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DIETARY *Nigella sativa* AND YEAST CELL WALL FOR REDUCING THE TOXICITY OF OCHRATOXIN A IN CULTURED NILE TILAPIA IN EGYPT.

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ABSTRACT

This study was conducted to investigate the toxic effects of Ochratoxin A (OTA) on mono-sex Nile tilapia *Oreochromis niloticus* in a feeding trial for 8 weeks and attempting to detoxify these drastic effects by using some dietary supplements. One percent of each of these supplements (yeast cell wall, and /or *Nigella sativa*) was added to 5mg OTA diet for fingerlings. The OTA contaminated diets significantly ($P<0.05$) decreased growth performance (live body weight, body weight gain and relative growth rate) and some tested blood parameters (total protein, albumin, globulin), but uric acid, creatinine and mortality rate were significantly increased by OTA. Either evaluated supplements significantly improved growth performance, blood parameters and mortality rate which negatively affected by OTA. The best results obtained with yeast cell wall plus *Nigella sativa* followed by *Nigella sativa and* yeast cell wall, respectively. The economic efficiency followed the same trend. It may be concluded that the tested supplements have the ability to alleviate the toxicity of OTA and improve the economic efficiency of fish.

Keywords: Ochratoxin A, Nile tilapia, cell wall of *Saccharomyces cerevisiae*, *Nigella sativa*

INTRODUCTION

Ochratoxin A (OTA) is a secondary metabolite produced mainly by Penicillium verrucosum in temperate climates and Aspergillus ochraceus and the rare Aspergillus carbonarius in warm and tropical regions, (EFSA, 2004 and Var *et al.*, 2009). This mycotoxin occurs in several parts of the world, contaminating different plant products, including cereals, coffee beans, nuts, cocoa, pulses, beer, wine, spices, and dried vine fruits (Mbarek *et al.*, 2007). OTA is a well-known nephrotoxic agent and has been associated with fatal human kidney disease, referred to as Balkan Endemic Nephropathy and with an increased incidence of tumors of the upper urinarytract (FAO/WHO, 2001). Several strategies have been investigated for lowering the ochratoxin content in agricultural products. These strategies can be classified into three main categories: prevention of ochratoxins contamination, decontamination or detoxification of foods contaminated with ochratoxins, and inhibition of the absorption of consumed ochratoxins in the gastrointestinal tract (Janos *et al.*, 2010).

The addition of mycotoxin binders to contaminated diets has been considered the most promising dietary approach to reduce effects of mycotoxins (Galvano *et al.*, 2001). The theory is that the binder decontaminates mycotoxins in the feed by binding them strongly enough to prevent toxic interactions with the consuming animal and to prevent

mycotoxin absorption across the digestive tract. Therefore, this approach is seen as prevention rather than therapy. Potential absorbent materials include activated carbon, aluminosilicates (clay, bentonite, montmorillonite, zeolite, phyllosilicates, etc.), complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, peptidoglycans, and others), and synthetic polymers such as cholestryamine and polyvinylpyrrolidone and derivatives.

Some scientific efforts were conducted to use dietary supplements which detoxify the drastic effects of aflatoxins on some animals such as, glucomannan (Karaman *et al.*, 2005), yeast cell wall mannanoligosaccaride (MOS) (Devegowda *et al.*, 1998), or Saccharomyces cerevisiae which were found to have beneficial effects during mycotoxicosis (Raju and Devegowda 2000), as well as chamomile (Abdelhamid *et al.*, 1985; Soliman and Badeaa 2002 and Ibrahim, 2004), and ginger (Vimala *et al.*, 1999 and Abdelhamid *et al.*, 2002).

Nigella sativa (N. sativa) seed, called as 'Black Seed' in English language, 'Al-Habba Al-Sauda' or ' Habba Al-Barakah' in Arabic is well known in the Middle East, Middle Asia and Far East. Proximate analysis of Nigella sativa seeds showed that its carbohydrates content ranged 23.5-33.2%, crude protein 20-27% and lipids 34.5-38.7% (Babyan *et al.*, 1978; Abdel-Aal and Attia, 1993; Hedaya, 1996 and Salem, 2001).

Most properties of Nigella sativa seeds are mainly attributed to quinone constituent compound. Thymoquinone, is the active quinone constituent of Nigella sativa seeds, which possesses therapeutic effects, such as antioxidant, anti- inflammatory, anticancer, antihistaminic (Kanter *et al.*, 2006), antibacterial effects (Abdel -Fattah *et al.*, 2000; Morsi, 2000 and Fahrettin *et al.*, 2008). Additionally, it has been shown that Nigella sativa has protective effect against ischemia reperfusion injury to various organs (El-Abhar *et al.*, 2003 and Bayrak *et al.*, 2008).

Elimination or reduction of the ochratoxin –producing fungi in grains is not always successful, particularly during the pre-harvest period. In turn, control of the established ochratoxicosis is of great importance and is a chief goal for many investigators. Therefore, the present study was designed to explore the effect of dietary ochratoxin on some hematological and biochemical biomarkers in Nile tilapia (Oreochromis niloticus) and the probable ameliorative effect of yeast cell wall and /or Nigella sativa on the toxicity of ochratoxin.

MATERIALS AND METHODS

The experimental work was carried out in the Aquaculture Research Lab., Abssa, Abo-Hamad, and Regional Center for Foods and Feeds, Agric. Res. Center, Ministry of Agric., Giza, Egypt. Five experimental groups were designed as follows, the 1st group fed basal diet (BD), the 2nd group fed BD with OTA (5mg/kg), the other groups (3-5) fed BD with OTA plus 1% yeast cell wall, 1% *Nigella sativa*, 1% yeast cell wall plus 1% *Nigella sativa*, respectively. Commercial pelleted diet product of the General Authority for Fish Resources Development. It consisted of fish meal, soybean meal, meat

meal, yellow corn, bone feedmill and a mixture of vitamins and minerals. The chemical composition of basal diet was determined according to A.O.A.C. (1980).

Healthy Nile tilapia fingerlings were kindly supplied from the fish hatchery of Aquaculture Research Lab., Abssa, Abo-Hamad. NS and yeast cell wall were purchased from local market, crushed then added to a ground commercial diet which was pelleted again.

The basal diet contained (on dry matter basis) 80.00, 29.00, 6.50, 4.93, 39.57, and 20.00% for OM, CP, CF, EE, NFE and ash, respectively. In each group, a total number of 30 fish (average body weight 10.05±0.10g) was used in 3 replicates glass aquaria (per treatment) of 10 Nile tilapia *Oreochromis niloticus* per aquarium. The dimensions of each aquarium were 150×150×150cm, these aquaria were supplied with dechlorinated tap water up to 80% of its highest and continuous aeration was adopted using an air pump and airstones. Fish wastes were filtered by siphon method each day and the water was completely changed every 3 days. Water temperature ranged from 25 to 27C. The fish were fed 2 times a day (900-and 1600h) at a rate of 3% of the total body weight. The fish were weighted every 2 weeks for 8 weeks. At the end of the experiment, blood samples were taken from the caudal vein of 6 fish for each treatment (2 fish /replicate). Serum was separated and stored at -20˚C for analysis for total protein, albumin, uric acid, creatinine using commercial kits from Diamond Diagnostics Company, Egypt.

OTA standard was obtained from Sigma-Aldrich (USA) as a crystalline powder form. Data of the trial were statically analyzed using the General Linear Model Program of SAS (1996).

RESULTS AND DISCUSSION

Nile tilapia *Oreochromis niloticus* may represent a sensitive model for mycotoxicosis, sience this fish is extremely vulnerable to toxic effects from various chemicals and poisons including aflatoxins B_1 (AFB_1).

1- Growth performance:

Data presented in Table (1) show that, ochratoxin A had significantly ($P<0.05$) negative effects on growth performance (live body weight, body weight gain and relative growth rate). These results agree with the findings of Santin *et al.* (2003) who reported that ochratoxin in diet significantly decreased feed intake and weight gain as compared to the control group. Each of the two supplement (yeast cell wall, *Nigella sativa*) had improving effect on body weight, body weight gain, feed consumption, feed conversion ratio, and weight gain. The cell wall of yeast is normally constituted of mannan oligosaccharides which have been showed improve in feed conversion of birds in some reports (Savage and Zakrzewska, 1997 and Fritts and Waldroup, 2003).

Most properties of *Nigella sativa* seeds are mainly attributed to quinone constituent. Quinonic alkaloids (thymoquinone) are likely to be involved in pharmaceutical properties (Daba and Abdel-Rahman, 1998 and Mansour *et al.*, 2001). It has been suggested that thymoquinone may act as an antioxidant agent and prevent membrane lipid peroxidation in tissues

(Mansour *et al.*, 2002). Also, hymoquinone inhibits bacteria and improves body function and performance. Fat soluble unidentified factors and essential fatty & amino acids display an essential role in growth performance, several macro and micro elements are responsible for regulating all vital functions in the body and improves the immunity and vitamins which have essential role in growth performance (thiamin, riboflavin, pyridoxine and niacin) as reported by various authors (Mohan *et al.*, 1996; William, 1999; Seleem and Riad, 2005 and Seleem *et al.*, 2007). Also, this improvement may be due to its contents which regulate digestion and absorption and fight the internal parasities (Nasr *et al.*,1996; Medenice *et al.*, 1997; Abdel-Azzem *et al.*, 1999 and Abd El-Hakim *et al.*, 2002).

Table (1): Effect of dietary OTA and addition of *Nigella sativa* (NS) and cell wall of *Saccharomyces cerevisiae* (CWSC) on Nile tilapia performance (Means±Sd)

Item	week	Treatment				
		Control	OTA	1%CWSC	1%NS	1%CWSC plus 1%NS
Live body Weight (g)	Initial	10.05±0.14	10.10±0.14	10.05±0.14	10.10±0.14	10.15±0.14
	2	11.22±0.14a	10.57±0.14b	11.08±0.14a	11.15±0.14a	11.04±0.14a
	4	13.03±0.17a	11.63±0.17b	12.58±0.17a	12.58±0.17a	12.79±0.17a
	6	15.22±0.19a	11.56±0.19c	13.88±0.19b	14.60±0.19a	14.78±0.19a
	8	17.77±0.14a	11.69±0.14d	14.87±0.14c	15.67±0.14b	15.92±0.14b
Body weight gain (g/2 weeks)	2	1.17±0.03a	0.47±0.03d	1.03±0.03b	1.05±0.03b	0.89±0.03c
	4	1.81±0.13a	1.06±0.13b	1.50±0.13a	1.43±0.13ab	1.75±0.13a
	6	2.19±0.31a	-0.07±0.31b	1.30±0.31a	2.02±0.31a	1.99±0.31a
	8	2.55±0.08a	0.13±0.08c	0.99±0.08b	1.04±0.08b	1.14±0.08b
	Average	1.93±0.14a	0.40±0.14c	1.20±0.14b	1.39±0.14b	1.44±0.14b
Relative growth rate %	2	11.64±0.37a	4.65±0.37d	10.26±0.37b	10.41±0.37b	8.70±0.37c
	4	16.10±1.17a	10.02±1.17b	13.52±1.17ab	12.85±1.17ab	15.80±1.17a
	6	16.81±2.58a	-0.60±2.58b	10.37±2.58a	16.13±2.58a	15.77±2.58a
	8	16.78±0.65a	1.13±0.65c	07.11±0.65b	7.15±0.65b	7.75±0.65b
	Average	15.34±1.15a	3.80±1.15c	10.32±1.15b	11.63±1.15b	12.02±1.15b
Surviving rate %		90.00±5.80a	70.0±5.8b	80.00±5.8ab	80.00±5.8ab	90.00±5.8a

a, b, c, d: Means in the same row bearing different litters significantly (P<0.05) differ.
Body weight gain= Final weight – Initial weight
Relative growth rate= Gain/ Initial weightX100

2- Blood parameters:

Total protein and albumin concentrations were significantly (P<0.05) decreased in fish fed Ochratoxin A contaminated diet (Table 2). In addition, yeast cell wall and *Nigella sativa* could partially counteract this decrease, but did not raise the protein levels back to the normal value. The hypoproteinemia and hypoalbuminemia may be attributed to three main causes: hepatic insufficiency, renal loss (protein-losing nephropathy), and gastrointestinal loss (protein-losing enteropathy) (Carlye-Rose, 2002). Moreover, OTA is found to be hepatotoxic and nephrotoxic (Saad, 2002), and increases the permeability of gastrointestinal tract (McLaughlin *et al.,* 2004) which explain the decrease of total protein and albumin with OTA treatments in the present study. Also, this finding was agreed with results obtained by

Coles, (1986) and Khalil (1998). This reduction may explain the inhibitory effect of OTA to protein synthesis (Ringot *et al*., 2006). The results showed significant (P<0.05) decrease of globulin with fish fed Ochratoxin A contaminated diet. There was significant (P<0.05) improvement with yeast and *Nigella sativa*. Globulin which is the building source of antibodies, which called immunoglobulin (White, 1986). So, globulin used as immune indicator and the decrease of its level in the present study with OTA treatments revealed the immunosuppressive effects of OTA. Elkafoury, (2006) reported an increase in fish serum proteins (total protein, albumin, globulin and A/G ratio) received yeast with diet.

Concentrations of creatinine and uric acid in serum of fish fed Ochratoxin A contaminated diet significantly (P<0.05) increased. These results agreed with the results obtained by Mansour *et al*., (2011). The increase of creatinine and uric acid in serum of ochratoxicosis fish may be attributed to renal disturbances associated with damage of proximal tubules and thickening of the glomerular basement membrane caused by OTA which lead to reduce the ability of kidney to produce concentrated urine (Marquadret, 1996). Moreover, kidney is the main target organ of OTA genotoxicity, where induced DNA single-strand breaks and DNA adducts in kidney (Pfohl-Leszkowicz *et al.,* 1993 and Hosseinzadeh *et al.,* 2007*).*

Table (2): Effect of dietary OTA and addition of *Nigella sativa* (NS) and cell wall of *Saccharomyces cerevisiae* (CWSC) on serum parameters of fish

Item	Treatment				
	control	OTA	1%CWSC	1%NS	%CWSC plus %NS
Total protein (g/dl)	4.07±0.01**a**	2.95±0.01**e**	3.60±0.01**d**	3.94±0.01**c**	3.99±0.01**b**
Index	100	72.48	88.45	96.81	97.79
Albumin (g/dl)	3.10±0.006**a**	2.40±0.006**c**	2.90±0.006**b**	3.11±0.006**a**	3.11±0.006**a**
Index	100	77.42	93.55	100.32	100.32
Globulin (g/dl)	0.97±0.009**a**	0.55±0..009**e**	0.70±0.009**d**	0.83±0.009**c**	0.87±0.009**b**
Index	100	56.70	72.16	85.57	89.69
Uric acid (mg/dl)	2.29±0.006**e**	3.48±0.006**a**	3.22±0.006**b**	3.11±0.006**c**	3.01±0.006**d**
Index	100	151.97	140.61	135.81	131.44
Creatinine (mg/dl)	1.13±0.006**d**	1.33±0.006**a**	1.23±0.006**b**	1.19±0.006**c**	1.18±0.06**c**
Index	100	117.70	108.85	105.31	104.42

3- Survival rate:

Ochratoxin might cause significant losses to Nile tilapia due to reduced performance and health problems in the exposed tilapia as was observed in the present study. The surviving rate in (Table 1) was significantly (P<0.05) decreased when fish fed Ochratoxin A contaminated died in comparison with control.

4- Economic efficiency:

The results in Table (3) showed that ochratoxin in diet decreased the feed intake and weight gain as compared to the control group. Tilapia exposed to ochratoxin had lower average feed intake and weight gain, these results agreed with the results obtained by Santin *et al*. (2003*).* All additives improved the economical efficiency which negatively affected by OTA. The

best improvement occurred with CWSC +NS followed by NS and CWSC, respectively.

It may be concluded that natural materials have the ability to alleviate the toxic effect of OTA and improve the economical efficiency of fish. The present study suggests that the yeast cell wall plus *Nigella Sativa* used in this investigation enhances fish tolerance to environmental stress and reduces ochratoxin toxicity.

Table (3) : Effect of dietary OTA and addition of *Nigella sativa* (NS) and cell wall of *Saccharomyces cerevisiae* (CWSC) on economical efficiency by fish.

Item	Treatment				
	control	OTA	1%CWSC	1%NS	%CWSC plus %NS
Total gain (g)[1]	7.72	1.59	4.82	5.54	5.77
Total feed intake (g)[2]	23.37	18.31	20.93	21.62	21.91
Total feed cost (piaster)[3]	5.84	4.58	5.65	5.66	6.18
Selling price (piaster)[4]	9.26	1.91	5.78	6.65	6.92
Net revenue (piaster)[5]	3.42	-2.67	0.13	0.99	0.74
Relative revenue (%) [6]	100	-78.07	3.80	28.95	21.64

1= final weight-initial weight.
2= final weight-initial weight /2X0.03X56 (8weeks).
3=total feed intake X price (price of 1 kg diet was 250,250,270, 262 and 282 piastres (pt)(price 2011). One kg of cell wall of *Saccharomyces cerevisiae* cost 2000 pt. *Nigella sativa* cost 1200 pt.
4= total gain X 1.2 (one kg 1200 *pt)*
5= selling price- feed cost
6= net revenue for treatment/ net revenue of control X100

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تقليل سمية الأوكراتوكسين أ عن طريق استخدام حبة البركة و جدر خلايا الخميرة الجافة في علائق السمك البلطي النيلي المستزرع في مصر

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اجريت تجربة لدراسة الأضرار الناتجة عن التسمم الأوكراتوكسيني بتغذية أسماك البلطي النيلي على عليقة ملوثة لمدة ٨ أسابيع ، و محاولة تقليل هذه الأضرار باستخدام بعض الإضافات و التي تشمل ١٪ من جدر خلايا الخميرة و/ أو حبة البركة للعليقة المضاف إليها ٥ ملليجرام سم الأوكراتوكسين/كم علف . أدت العليقة الملوثة بسم الأوكراتوكسين الى نقص معنوي في معدلات اداء الأسماك (وزن الجسم الحي، عائد وزن الجسم، معدل النمو) ، أيضا بعض قياسات الدم (البروتين الكلي، الألبيومين، جلوبيولين) و زيادة معنوية في (حامض اليوريك، الكرياتنين و معدلات النفوق) .حسنت المواد المضافة من كفاءة النمو و قياسات الدم ، وقللت من معدلات النفوق الناتجة من التأثير السلبي للتغذية على سم الأوكراتوكسين. أفضل النتائج تم التحصل عليها عند التغذية على عليقة مضاف إليها جدر الخميرة الجافة مع كسب حبة البركة ، يليها المجموعات المفذاة على حبة البركة فقط . ثم جدر الخميرة الجافة ، و يأخذ العائد الاقتصادي نفس هذا الإتجاه.

يستخلص من هذه الدراسة أن المواد المضافة المختبرة لها القدرة على تقليل الأثر السام للأوكراتوكسين أ و تحسين الكفاءة الاقتصادية للسمك.

قام بتحكيم البحث

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