Ameliorate the Drastic Effect of Ochratoxin A by using Yeast and Whey in Cultured Oreochromus niloticus in Egypt.

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Abstract: Ochratoxin A is one of the most important mycotoxins in fish feed. In the present study the effects of OTA on cultured *Oreochromus niloticus* were evaluated. Trials for ameliorate the drastic effect of OTA were done by using active life yeast and whey. The results indicted that significant ($p<0.05$) decrease in RBCS, WBCS, phagocytic activity and phagocytic index were occurred in both levels of OTA. Hypoalbuminemia, hypoproteinemia, decrease of globulin, and antibody titer as well as increase of liver enzymes, creatinine and uric acid were noticed. The histopathological examination showed that OTA caused diffuse hydropic degeneration and advanced fatty changes in liver. Tubular necrosis and hydropic degeneration of the kidneys were observed .The activation of melano macrophage centers (MMCs) were recorded. The results proved that OTA produce serious physiological, immunological and pathological effects on, *O.niloticus*. Morovere active life yeast and whey were succeed to neutralize the drastic toxic effects of OTA.

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1. Introduction

Ochratoxin is a group of secondary metabolites produced by fungi of two genera: *Penicillium* and *Aspergillus*, this group include Ochratoxin A; Ochratoxin B; Ochratoxin C; Ochratoxin α, and the most toxic member is Ochratoxin A (OTA) (Ringot *et al.,* 2006).

Manning *et al.,* (2005) indicated that juvenile channel catfish fed OTA had greater mortality when challenged with *Edwardsiella ictaluri* compared with control group. Saad (2002) reported that OTA has immunosuppressive effect on *O. niloticus* and Common carp in acute (50 µg/kg fish) and chronic toxicity (10 µg/kg fish).

The role of microorganisms on detoxification of OTA has a lot of concern because they promote the hydrolysis of OTA to its nontoxic form (Ochratoxin α (OTα)) in case of ruminant (Sreemannarayana *et al.,* 1988) and non ruminant (Madhyastha *et al.*, 1992).

In many studies on OTA detoxification by yeast showed antagonistic effect on the production of OTA by fungi. Petersson *et al.*, (1998) showed that *Saccharomyces cerevisiae* inhibit production of toxin from *Penicillium verrucosum*. Péteri *et al.* (2007) found that yeast strain, *Phaffia rhodozyma,* degraded more than 90% of OTA in 15 days at 20°C where hydrolysis it to OTα.

Moreover, yeast enhanced immune response of treated fish (Elkafoury, 2006; Reyes-Becerril *et. al.,* 2008). Useful microflora in the intestine such as Lactobacillus and Bifidobacterial can utilize the lactose for proliferation (Naghton *et al.,* 2001).

The proliferation of this species causes increase in the acidity of intestine by producing lactic acid and short-chain fatty acids formed unsuitable environment to pathogen bacteria like *Salmonida* and *Escherichia coli* (Juven *et al.,* 1991). This competition leads to excluding harmful bacteria out of the gut (Nurmi and Rantal, 1973). Consequently digestion and absorption increased and feed utilization improved (Tellez *et al.,* 1993). No available studies conducted to investigate the effect of whey on fish.

Moreover, whey protein concentrates enhanced ex-vivo lymphoid cell proliferative responses and increased in vivo antibody production (Knowles and Gill, 2002).

The aim of the present study is to investigate the effects of OTA on cultured *O. niloticus* and attempt to ameliorate the drastic effect of OTA by using yeast and whey as diet supplementations.

2. Materials and Methods:

Apparently healthy 210 *O. niloticus* with an average body weight of 40 ±5 g/fish were used. Fish was obtained from private fish farm in Alexandria governorate and kept for 21 days in circular fiberglass tanks (800L) for acclimatization and fed on a diet contained 30 % crude protein.

Water temperature was ranged 25–27C. Continuous aeration was maintained in each tank using an electric air pumping compressor.

The 210 *O. niloticus* fish were randomly allotted in fourteen fiberglass tanks (two tanks/treatment) with fifteen fish per tank. The fish treated by Ochratoxin A (OTA) in two doses according to Saad (2002), 80 µg/kg fish as low dose (LOTA) and 160 µg/kg fish as high dose (HOTA). The OTA doses performed by stomach intubations once in day zero of the experiment in all fish groups by dissolving OTA in chloroform according to Trucksess and Pohland (2001) then dissolved in corn oil (Abdel-Wahhab *et al.,* 2005) and left to evaporate the chloroform before using. The individual stomach) intubations performed by using syringe attached with butterfly cannula to get the doses through the stomach of the fish (Abdel-Wahhab *et al*., 2005). Fish in control group which fed basal diet received 0.5 ml corn oil.

Yeast (Tonilisat®): Active live yeast (China Way Corporation, Taiwan kindly supplied by EL Zahra Vetrinary), *Saccharomyces cerevisiae*, (8 X 10^9 cells/gram) was used. The yeast added in the ration by incorporating 0.5 kg/ton ration after coating it with oil according to (Elkafoury, 2006). Fish were kept under daily observation for 8 weeks.

Whey: Whey powder (Dairy Farmers Company of America New Wilmington, PA 16142 U.S.A) free fats were used in the experiment. The whey incorporated into the diet at 14%. The whey contained 11, 62, 0.5 and 11% of Protein, Lactose, Fiber and Ash, respectively.

Seven experimental treatments were designed as follows: the basal diet (BD), BD with LOTA dose (80 µg OTA/kg fish), BD with HOTA dose (160 µg OTA/ kg fish), AY diet (0.5 g/kg diet) and LOTA dose,AY diet (0.5 g/kg diet) and HOTA dose, W diet (14% of diets) and LOTA dose and W diet (14% of diets) and HOTA dose.

Every two weeks, blood samples were taken from the caudal vasculature of - fish after anesthetized with MS222 (ten fish/treatment) for hematological assay and serum separation. Total red blood cell (RBCs), white blood cell (WBCs) were performed according to the methods of Anderson and Siwicki (1995) and Hesser (1960) respectively.

Determination of phagocytic activity and phagocytic index:

Phagocytic activity was determined according to Kawahara *et al.* (1991) and Safinaz, (2001). Phagocytosis was estimated by determining the proportion of macrophages which contained intracellular yeast cells in a random count of 300 phagocytes and expressed as percentage of phagocytic activity (PA). The number of phagocytized organisms was counted in the phagocytic cells and called phagocytic index.

Clinico-biochemical determination was used to examine total protein, albumin, globulin and albumin/globulin ratio, alkaline phosphatase, glutamic-oxaloacetic transaminase, uric acid and creatinine were done according to Saad (2002) and Safinaz (2001) by using commercial kits (Biodiagnostic , Cairo, Egypt).

Evaluation of immune response of *O. niloticus* against *Aeromona. hydrophila* bacterin.

Aeromonus hydrophila isolate was used in the bacterin preparation according to the method described by (Sakai *et al.,* 1984)

The preparation of bacterin for injection was carried out according to the method of Badran (1990). The formalin inactivated bacterin cells were mixed with an equal volume of 0.85% sterile saline. Bacterial number was adjusted to Fit MacFarlan's No. 2

At the 4th week one hundred and five *O. niloticus* fish exposed to both dose of OTA and control were inoculated intraperitoneally (IP) with 0.2 ml/fish of formalin inactivated bacterin. One hundred and five *O. niloticus* fish were similarly injected IP with 0.2 ml/fish sterile saline. After 2 weeks, the injected fish received booster dose from bacterin. After 1, 2, 3 and 4 weeks post-injection with inactivated bacterin blood collection was carried out from the caudal vasculature of inoculated fish after anesthetized with MS222 for antibody determination by microagglutination test according to the method described by Badran (1990).

Histopathological studies:

At the end of experiment specimen from kidneys, spleens and livers were removed from fish of the experimental groups and rapidly placed in adequate amount of 10% neutral buffered formalin for at least 24 hrs and used for histopathological studies according to Culling (1983).

Statistical analysis:

Statistical analysis of the experimental results was conducted according to SPSS (version 16.00). Duncan's (1955) multiple range test was carried out to test the significance levels among means of treatments.

3. Results:

The effects of OTA, yeast and whey on red blood cells (RBCs), white blood cells (WBCs) count PA and PI are demonstrated in (Table 1). The red blood cells count differ significantly (P <0.05) all over experimental period, where OTA presented severe decrease of RBCs especially with HOTA dose and showed anemia. Meanwhile, addition of yeast and whey with both OTA doses increased RBCs count and improved the body health condition.

Significant (P< 0.05) differences were observed after two weeks of treatment and showed decrease of WBCs count with LOTA and HOTA doses significantly than control group and reduced insignificantly than yeast and whey treatments all over the experimental period.

The significant (P<0.05) differences of PA were observed at week four until the end of the experiment. The PA of HOTA dose reduced significantly than other treatments. Meanwhile, insignificant (P>0.05) differences were observed among LOTA dose and detoxification treatments.

The phagocytic index differ significantly from the second week of treatment, where HOTA dose recorded the lowest significant (P<0.05) PI and showed insignificant (P> 0.05) differences with LOTA dose and HOTA dose plus whey all over the experiment. The addition of yeast ameliorate the drastic effect of OTA significant (P<0.05) on PI especially with LOTA dose. Meanwhile, slightly improve of PI observed with HOTA dose plus yeast and LOTA dose plus whey.

The significant effects of OTA, yeast and whey on total protein (Table 2) observed at week six to eight and showed significant (P<0.05) decrease of total protein with both LOTA and HOTA doses treatments. Meanwhile, the addition of yeast increased total protein values with both LOTA (significant P<0.05) and HOTA doses. Whey addition increased total protein but not significantly (P> 0.05) with both LOTA and HOTA doses.

Regarding to albumin level, significant effects was observed at week six where each OTA treatments and detoxification treatments showed significant (P> 0.05) decrease of albumin value (hypoalbuminemia) than control group although yeast and whey improved albumin levels insignificantly (P<0.05) than LOTA and HOTA doses.

Insignificant (P> 0.05) decrease of globulin with LOTA and HOTA doses and increased in case of yeast and whey until sixth week were found. Meanwhile, at eighth week globulin decrease significant (P<0.05) with LOTA dose and HOTA dose.

The results of antibody titer of *O. niloticus* after vaccination with *A. hydrphila* and exposed to OTA and detoxification agents (yeast and whey) were 4, 2.67±0.33, 2±0.00, 3.33±0.33, 3±0.00, 3.67±0.33 and 3.00±0.00 in case of control, LOTA, HOTA, LOTA plus yeast, HOTA plus yeast, LOTA plus whey and HOTA plus whey respectively. The results indicated that significant (P> 0.05) differences were observed among other treatments and control group. However, the addition of yeast and whey to the diet increased significantly antibody titare.

Data presented in (Table 3) showed the effect of OTA, yeast and whey on the liver and kidneys function. Significant differences of GOT were observed at the sixth week of treatment where GOT values with LOTA and HOTA doses increased significantly than control and yeast supplementation treatments.

In the same time the levels of liver enzymes in case of OTA plus yeast were less than OTA only.

The results of creatinine and uric acid showed increase especially with HOTA dose than other treatments. Moreover, the addition of yeast and whey decreased creatinine and uric acid levels especially with LOTA dose.

The histopathological examination in the present study showed that LOTA dose after 8 weeks from treatment caused diffuse hydropic degeneration of hepatic cells, congestion of hepatic sinusoids and mild incidence of melanomacrophage centers (MMCs). Moreover, the posterior kidney showed mild acute cellular swelling and MMCs activationwere observed in kidney and spleen (Fig. 1, 2 and 3).

In case of HOTA dose diffuse advanced fatty changes appeared as Signet ring, atrophied of hepatic cells and activation of MMCs in pancreatic islets were recorded. In kidney, infiltration and activation of MMCs and acute tubular necrosis were recorded. Severe infiltration of MMCs to extent that total replaced of the splenic tissues were also noticed (Fig. 4, 5 and 6).

Regarding to addition of yeast to diets the drastic effects of OTA on hepatopancreas and kidneys in LOTA treatment were similar as control.

HOTA dose showed acute cloudy swelling, tubular necrosis and mild activation of MMCs. Also spleen in LOTA dose didn't affected but in HOTA dose spleen showed mild activation of MMCs (Fig. 7, 8 and 9).

Addition of whey to fish diets with OTA showed mild congestion and hydropic degeneration of liver cells in case of LOTA. Meanwhile, with HOTA dose the alteration appeared as mild fatty changes, focal lymphocytic aggregation, enlargement and hyper activation of MMCs. Kidney in LOTA dose showed slight acute cellular swelling of tubular epithelial lining with mild MMCs infiltration. The effect of HOTA dose with whey on posterior kidney

appeared as focal tubular necrosis replaced by inflammatory cells. In spleen the alteration is activation of MMCs in both OTA doses but the severity increased with HOTA dose (Fig. 10, 11 and 12).

Table (1): Effect of Ochratoxin A (OTA), yeast and whey on red blood cells (RBCs), white blood cells (WBCs) phagocytic activity (PA) and phagocytic index (PI) of blood of *O. niloticus* through out experimental period ($\overline{X}$ ±SE)

Items	Treatments	Week 2	Week 4	Week 6	Week 8	Total Mean
Total protein (g/dl)	Control	4.39±0.21	4.37±0.10	4.39±0.12^{a}	4.46±0.06^{a}	**4.40±0.06^{a}**
	LOTA dose	4.14±0.31	4.05±0.22	3.73±0.08^{cd}	3.55±0.09^{bc}	**3.87±0.11^{BC}**
	HOTA dose	3.91±0.10	3.69±0.17	3.42±0.04^{d}	3.06±0.06^{d}	**3.52±0.09^{D}**
	LOTA dose + yeast	4.34±0.17	4.29±0.12	4.12±0.13^{ab}	3.84±0.14^{b}	**4.15±0.08^{AB}**
	HOTA dose + yeast	4.31±0.32	4.14±0.06	3.88±0.14^{bc}	3.44±0.08^{bcd}	**3.94±0.12^{BC}**
	LOTA dose + whey	4.22±0.18	4.20±0.18	4.02±0.20^{bc}	3.65±0.25^{bc}	**4.02±0.11^{BC}**
	HOTA dose + whey	4.18±0.12	3.98±0.25	3.70±0.03^{cd}	3.42±0.10^{cd}	**3.82±0.10^{C}**
	Total Mean	**4.21±0.08^{A}**	**4.10±0.07^{AB}**	**3.90±0.07^{B}**	**3.63±0.09^{C}**	**3.96**
Albumin (g/dl)	Control	3.10±0.11	3.16±0.21	3.06±0.10^{a}	3.13±0.08^{a}	**3.11±0.06^{A}**
	LOTA dose	3.03±0.23	2.87±0.03	2.73±0.06^{b}	2.52±0.04^{c}	**2.79±0.07^{B}**
	HOTA dose	2.95±0.04	2.76±0.14	2.65±0.08^{b}	2.47±0.05^{c}	**2.71±0.06^{B}**
	LOTA dose + yeast	3.09±0.25	2.97±0.13	2.81±0.14^{b}	2.73±0.05^{b}	**2.90±0.08^{B}**
	HOTA dose + yeast	3.04±0.33	2.87±0.09	2.75±0.06^{b}	2.76±0.07^{b}	**2.84±0.09^{B}**
	LOTA dose + whey	3.06±0.12	2.92±0.09	2.79±0.07^{b}	2.73±0.06^{b}	**2.87±0.05^{B}**
	HOTA dose + whey	3.00±0.04	2.81±0.06	2.67±0.04^{b}	2.52±0.06^{c}	**2.75±0.05^{B}**
	Total Mean	**3.04±0.06^{A}**	**2.91±0.05^{AB}**	**2.78±0.04^{BC}**	**2.69±0.04^{B}**	**2.85**
Globulin (g/dl)	Control	1.29±0.17	1.21±0.22	1.34±0.11	1.33±0.11^{a}	**1.29±0.07^{A}**
	LOTA dose	1.11±0.38	1.18±0.22	0.99±0.12	1.03±0.12^{ab}	**1.08±0.11^{A}**
	HOTA dose	0.96±0.10	0.92±0.26	0.77±0.11	0.59±0.07^{b}	**0.81±0.08^{B}**
	LOTA dose + yeast	1.25±0.22	1.33±0.23	1.31±0.07	1.11±0.17^{ab}	**1.25±0.09^{A}**
	HOTA dose + yeast	1.27±0.07	1.27±0.12	1.12±0.12	0.73±0.13^{b}	**1.10±0.08^{A}**
	LOTA dose + whey	1.16±0.24	1.29±0.27	1.24±0.26	0.92±0.31^{ab}	**1.15±0.13^{A}**
	HOTA dose + whey	1.18±0.15	1.17±0.21	1.03±0.03	0.89±0.10^{ab}	**1.07±0.07^{A}**
	Total Mean	**1.17±0.07^{A}**	**1.19±0.08^{A}**	**1.12±0.06^{AB}**	**0.94±0.07^{B}**	**1.11**
A/G Ratio	Control	2.55±0.36	3.01±0.74	2.33±0.20	2.42±0.26	**2.58±0.21**
	LOTA dose	4.00±1.54	2.68±0.46	2.87±0.34	2.60±0.44	**3.04±0.41**
	HOTA dose	3.17±0.34	4.18±1.47	3.84±0.87	4.48±0.71	**3.92±0.44**
	LOTA dose + yeast	2.75±0.55	2.53±0.57	2.16±0.17	2.66±0.48	**2.53±0.22**
	HOTA dose + yeast	2.43±0.33	2.36±0.35	2.55±0.31	4.46±1.34	**2.95±0.40**
	LOTA dose + whey	3.00±0.58	2.63±0.62	2.67±0.68	4.50±1.54	**3.20±0.47**
	HOTA dose + whey	2.69±0.36	2.74±0.62	2.59±0.11	2.95±0.39	**2.74±0.19**

Values in the same item with different letters are significantly different.
LOTA dose (80 µg OTA/ kg fish). HOTA dose (160 µg OTA/ kg fish).

Table (2): Effect of Ochratoxin A (OTA), yeast and whey on the total protein, albumin, globulin and albumin / globulin ratio (A/G Ratio) in serum of *O. niloticus* through out experiment

Items	Treatments	Week 2	Week 4	Week 6	Week 8	Total Mean
Total protein (g/dl)	Control	4.39±0.21	4.37±0.10	4.39±0.12^{a}	4.46±0.06^{a}	**4.40±0.06^{a}**
	LOTA dose	4.14±0.31	4.05±0.22	3.73±0.08^{cd}	3.55±0.09^{bc}	**3.87±0.11^{BC}**
	HOTA dose	3.91±0.10	3.69±0.17	3.42±0.04^{d}	3.06±0.06^{d}	**3.52±0.09^{D}**
	LOTA dose + yeast	4.34±0.17	4.29±0.12	4.12±0.13^{ab}	3.84±0.14^{b}	**4.15±0.08^{AB}**
	HOTA dose + yeast	4.31±0.32	4.14±0.06	3.88±0.14^{bc}	3.44±0.08^{bcd}	**3.94±0.12^{BC}**
	LOTA dose + whey	4.22±0.18	4.20±0.18	4.02±0.20^{bc}	3.65±0.25^{bc}	**4.02±0.11^{BC}**
	HOTA dose + whey	4.18±0.12	3.98±0.25	3.70±0.03^{cd}	3.42±0.10^{cd}	**3.82±0.10^{C}**
Total Mean		**4.21±0.08^{A}**	**4.10±0.07^{AB}**	**3.90±0.07^{B}**	**3.63±0.09^{C}**	**3.96**
Albumin (g/dl)	Control	3.10±0.11	3.16±0.21	3.06±0.10^{a}	3.13±0.08^{a}	**3.11±0.06^{A}**
	LOTA dose	3.03±0.23	2.87±0.03	2.73±0.06^{b}	2.52±0.04^{c}	**2.79±0.07^{B}**
	HOTA dose	2.95±0.04	2.76±0.14	2.65±0.08^{b}	2.47±0.05^{c}	**2.71±0.06^{B}**
	LOTA dose + yeast	3.09±0.25	2.97±0.13	2.81±0.14^{b}	2.73±0.05^{b}	**2.90±0.08^{B}**
	HOTA dose + yeast	3.04±0.33	2.87±0.09	2.75±0.06^{b}	2.76±0.07^{b}	**2.84±0.09^{B}**
	LOTA dose + whey	3.06±0.12	2.92±0.09	2.79±0.07^{b}	2.73±0.06^{b}	**2.87±0.05^{B}**
	HOTA dose + whey	3.00±0.04	2.81±0.06	2.67±0.04^{b}	2.52±0.06^{c}	**2.75±0.05^{B}**
Total Mean		**3.04±0.06^{A}**	**2.91±0.05^{AB}**	**2.78±0.04^{BC}**	**2.69±0.04^{B}**	**2.85**
Globulin (g/dl)	Control	1.29±0.17	1.21±0.22	1.34±0.11	1.33±0.11^{a}	**1.29±0.07^{A}**
	LOTA dose	1.11±0.38	1.18±0.22	0.99±0.12	1.03±0.12^{ab}	**1.08±0.11^{A}**
	HOTA dose	0.96±0.10	0.92±0.26	0.77±0.11	0.59±0.07^{b}	**0.81±0.08^{B}**
	LOTA dose + yeast	1.25±0.22	1.33±0.23	1.31±0.07	1.11±0.17^{ab}	**1.25±0.09^{A}**
	HOTA dose + yeast	1.27±0.07	1.27±0.12	1.12±0.12	0.73±0.13^{b}	**1.10±0.08^{A}**
	LOTA dose + whey	1.16±0.24	1.29±0.27	1.24±0.26	0.92±0.31^{ab}	**1.15±0.13^{A}**
	HOTA dose + whey	1.18±0.15	1.17±0.21	1.03±0.03	0.89±0.10^{ab}	**1.07±0.07^{A}**
Total Mean		**1.17±0.07^{A}**	**1.19±0.08^{A}**	**1.12±0.06^{AB}**	**0.94±0.07^{B}**	**1.11**
A/G Ratio	Control	2.55±0.36	3.01±0.74	2.33±0.20	2.42±0.26	**2.58±0.21**
	LOTA dose	4.00±1.54	2.68±0.46	2.87±0.34	2.60±0.44	**3.04±0.41**
	HOTA dose	3.17±0.34	4.18±1.47	3.84±0.87	4.48±0.71	**3.92±0.44**
	LOTA dose + yeast	2.75±0.55	2.53±0.57	2.16±0.17	2.66±0.48	**2.53±0.22**
	HOTA dose + yeast	2.43±0.33	2.36±0.35	2.55±0.31	4.46±1.34	**2.95±0.40**
	LOTA dose + whey	3.00±0.58	2.63±0.62	2.67±0.68	4.50±1.54	**3.20±0.47**
	HOTA dose + whey	2.69±0.36	2.74±0.62	2.59±0.11	2.95±0.39	**2.74±0.19**

Values in the same item with different letters are significantly different.
LOTA dose (80 µg OTA/kg fish). HOTA dose (160 µg OTA/kg fish).
Yeast (0.5 g/kg diet). Whey (14% of diets).

Table (3): Effect of Ochratoxin A (OTA), yeast and whey on the glutamic-oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), Creatinine and Uric acid of *O. niloticus* through out experimental period ($\overline{X}$ ± SE).

Items	Treatments	Week 2	Week 4	Week 6	Week 8	Total Mean
GOT (units/ml)	Control	24.67±4.06	28.33±5.07	29.67±2.51^{b}	36.00±3.46^{c}	**29.67± .07**
	LOTA dose	26.33±5.49	30.83±4.80	33.33±0.29^{ab}	44.83±2.13^{ab}	**33.33±2.39**
	HOTA dose	28.00±1.15	34.33±2.67	35.78±1.61^{a}	49.00±2.08^{a}	**35.78±2.06**
	LOTA dose + yeast	21.50±3.62	26.33±4.70	29.61±0.46^{b}	38.67±2.40^{bc}	**29.61±2.64**
	HOTA dose + yeast	24.50±1.04	27.00±2.75	31.05±1.01^{b}	41.00±1.53^{bc}	**31.06±2.13**
	LOTA dose + whey	23.67±0.73	30.83±4.64	32.11±0.86^{ab}	41.83±1.92^{abc}	**32.11±2.24**
	HOTA dose + whey	24.67±4.42	30.33±0.88	32.78±0.24^{ab}	45.33±2.60^{ab}	**32.78±2.44**
Total Mean		**24.76±1.16^{C}**	**29.71±1.36^{B}**	**32.05±0.60^{B}**	**41.67±1.11^{A}**	**32.05**
ALP (IU/L)	Control	21.60±2.18	17.62±2.14	18.58±1.49^{b}	18.06±1.48^{c}	**18.97±0.92**
	LOTA dose	20.43±0.75	19.52±3.42	21.98±0.40^{ab}	23.32±1.33^{ab}	**21.31±0.92**
	HOTA dose	23.19±0.60	20.54±1.44	23.63±0.54^{a}	25.10±1.58^{a}	**22.32±0.89**
	LOTA dose + yeast	20.48±0.44	18.49±0.49	21.79±0.88^{ab}	20.40±0.74^{bc}	**20.29±0.45**
	HOTA dose + yeast	22.13±3.22	18.14±3.26	21.51±1.58^{ab}	22.32±1.13^{ab}	**21.02±1.18**
	LOTA dose + whey	20.00±1.86	18.70±3.24	20.38±0.79^{ab}	20.45±1.14^{bc}	**20.68±0.90**
	HOTA dose + whey	21.86±2.64	19.62±0.97	23.07±1.95^{a}	23.47±1.29^{ab}	**22.00±0.90**
Total Mean		**21.38±0.66^{A}**	**18.95±0.79^{B}**	**21.56±0.52^{A}**	**21.87±0.63^{A}**	**20.94**
Creatinine (mg/dl)	Control	0.78±0.03	0.62±0.11	1.59±0.14^{c}	1.76±0.33	**1.31±0.21**
	LOTA dose	1.10±0.25	1.61±0.62	2.10±0.04^{bc}	3.06±0.31	**1.97±0.27**
	HOTA dose	1.32±0.46	1.83±0.32	3.63±0.32^{a}	3.50±0.28	**2.57±0.34**
	LOTA dose + yeast	1.10±0.09	1.34±0.47	1.66±0.30^{c}	2.72±0.52	**1.71±0.25**
	HOTA dose + yeast	1.07±0.21	1.36±0.17	2.43±0.37^{bc}	3.02±0.14	**1.97±0.26**
	LOTA dose + whey	1.08±0.30	0.64±0.35	1.84±0.46^{c}	2.57±0.97	**1.53±0.33**
	HOTA dose + whey	0.92±0.39	1.10±0.27	2.80±0.08^{ab}	3.17±1.10	**2.00±0.40**
Total Mean		**1.05±0.10^{C}**	**1.22±0.15^{C}**	**2.29±0.18^{B}**	**2.90±0.22^{A}**	**1.87**
Uric acid (mg/dl)	Control	0.83±0.26	1.26±0.26	2.25±0.45	2.19±0.31^{c}	**1.63±0.23**
	LOTA dose	0.86±0.34	1.56±0.27	3.00±0.42	3.86±0.62^{ab}	**2.32±0.40**
	HOTA dose	2.05±0.47	2.45±0.53	3.55±0.59	4.14±0.43^{a}	**3.05±0.33**
	LOTA dose + yeast	0.93±0.23	1.29±0.27	3.04±0.41	2.98±0.17^{abc}	**2.06±0.31**
	HOTA dose + yeast	0.83±0.35	1.62±0.37	2.65±0.17	3.02±0.37^{abc}	**2.03±0.29**
	LOTA dose + whey	1.12±0.09	2.24±0.33	2.88±0.18	2.25±0.52^{c}	**2.13±0.24**
	HOTA dose + whey	1.51±0.41	2.13±0.48	2.73±0.21	2.51±0.33^{bc}	**2.22±0.21**
Total Mean		**1.16±0.14^{C}**	**1.79±0.15^{B}**	**2.87±0.15^{A}**	**2. 99±0.21^{A}**	**2.20**

Values in the same item having different letters are significantly different. LOTA dose (80 µg OTA/kg fish). HOTA dose (160 µg OTA/kg fish). Yeast (0.5 g/kg diet). Whey (14% of diets).

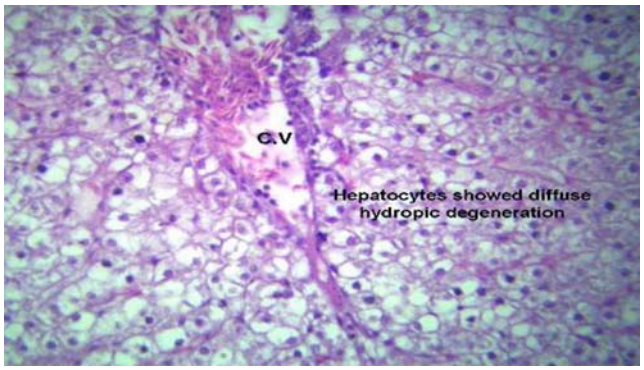

Figure 1: Liver of *O. niloticus* exposed to LOTA dose showing diffuse hydropic degeneration of hepatic cells. H&E. (X 250)

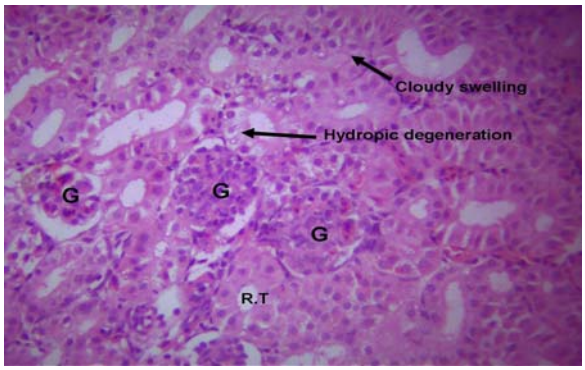

Figure 2: Kidney of *O. niloticus* exposed to LOTA dose showing mild acute cellular swelling. H&E. (X 250)

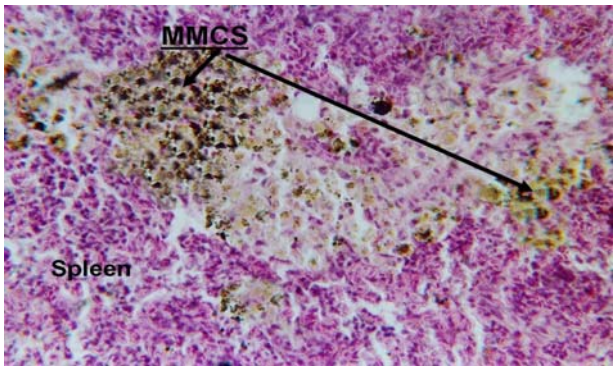

Figure 3: Spleen of *O. niloticus* exposed to LOTA dose showing activation of MMCS. H&E. (X 250)

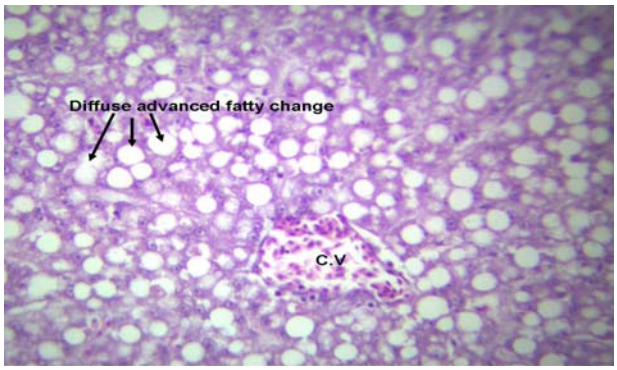

Figure 4: Liver of *O. niloticus* exposed to HOTA dose showing diffuse advanced fatty changes characterized by hepatic cells appear as signet ring. H&E. (X 250)

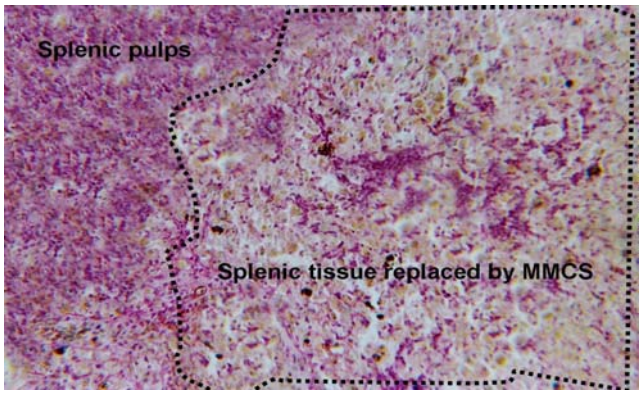

Figure 5: Spleen of *O. niloticus* exposed to HOTA dose showing severe infiltration of the splenic pulps with MMCS to extent that total replacement of the splenic tissues. H&E. (X 160)

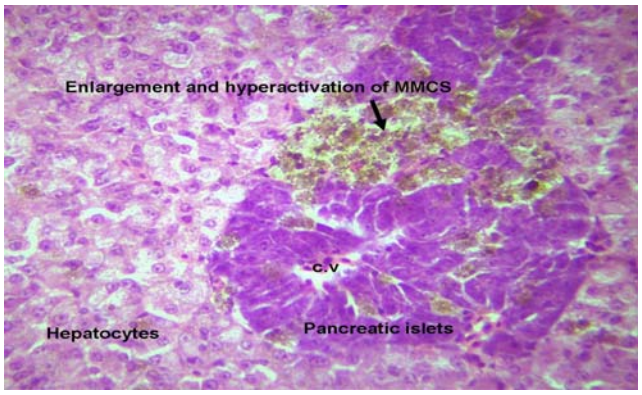

Figure 6: Liver of *O. niloticus* exposed to HOTA dose showing severe infiltration of MMCS in pancreatic islets. H&E. (X 250)

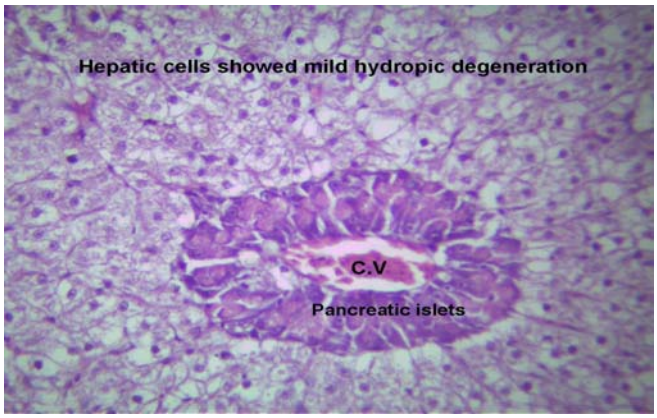

Figure 7: Liver of *O. niloticus* exposed to HOTA dose plus yeast showing mild hydropic degeneration of the hepatic cells. H&E. (X 250)

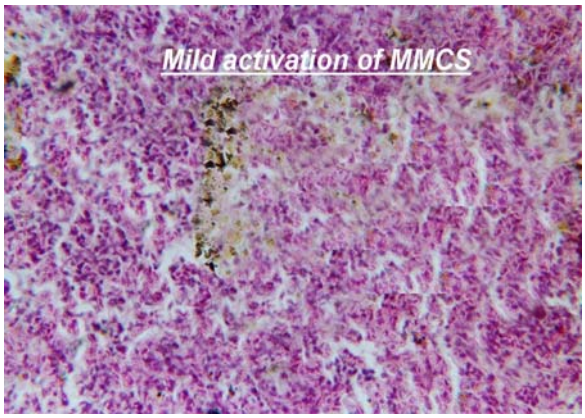

Figure 8: Spleen of *O. niloticus* exposed to HOTA dose plus yeast showing mild activation ofMMCS. H&E. (X 160))

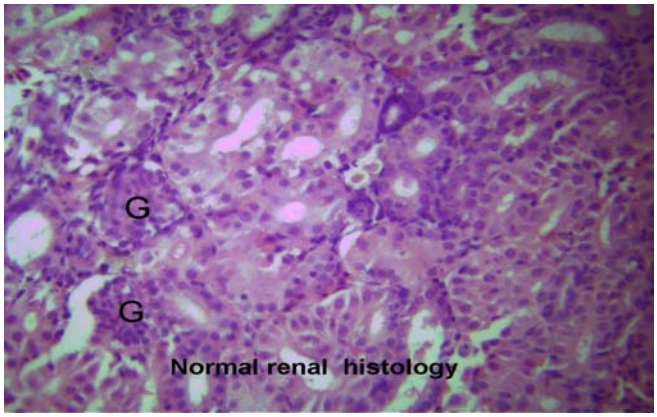

Figure 9: Kidney of *O. niloticus* exposed to LOTA dose plus yeast showing normal renal architecture and histology. H&E. (X 250)

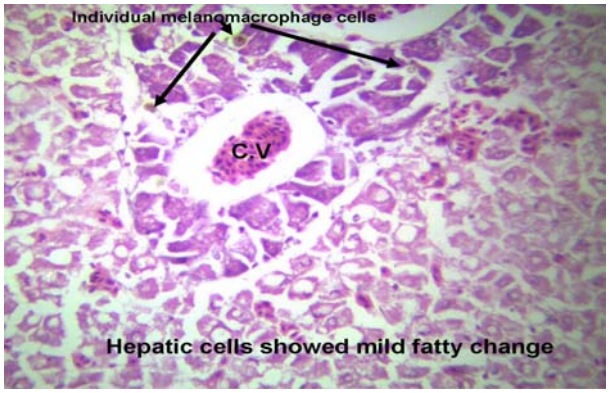

Figure 10: Liver of *O. niloticus* exposed to HOTA dose plus whey showing mild fatty change of hepatic cells beside individual infiltration of MMC plus in pancreatic islets. H&E. (X 250)

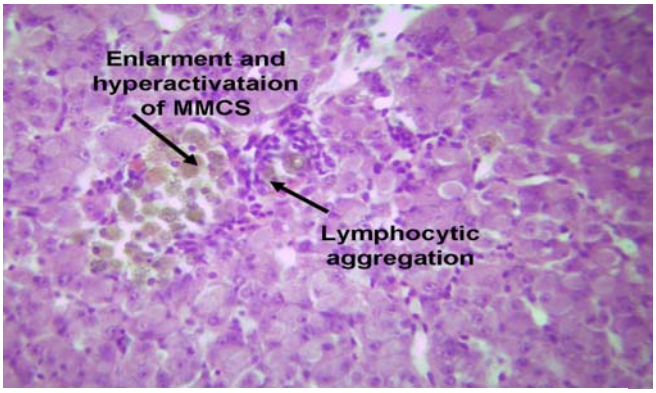

Figure11: Hepatopancreas of *O. niloticus* exposed to HOTA dose plus whey showing focal lymphocytic aggregation and enlargement and hyperactivation of MMCS. H&E. (X 250)

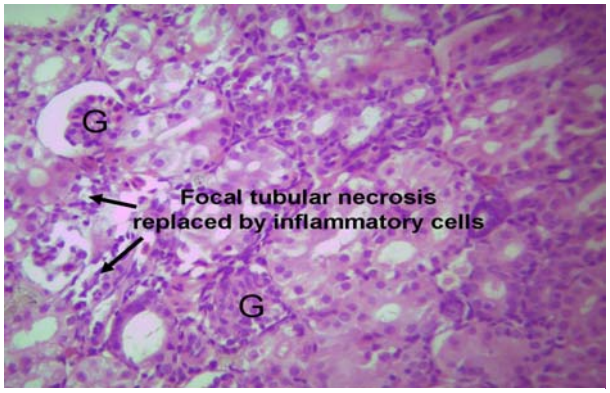

Figure 12: Posterior kidney of *O. niloticus* exposed to HOTA dose plus whey showing focal tubular necrosis replaced by inflammatory cells. H&E. (X 160)

4. Discussion:

The reduction of RBCs count which observed in the present study may be due to destruction of mature RBCs and inhibition of erythrocyte production due to reduction of haeme synthesis by ochratoxicosis. Also, the decrease in the RBCs may related to the elimination of RBCs from circulation as a result of ochratoxin – induced extravasations of the blood (Jordan *et al.*, 1977).

Moreover, Shalaby (2004) found a significant reduction in RBCs of *O. niloticus* feed contaminated diet with OTA.

Decrease of WBCs count with LOTA and HOTA doses significantly than control group and reduced insignificantly than yeast and whey treatments all over the experimental period were noticed. The decrease of WBCs may be due to the immunosuppressive effects of OTA. Saad (2002) reported lymphopenia in case of acute and chronic exposure of *O. niloticus* to OTA.

This change usually associated with acute stage of haemolytic anemia (Chang *et al.,* 1979) and destructive effects of OTA on spleen, kidney and liver (Smith and Hamilton, 1970). Moreover, Easa (1997) confirmed these results by recording depletion of hematopiotic elements due to the effects of OTA.

The PA of HOTA dose reduced significantly than other treatments. Meanwhile, insignificant (P >0.05) differences were observed among LOTA dose and detoxification treatments. Control showed the highest significant (P<0.05) PA value.

The phagocytic index differ significantly from the second week of treatment, where HOTA dose recorded the lowest significant (P<0.05) PI and showed insignificant (P> 0.05) differences with LOTA dose and HOTA dose plus whey all over the experiment. The addition of yeast ameliorate the drastic effect of OTA significant (P<0.05) on PI especially with LOTA dose. Meanwhile, slightly improve of PI observed with HOTA dose plus yeast and LOTA dose plus whey.

Saad (2002) who found that OTA (10,000 ng/kg fish) decreased phagocytic activity and phagocytic index in *O. niloticus* after eight weeks of treatments.

The decrease of PA and PI by OTA may be due to the stress effect of OTA on *O. niloticus* (Pickering, 1981). This lead to increase level of serum cortisol which leads to suppression of phagocytosis process (Khalil, 1998).

The increase of Phagocytic activity by addition of yeast may be attributed to enhancing the phagocytic and oxidative activities of kidney phagocytic cells Sakai et,al (2001).

The significant effects of OTA, yeast and whey on total protein observed at week six to eight and showed significant (P<0.05) decrease of total protein with both LOTA and HOTA doses treatments. Meanwhile, the addition of yeast increased total protein values with both LOTA (significant P<0.05) and HOTA doses. Whey addition increased total protein but not significantly (P> 0.05) with both LOTA and HOTA doses.

In general, OTA disruptive total protein level in *O. niloticus* and addition of yeast and whey improved total protein level especially with HOTA dose. Moreover, total protein levels decreased significant (P<0.05) with term of exposure.

OTA and detoxification treatments showed significant (P> 0.05) decrease of albumin value (hypoalbuminemia) than control group. Although LOTA and HOTA doses decreased albumin level significant (P<0.05) than control, yeast treatments and whey with LOTA dose.

The results showed insignificant (P> 0.05) decrease of globulin with LOTA and HOTA doses and significant (P> 0.05) increase with yeast and whey until sixth week. Meanwhile, at eighth week globulin decrease significant (P <0.05) with LOTA and HOTA dose.

The reduction of plasma total protein may be due to liver damage caused by OTA where all plasma protein synthesis usually occurs in liver except gamma globulins which are produced by lymphocytes (Coles, 1986 and Khalil, 1998). This reduction may be interpreted to the inhibitory effect of OTA to protein synthesis (Ringot *et al*., 2006).

The hypoproteinemia and hypoalbuminemia my be attributed to three main causes: hepatic insufficiency, renal loss (protein-losing nephropathy), and gastrointestinal loss (protein-losing enteropathy) Carlye-Rose, (2002). Moreover, OTA found to be hepatotoxic (Gagliano *et al.* 2006), nephrotoxic (Saad, 2002), and increase the permeability of gastrointestinal tract (McLaughlin *et al.,* 2004) which interpreted the decrease of total protein and albumin with OTA treatments in the present study.

Globulin is the building source of antibody where 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. Elkafory (2006) reported increase in fish serum proteins (total protein, albumin, globulin and A/G ratio) received yeast with diet.

Antibody titer reduced significantly with HOTA dose than control group. Insignificant (P >0.05) differences were observed among other treatments and control group. In eighth week (the

forth week after vaccination) a significantly ($P< 0.05$) decreased of antibody titration was observed with LOTA and HOTA doses compared to other treatments. However, the addition of yeast and whey to the diet increase significantly antibody titration especially with HOTA dose.

Regarding to the overcome of detoxification agents to OTA on antibody titer where significantly increase of antibody titer was observed with yeast supplementation. Yoshida *et al.* (1995) showed that *Saccharomyces cerevisiae* was a source of nucleic acids and β-1,3-glucans which have been recognized to effectively enhance immune functions of African catfish. Also, Anderson *et al.* (1995) reported that Baker's yeast, *S. cerevisiae*, contains various immunostimulating compounds such as β-glucans, nucleic acids and mannan oligosaccharides. Moreover, Glucan treatment in fish enhanced the expression of interleukin 1 and complement activity Engstad *et al.* (1992).

In case of whey, which increase antibody titer may be due to whey act as source of biologically active molecules. Several of which are known to impact on the immune system (Knowles and Gill 2002). The biological components of whey protein, including lactoferrin, beta-lactoglobulin, alpha-lactalbumin, glycomacropeptide, and immunoglobulins, demonstrate a range of immune-enhancing properties (Horton, 1997).

Also, whey protein concentrates found to be enhanced humoral immunity, with significantly elevated serum and intestinal tract antibody responses to orally administered antigens (Rutherfurd-Markwick, *et al.,* 2005).

The significant differences of GOT observed at the sixth week of treatment where GOT values with LOTA and HOTA doses increased significantly than control and yeast supplementation treatments. Alkaline phosphatase showed significantly ($P<0.05$) different at sixth week where control group decreased significantly than other treatments.

The increase of serum transaminases may reflect myocardial and hepatic toxicity leading to extensive liberation of the enzymes in to blood circulation (Fuchs *et al.,* 1986). These results agreed with Saad (2002) who found significant increase of serum aspartat aminotransferase and alkaline phosphatase with OTA treatment on *O. niloticus*

The increase of liver function enzymes in case of LOTA and HOTA doses may be due to the toxic effect of toxin in liver cells. Moreover the liver used to be the site of detoxification of the OTA to 4(*R*)-and 4(*S*) - hydroxyochratoxin A (Stormer and Pederson, 1980). In the same time the level of liver enzymes in case of OTA plus yeast were less than OTA only. This may be indicated that yeast decreased the toxic effect of OTA on liver and in the same time increase liver function.

The increase of creatinine and uric acid in serum of ochratoxicosis fish my be attributed to renal disturbance 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).

Saad (2002) found that in case of OTA on acute and chronic toxicity in *O. niloticus* causes severe destruction of the proximal tubules of the posterior kidney and hydropic degeneration of the tubular epithelium.

Creatinine is a protein produced by muscle and released into the blood hence removed by the kidney and the increase of creatinine levels indicated to decrease of kidney function (Zotti *et al.,* 2008).
The histopathological alteration which confirmed in case of LOTA and HOTA in the form of activation of melanomacrophage centers in liver and spleen atrophied of hepatic cells, severe fatty changes, and cellular degeneration of kidneys could be attributed to the toxic effects of OTA (Saad 2006).

Similar results obtained by Manning *et al.,* (2003) in case of catfish fed dietary concentrations of 2.00 to 8.00 mg OTA/kg which revealed increase incidence and activation of MMCs centers in hepatopancreatic tissue and posterior kidney.

Orrenius and Bellomo (1986) demonstrated that lipid perox idation which caused by OTA may be an early event in hepatotoxicity, which results in structural changes in the cell membrane and allow an influx of cellular calcium to cause changes in metabolic activity within the cell and ultimately cause cell necrosis.

Also the activation of the MMCs considered as indicative on the degree of the tissue damage (Roberts, 2001).

Regarding to addition of yeast to diets of ochratoxicosis fish elimenate the drastic effects of OTA on hepatopancreas. Also spleen in LOTA dose didn't affected but in HOTA dose spleen showed mild activation of MMCs .

Addition of whey to toxicated fish diets affects the histological findings as follow; Hepatopancreas in LOTA dose are congestion and hydropic degeneration. Meanwhile, with HOTA dose the alteration appeared as mild fatty changes, focal lymphocytic aggregation, enlargement and hyper activation MMCs. Posterior kidney in LOTA dose showed acute cellular swelling of tubular epithelial lining with mild MMCs infeltiration. The effect of

HOTA dose on posterior kidney appeared as focal tubular necrosis replaced by inflammatory cells. In spleen the alteration is activation of MMCs in both OTA doses but severity increased with HOTA dose.

The histopathological examination results concluded that yeast more effective than whey in minimize the destructive effect of ochratoxin in the most affected organs (hepatopancreas, kidney and spleen) especially at the LOTA dose.

Moreover, yeast reduce the presence of potentially pathogenic bacteria by competitive exclusion and causes intestinal microbial balance of the host organism and confer various beneficial effects include immunostimulation and enhance disease resistance (Gatlin *et al.,* 2006).

The detoxification effect of yeast on OTA may be revealed to the ability of yeasts to secrete an enzyme related to carboxypeptidases which convert OTA to OTα (non toxic form) (Péteri *et al.*, 2007) by the cleavage of the peptide bond between isocoumarin and phenylalanine in OTA moiety (Marquardt, 1996). Furthermore, yeast cell wall was an effective adsorbent for OTA (Ringot *et al.*, 2007) which may reduce OTA absorption from the fish gastro intestinal tract and excluded with feces.

Molnar *et al.* (2004) found that yeast strain of the genus *Trichosporon* from the hindgut of the termite, refers to important characteristics to detoxify mycotoxins such as OTA. Since, fish gastric microorganisms able to transform mycotoxin to non toxic form in various environmental conditions (Guan *et al*., 2009). Moreover, yeast showed antagonistic effects to OTA production and growth of OTA producing fungi (Petersson *et al.,* 1998 and Masoud & Kaltoft, 2006).

In conclusion, OTA proved to produce drastic effects on physiological and pathological levels of *O. niloticus.* Meanwhile, active yeast and Sweet whey were successed to neutralize the drastic toxic effects of OTA.

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