences among the initial body weights of the different dietary groups of fish. While, there were significant ($P<0.05$) differences among various group of fish concerning final body weight, AWG, ADG and relative growth rate (RGR) of experimented fish. Being the best values in favor of T6 (ochratoxin-A contaminated diet plus dried ginger), which seem even more better than the control (uncontaminated diet, T1) and significantly ($P<0.05$) better than ochratoxin-A contaminated diet (T2), ochratoxin-A contaminated diet plus Basil (T4) or curcuma (T5) and black seed (T3). Curcuma gives the lowest effect on fish growth and performance, ochratoxin-A contaminated diet gives the worst growth performance. In this context, ochratoxins and fumonisins being of particular interest, although it has to be mentioned that the extent of harm each toxin (group) can cause is highly species-dependant (Erber and Binder, 2004). Growth rates (Average ,SGR, RWG) and immunity in all treatments supplied with natural products and also reducing the ochratoxin toxicity due to their antimicrobial activity (Shalaby *et al*., 2006)

Our present study shows improving in growth (average ,SGR, RWG) and immunity in all treatments supplied with natural products and also reducing the ochratoxin toxicity due to their antimicrobial activity this agree with (Shalaby *et al*., 2006) which found herbs or spices have reported to promote various functions like growth, appetite stimulation, antistress (Citarasu, 2010), immune functions (Ergün *et al*., 2011).

Ginger contains the compound caprylic acid, which has potent antifungal properties and improving growth performance (Grzanna *et al*., 2005).Black seed, liquorice, garlic, onion, fenugreek seeds, basil seeds and roquette seeds were used in detoxification of mycotoxin and increasing growth rate (Salem et al., 2010 and El-Dakar et al., 2005).Turmeric (Curcuma longa) is an extensively used spice, food preservative and coloring material which has biological actions and medicinal applications (Burt, 2004). The active and main ingredient found in turmeric is curcumin, which was found to have antioxidant (Karami et al., 2011) and antibacterial activities (Negi *et al*., 1999). Additionally, Soni *et al*. (1997) proved the protective effect of turmeric as feed additives on aflatoxin induced mutagenecity and hepatocarcinogenicity which in turn increasing growth .

Feed utilization:

All criteria studied and presented in Table (1) showed that , T6 was the best ($p<0.05$) treatment (even than the control, T1) concerning FI, FCR and FE in tilapia fish, there is no significant difference between T1, T4 and T3 in data of FI and FE. FCR in T2 gives the worst result. Similar negative effects of ochratoxin on feed utilization parameters of tilapia fish In my study natural additives give excellent feed utilization efficiency as they reduce ochratoxin toxicity that improve feed utilization an improve feed quality this is due to their antimicrobial activity this agree with (Bassett, 2000 and Hertrampt, 2001) that haves researches suggested that some aromatic plants and their component could improve feed intake, feed conversion ratio and carcass yield. Abdelhamid , (2010) who showed that the diet containing Alpinia as a medicinal plant produced the best FCR parameters compared with other medicinal plant sources (Ginger , Cresson and Lpecdcuanha) .

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Table 1: Growth performance and feed utilization of experimented tilapia fish as affected by dietary treatments for 12 weeks.

Treatments.	T1	T2	T3	T4	T5	T6
Initial wt (g)	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00	8.0±0.00
Final wt (g)	39.36^{e}±1.17	35.31^{f}±0.06	47.01^{c}±0.51	48.86^{b}±0.43	44.08^{d}±0.51	49.71^{a}±0.13
Weight gain (g)	31.36^{e}±1.16	27.32^{f}±0.05	38.91^{c}±0.50	40.79^{b} ±0.43	36.35^{d}±0.50	41.71^{a}±0.13
Daily weight gain(g/fish/d)	0.38^{e}±0.010	0.33^{f}±0.001	0.47^{c}±0.010	0.49^{b}±0.01	0.43^{d}±0.010	0.50^{a}±0.010
Feed Intake	50.22^{e}±0.70	45.81^{f}±0.03	54.11^{b}±0.72	53.04^{c}±0.25	51.95^{d}±0.30	55.30^{a}±0.08
FE	62.33^{e}±3.32	56.76^{f}±0.47	71.92^{c}±0.73	76.92^{b}±1.15	69.96^{d}±1.67	78.32^{a}±0.41
FCR	1.61^{b}±0.02	1.76^{a}±0.01	1.39^{d}±0.01	1.30^{e}±0.01	1.43^{c}±0.01	1.28^{f}±0.01

Means followed by the same superscript in the same row are not significantly different according to Duncan's multiple range test (P<0.05).

T1= control diet.
T2= diet1 + OCTA (600 µ kg-1).
T3= diet1 + OCTA (600 µ kg^{-1}) +Black seed (10g kg^{-1}).
*T4= diet1 + OCTA (600 µ kg^{-1}) +*Basil *(10g kg^{-1}).* .
T5= diet1 + OCTA (600 µ kg^{-1}) + curcuma *(10g kg^{-1}).*
T6= diet1 + OCTA (600 µ kg^{-1}) + dried ginger(10g kg^{-1}).

Chemical analysis of the whole body:

Data of the proximate chemical analysis of whole body of the tested tilapia fish are given in Table (2).. The highest CP and lowest EE were determined in (T6), which is better than control (T1). The best treatment (dried ginger) show differ significantly (P<0.05) in ash content of fish than in the other dietary treatments. Abdelhamid *et al*., (2012). indicated that proximate analysis of the fish, the dry matter (DM) and crude protein (CP) percentages increased but the ether extract (EE) and or ash contents decreased comparing with the analysis before the start of the experiment. However, the dietary inclusion of guava or camphor leaves meal significantly (P≤0.05) increased each of CP, EE and energy content (EC) of the fish body comparing with the control. Yet, DM and ash contents did not reflect any significant (P≥0.05) alteration due to the dietary additive.

Table 2: Proximate chemical analysis of the whole tilapia body as affected by the experimental diets

		% on Dry matter basis		
Treat.	Moisture %	Ash	Ether Extract	CP
T1	73.07^{b}±0.44	16.2^{b}±0.10	17.58^{b}±0.11	57.6^{ab}±0.24
T2	73.96^{a}±0.22	16.77^{a}±0.06	18.62^{a}±0.13	60.79^{ab}±0.20
T3	73.08^{b}± 0.01	16.15^{c} ± 0.09	17.58^{b} ± 0.09	60.94^{a} ± 0.01
T4	73.12^{ab} ±0.01	16.12^{c}± 0.09	17.53^{b} ± 0.07	60.9^{a} ± 0.02
T5	73.45^{ab} ± 0.01	16.2^{b} ± 0.07	17.68^{a} ± 0.05	60.7^{ab} ± 0.01
T6	73.8^{ab} ± 0.06	16.21^{b} ± 0.05	17.73^{c} ± 0.09	60.6^{ab} ± 0.02

Means (in the same column) superscripted with different letters significantly different (P<0.05) differ.

Abdelhamid *et al*. (2004) found that aflatoxin B1 significantly reduced DM and CP contents of the *O. niloticus* fish carcass but, it significantly increased EE and ash contents of the fish. Meanwhile, they added that dietary addition of clay or egg shell and shrimp wastes to the AFB1 including diets improved this picture In accordance with the present findings, Abdelhamid *et al*. (2002) reported that the aflatoxic diets significantly (P<0.01) reduced the fish flesh crude protein content but increased its fat and ash contents proportional to the dietary levels of the aflatoxin.

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Blood biochemistry:

Liver function indicators were studied in the tested fish plasma Data of these plasma biochemical criteria are given in Table (3). All toxic diets with different additives (T3, T4, T5 and T6) also control (T1) used decreased the values of tested parameters [aspartate transaminase (AST), alanine transaminase (ALT)] than in the toxic diet without additives (T2). In same trend, OCTA increases uric acid and creatnine similar effects of OCTA on blood parameters of tilapia fish were recorded also Talpur *et al.*, (2013). showed that the hemoglobin content was significantly higher in treated groups with ginger than the control. Also, there was a consistent increase in Hct % in ginger fed groups attributed as a result of immunostimulatory effect of ginger and slight decrease in Hct % in post-challenge, which may be impairing the osmoregulation owing to pathogenic stress, these findings totally agree with present study.Dorucu *et al*. , (2009). who reported significant increase in serum protein and total immunoglobulin levels in rainbow trout fed with basal diet incorporated with 1, 2.5 and 5% annual flowering plant, N. sativa (Black seed) compared to the control group. Saied and Al Al-Nasry (2010)who reported that dietary supplementation of Curcuma increased serum total protein, and decreased serum total triglycerides and cholesterol compared to control. Zolfaghari and Firouzbakhsh (2013) stated that adding basil aqueous extract to rainbow trout diet may improve the positive physiological characteristics.

Table 3: Plasma biochemical parameters of *O. niloticus* at the end of the experiment as affected by experimental diets

	AST(IU)	ALT(IU)	Creatinine (mg/100ml)	Uric acid (mg/100ml)
T1	**26.46c±0.49**	**19.86f ± 0.2**	**0.33c ± 0.02**	**1.36d ± 0.02**
T2	**34.94a±3.78**	**34.58a ± 0.2**	**0.68a±0. 2**	**1.88a ± 0.02**
T3	**15.5d ± 0.2**	**17.3d ± 0.2**	**0.27d ± 0.6**	**1.29e ± 0.02**
T4	**14.73e ± 0.3**	**15.35e ± 0.1**	**0.25 d ± 0. 2**	**1.28e ± 0.09**
T5	**24.31d ± 0.3**	**27.05c ± 0.5**	**0.55b ± 0. 3**	**1.56c ± 0.02**
T6	**29.57b ± 0.3**	**28.81b ± 0.3**	**0.54b ± 0. 1**	**1.63b ± 0.09**

Means followed by the same superscript in the same column are not significantly different according to Duncan's multiple range test (P<0.05).

Zotti *et al*.,(2008) the increase of creatinine and uric acid in serum of ochratoxicosis fish may be attributed to renal disturbance associated with damage of proximal tubules and thickening of the glomerular basement membrane caused by OCTA which lead to reduce the ability of kidney to produce concentrated urine (Marquadret, 1996).

Table 4: Total protein, Albumin and Globulin of *O. niloticus* at the end of the experiment as affected by experimental diets

	Total protein (g/100ml)	Albumin (g/100ml)	Globulin (g/ml)
T1	**1.53b ± 0.02**	**1.59^{c}±0.13**	**1.34^{ab}±0.15**
T2	**1.32c ± 0.02**	**1.80^{a}±0.08**	**1.10^{b}±0.07**
T3	**1.55b ± 0.01**	**1.60^{b}±0.03**	**1.56^{a}±0.05**
T4	**1.61a ± 0.09**	**1.63^{b}±0.15**	**1.34^{ab}±0.23**
T5	**1.36c ± 0.09**	**1.62^{b}±0.05**	**1.35^{ab}±0.07**
T6	**1.36c ± 0.09**	**1.54^{c}±0.06**	**1.42^{a}±0.02**

Means followed by the same superscript in the same column are not significantly different according to Duncan's multiple range test (P<0.05).

CONCLUSIONS

Ochratoxin has hazard effect on fish growth and performance. It could be recommended for the beneficial using of Species specially Ginger and to reduce the toxic effects of OCTA contaminated fish diets.

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