# THE OPPOSING EFFECT OF ASCORBIC ACID (VITAMIN C) ON OCHRATOXIN TOXICITY IN NILE TILAPIA (*Oreochromis niloticus*)

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## Abstract

The opposing effect of ascorbic acid against ochratoxin induced perturbations in some haematological and biochemical parameters in Nile tilapia (*Oreochromis niloticus*) was investigated. This study was carried out to evaluate the ability of a minimum level (500 mg/ kg diet) of vitamin C to counteract the toxicity of two levels of ochratoxin (400 and 600 µg/ kg diet). Nile tilapia (50- 60 g/ fish) was assigned to five treatments with three replicates each. The first treatment was fed normal diet and kept as control. The second and third ones were fed normal diet containing 400 and 600 µg of ochratoxin/kg diet, respectively. The fourth group was fed normal diet containing 400 ug of ochratoxin and 500 mg of vitamin C /kg diet. The fifth group was fed normal diet with 600 µg of ochratoxin and 500 mg of vitamin C/kg diet.

The obtained results showed a significant decrease in erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit value (Hct) in fish exposed to the low or high level ochratoxin and the vitamin C enhanced the blood parameters in fish exposed to both levels of ochratoxin to values close to those of control fish. The high level of ochratoxin (600 µg/ kg diet) induced a significant decrease in mean corpuscular volume (MCV), mean corpuscular haemaglonin (MCH) and mean corpuscular haemaglonin concentrate (MCHC) blood indices. Moreover, the plasma total protein, total lipids and lactate dehyrogenase were decreased significantly in response to the two doses of ochratoxin. On other hand, the glucose concentration was increased significantly in fish fed the low or high level of ochratoxin, whereas the plasma glucose concentration, total lipids and lactate dehyrogenase in the fish group fed ochratoxin with vitamin C became similar to those of the control.

The total protein content and the activity of aspartate aminotransferase (AST) in the liver and muscle were significantly decreased in fish fed on the two levels of ochrayoxin. On the other hand, the activity of alanine aminotransferase (ALT) in the liver and muscle was increased significantly in the fish group exposed to the high dose of ochratoxin. Vitamin C administration into the ochratoxin toxicated fish normalized the total protein content and AST activity to become similar to those of the control group.

## Introduction

Fish are considered one of the important food sources for human beings because their flesh contains a high percentage of protein, calcium and phosphorus. So, there is an increased attention given to fish farms and their diseases. Mycotoxins are among the most common contaminants in animal feed, causing great economic loss in both the livestock industry and fish, especially in aquaculture (Jantrarotai and Lovell, 1990). Mycotoxin may cause pathological and undesirable physiological responses in man and animals (Tetushia, 1990). Ochratoxin is one of these mycotoxins, produced as a secondary metabolite from *Aspergillus ochraceus* (Hesseltine *et al*., 1972). Aflatoxins are hepatotoxic and carcinogenic mycotoxins that are produce by *Aspergillus flavus* and *A. parasticus* (Patterson, 1977; Carlson *et al*., 2001). Mycotoxins are extremely carcinogenic and responsible for the wide spread occurrence of hepatic carcinoma of farmed rainbow trout fed at high rate of the toxin (Carlson *et al.,* 2001). The LD50-96 h of ochratoxin for the rainbow trout by intraperitoneal injection was 4.70 mg/ kg of feed (Dodter *et al*., 1972; Lovell, 1990). Low levels of ochratoxin in feed for few weeks caused reduction in the RBCs, Hb, Hct, total serum protein, cholesterol and body weight of chicks, rabbits and rats, whereas the AST, ALT, creatinine, uric acid and serum glucose levels were increased (Farshid and Rajan, 1995.; Ramadevi *et al*., 1998; Saleem and Khafajii, 2001).

Studies performed on blood usually reflect the toxicity of ochratoxin. In this connection, several studies, exhibiting different results, were carried out to determine the toxic effects of ochratoxin (Subramanian *et al*., 1989; Ramadevi *et al*., 1998; Mousa and Khattab, 2003).

Ascorbic acid (vitamin C) is an essential nutrient in aqua feeds and is an indispensable nutrient required to maintain the physiological processes of different animals including fishes (Tolbert, 1979). Most fish, including tilapia, are not capable of vitamin C biosynthesis (Chotterjee, 1973) due to the absence of the enzyme L- gulonolactone oxidase necessary for ascorbic acid synthesis (Wilson, 1973). Vitamin E and vitamin C play important roles in animal health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stresses (Chew, 1995). It has been suggested that the antioxidant function of these micronutrients could enhance immunity by preserving the functional and structural integrity of important immune cells. In this respect, the need for specific nutrients may be increased during infection which could require the feeding on diets formulated for optimal immune competence rather than growth and survival. Supplementation of vitamin C enhanced antibody production against *Edwarsiella ictaluri* in channel cat fish (Li and Lovell, 1985).

Vitamin C has an important role the intracellular and extracellular processing and assembly of collagen from its precursors. Collagen is a principal constituent of skin, scales, mucous, cartilaginous tissues, bones and in conjunctive tissue formation, which involves all organs of the body (Mc-Dowell, 1989). Ascorbic acid was also reported to have curative effects due to its properties, by many authors. Ghazaly (1994) reported that ascorbic acid reduced the mortality, lowered metal content of tissue and prevented the inhibition of blood

AST and LDH activities of *Tilapia zillii* after exposure to mercury. Abdel-Tawwab *et al*. (2001) found that a high level of ascorbic acid enhanced the weight gain, specific growth rate and survival rate in Nile tilapia exposed to sublethal dose of mercury.

Elimination or reduction of the ochratoxin –producing fungi in grains is not always successful, particularly during the preharvest period. In turn, control of 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 haematological and biochemical biomarkers in Nile tilapia (*Oreochromis niloticus*) and the probable ameliorative effect of dietary vitamin C on the toxicity of ochratoxin.

## *Materials and methods*

Nile tilapia *Oreochromis niloticus* used in the present study were obtained from the Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammed, Sharkia. The average weight of fish was 50–60 gm /fish and the acclimated in laboratory conditions for 2 weeks before the initiation of the experimental work. Dietary L- ascorbic acid in the form of ascrobyl monophosphate magnesium salt (1 mg of ascorbic acid is equivalent to approximately 2.4 mg of ascorbyl monophosphate magnesium salt (Sigma, St Louis, MO, USA) was supplemented at a level of 500 mg/Kg diet.

Fish were distributed in fifteen glass aquaria of a 100-liter capacity each at a rate of 10-fish/aquarium. These aquaria were supplied with dechlorinated tap water with continuous aeration. The aquaria were divided into five groups with three replicates per group. The first group was fed normal diet and kept as a control. The second and third groups were fed normal diet containing 400 or 600 µg of ochratoxin/kg diet, respectively. The fourth was fed normal ration contained 400 µg of ochratoxin and 500 mg vitamin C/kg diet. The fifth group was fed normal diet containing 600 µg ochratoxin and 500 mg vitamin C/kg diet. Fish in these groups were fed at a rate of 3 % of live body weight with pellet fish diet (32 % CP) twice daily for 90 days.

Semi–dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of water.

At the end of the experiment, blood samples were taken from the caudal vein of non-anaesthetized fish by sterile syringe containing EDTA as an anticoagulant. The blood was used for erythrocyte count (Dacie and Lewis, 1984), haemoglobin content (Vankanpen, 1961) and haematocrit value (Britton, 1963).

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (1984).

Plasma was obtained by centrifugation at 3000 rpm for 15 min and the non-haemolyzed plasma was stored in a deep freezer at -20 °C pending analysis. Plasma protein content was determined by Biuret method described by Wotton (1964). Glucose concentration was measured according to Trinder (1969) using Boehring Mannheium kits. Total lipids were determined colorimetrically using a kits supplied by El Nasr Pharmaceutical Chemical, Co. according to Joseph *et al.* (1972). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically using kits supplied by Diamond Diagnostics according to Reitman and Frankel (1957). Lactate dehydrogenase was measured using kits supplied by Diamond Diagnostics according to the method of Cobaud and Warblewski (1958).

The data were analyzed statistically using Duncan's multiple range test to determine differences among means (Duncan, 1955) and using T-test (Harold and Larson, 1982) to compare the means of treated groups against that of the corresponding control.

## Results

### *Haematological variables*

The results of erythrocyte count (RBCs), haemoglobin content (Hb) and haematocrit % (Hct) obtained from fish fed on diet containing 400 or 600 µg of ochratoxin with or without ascorbic acid (vitamin C) are given in Table 1. This shows that the two levels of ochratoxin (400 and 600 µg of ochratoxin/kg diet) induced a reduction in all blood parameters examined, and were significantly different from the control values. The erythrocyte count was decreased significantly in fish fed with diet containing the low or high dose of ochratoxin. On the other hand, the treatment with ochratoxin and vitamin C improved these parameters and the erythrocyte count, Hb content, Hct values were not significantly different from the corresponding content values in the group treated with the lower dose of ochratoxin and vitamin C. In the group treated with higher dose of ochratoxin and vitamin C, the erythrocyte count and Hct value were not significantly different from the control with some exceptions, which differed in some point obtained from treatment with ochratoxin alone.

The results blood indices calculated form the mean values of blood parameters for the aforementioned treatments are given in Table 2. Data showed that the MCV and MCHC were reduced significantly in the fish treated with the higher level of ochratoxin. Also, the MCH was decreased significantly in the fish treated with the two levels of ochratoxin when compared with the control value. The vitamin C maintained the MCV and MCH at levels close to those of the control in the fish fed the two doses of ochratoxin + 500 mg vitamin C/ kg diet.

### *Biochemical parameters*

Data presented in Table 3 indicated that treatment of *Orechromius niloticus* with ochratoxin induced a highly significant increase in plasma glucose as compared to the control group. However, with the ochratoxin plus vitamin C treatments, there were no

marked differences from the control value. Ascorbic acid decreased the glucose concentrations in fish fed diet with ochratoxin to be similar to that of control group.

The mean value of plasma total lipids of the Nile tilapia fed on ochratoxin and ochratoxin with ascorbic acid for 30 days are shown in Table 3. It can be observed that the fish fed on the lower dose of ochratoxin showed a significant decrease as compared with the control. The total lipids in fish fed diet containing the two levels of ochratoxin with vitamin C were similar to those (lipids in plasma) of the control fish.

The level of plasma lactate dehydrogenase (LDH) in the control fish was 177.30 6.70 IU/L. This level was significantly decreased to 149.5 ± 6.27 and 133.5 ± 3.14 IU/L ($P<0.001$) in fish fed with the lower and higher doses of ochratoxin respectively. Vitamin C enhanced the lactate dehydrogenase in fish fed on the diet containing ochratoxin to be similar to that of the control group.

As shown in Table 3, the plasma protein was reduced significantly in both fish groups treated with the lower and higher levels of ochratoxin. In addition, vitamin C could partially counteract this decrease, but did not raise the protein levels back to the normal value.

It can be seen from data in Table 4 that the mean values of liver protein were significantly decreased in fish fed on the higher dose of ochratoxin. The hepatic AST activities were decreased significantly in fish fed ration containing the two levels of ochratoxin. The enzyme activities in these groups tended to match the control value in the fish group fed on the low dose of ochratoxin plus 500 mg vitamin C. The assessment of hepatic enzyme activities of ALT after treatment with the lower or higher dose of ochratoxin revealed a significant increase when compared to the control value Table 4, but vitamin C curtailed these increases back to levels not significantly different from the control.

A significant reduction in the total protein of muscle was observed in Nile tilapia fed on diet containing 400 or 600 µg ochratoxin/kg diet (Table 5). Ascorbic acid increased the total protein content in muscle of fishes fed on the low dose of ochratoxin . As shown in Table 5, the muscle AST activity was significantly decreased in fish fed on the lower or higher level of ochratoxin. The AST activity in the muscle of fish fed on the two levels of ochratoxin + 500 mg of ascorbic acid became similar to that of the control fish. On the other hand, the muscle ALT activitiy revealed the highest significant elevation in fish fed on the high dose of ochratoxin with or without vitamin C.

## Discussion

The importance of haematology in diagnosis of fish diseases and assessment of the effect of ochratoxin has been widely accepted. The reduction of erythrocyte count (RBCs), haemoglobin count (Hb) and haematocrit value (Hct) in response to ochratoxin toxicity

observed in the present experiment may be attributed to destruction of mature RBCs and inhibition of erythrocyte production due to reduction of haeme synthesis by ochratoxicosis. Also, the decrease in the RBCs, Hb and Hct may be related to the elimination of RBCs from circulation as a result of ochratoxin – induced extravasation of the blood (Gordan *et al.* 1977). These results are in agreement with those of Ramadevi *et al*. (1998) who found a decrease in RBCs, Hb and Hct in the blood of broiler chicks after ochratoxin intoxication. Shalaby (2001) found that the RBCs, Hb and packed cell volume (PCV) were decreased significantly in Nile tilapia poisoned by mercury. Also, Mousa and Khattab (2003) found a decrease in RBCs, Hb and Hct in the blood of the African catfish (*Clarias gariepinus)* after ochratoxin intoxication. The obtained results revealed that toxicated fish were recovered when fed dietary ascorbic acid as the RBCs, Hb and Hct in fish fed with diet containing ochratoxin and vitamin C became similar to those of the control fish.

The calculated blood indices MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals (Coles, 1986). The perturbations in these blood indices maybe attributed to a defense reaction against toxicity of ochratoxin through the stimulation of erythropiosis or may be related to the decrease in RBCs, Hb and Hct due to the exaggerated disturbances that occurred in both metabolic and hemopoietic activities of fish exposed to sublethal concentrations of pollutants (Mousa, 1999).

In the present study, plasma glucose concentration was highly increased in fish poisoned by ochratoxin (400 or 600 µg of ochratoxin/kg diet). The increase in blood glucose might have resulted from an increase in plasma catecholamines and corticosteroid hormones (Pickering, 1981). Moreover, the hyperglycemia induced by any toxicant might be explained by the inhibition of the neuroeffector sites in the adrenal medulla leading to hypersecreation of adrenaline, which stimulates the breakdown of glycogen to glucose (Gupta, 1974).

One of the important functions of serum protein is the maintenance of osmotic balance between the circulating blood and the tissue fluids (Haper *et al.,* 1977). The influence of toxicants on the total protein concentration of fish has been also taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy.

The quantitative determination of the total protein in plasma, muscle and liver reflects the liver capacity of protein synthesis and denotes the osmolaity of the blood and the renal impairments. So, it is of a valuable factor in the diagnosis of toxicity in fish. It the present study the total plasma protein, muscle and liver protein were decreased significantly and the effect was dose dependent. This decrease might have been attributed to several pathological processes including plasma dissolution, renal damage and protein elimination in the urine, a decrease in liver protein synthesis, alteration in hepatic blood flow and/ or hemorrhage into the peritoneal cavity and intestine (Salah El-Deen *et al.,* 1996). These results agree with those of Saleem and Khafajii (2001) who found a significant decrease in serum protein in the rabbit after toxication with mycotoxin. Kopp and Hetesa (2000) found that the total protein in common carp (*Cyprinus carpio*) was significantly reduced after exposure to the cyanobacteria *Microcysts aeruginosa* and *Anabaena flos-aquae*. Also, Mousa and Khattab

(2003) showed that the plasma protein and liver protein were decreased significantly in ochratoxin poisoned fish. The decrease in plasma and tissue protein may occur due to the increase of protein breakdown as a result of stimulated corticosteroid hormones which to provide amino acids for enhance the breakdown of proteins and gluconeogenesis to provide glucose to compensate for increase in energy demands under stressful condition.

Lipids because of their rapid metabolic transformation are considered transient body material, but they represent the major source of stored chemical energy and their or absence reflects the physiology capacity of fish (Schreck and Moyle, 1990). The influence of stress on lipid metabolism in fishes was studied by several authors (Abo-Hegab *et al,* 1993 and El-Nagar *et al.,* 2000). The reduction in total lipid in plasma of Nile tilapia fed on diet containing ochratoxin alone. It is in agreement with El-Sayed *et al.,* (1996) who illustrated that the decrease in body protein and lipid in appropriate habitat was a direct of utilization of body protein and/or fat as a an energy supply to meet the increase in physiology demands.

In this investigation a significant decrease in the plasma lactate dehdrogenase (LDH) was detected in Nile tilapia after toxication with ochratoxin. These results are in agreement with those of Subramanian *et al*. (1989) who found a decrease in LDH in the blood of rat after ochratoxin intoxication (100 µg/ day for 8 weeks). On the other hand, vitamin C enhanced plasma lactate dehydrogenase in fish fed on low or high dose of ochratoxin to reach levels similar to that of the control fish.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) belong to the plasma non-functional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs. Their presence in blood plasma may give information on tissue injury or organ dysfunction (Wells *et al.,* 1986). Monitoring of liver enzymes leakage into the blood has proved to be a very useful tool in liver toxic studies (Salah El-Deen and Rogeps, 1993). The diagnostic significance of aminotransferase (AST and ALT) has been well recognized. These enzymes are liberated into the blood in pathological situations and therefore are of clinical importance. In the environmental studies also blood and tissues level of AST and ALT have been measured to assess the toxic impact of aflatoxicosis and ochratoxicosis (Ellakany and Gaafar, 2002). The present observation on *Oreochromis niloticus*, revealed a marked reduction in AST in liver and muscle in response to the lower or higher level of ochratoxin. The reduced levels of aminotransfersase in various organs may be a result of tissue damage and consequently the reduction of enzyme biosynthesis for reasons related to the presence of ochratoxin. Mousa and Khattab (2003) found inhibition of AST activities in liver of African catfish after intoxication with dietary ochratoxin. Also, Abdel-Tawwab *et al.* (2001) found similar results in liver AST of Nile tilapia after exposure to mercury. These authors ascribed this effect to liver necrosis caused by the toxicant that led to leakage of the enzyme from liver into the blood and / or the actual inhibition of liver enzyme activity or synthesis. On the other hand, the ALT activities in liver and muscle were found to increase during the time course of endogenous cortisol elvation induced by ochratoxin intoxication. These results are in agreement with those of Abo-Hegab (1985) who found as increase in ALT in common carp after exposure to salt water.

The results obtained herein, revealed that the tissue injury in toxicated fish recovered when they were fed dietary ascorbic acid because the AST and ALT activities in fish exposed to the lower or higher dose of ochratoxin + vitamin C became similar to those of control fish. These results agree with those of Abdel-Tawwab *et al*. (2001). Therefore, it could be concluded that dietary ascorbic acid is efficient in the reduction of ochratoxin toxicity. This is probably ascribed to the properties of vitamin C closely related to the immunological system performance, antioxidant action, maintenance of the integrity and fluidity of biological membranes (Barke, 1992) and control of oxidizing reaction of fatty acids. The latter function is particularly important in keeping cellular respiration and avoiding cell death (Verthac and Gabaudan, 1994). Moreover, it was reported that high levels of ascorbic acid are efficient in reduction of toxicity, preventing disease and enhancing fish tolerance to environmental stress (Abdel-Tawwab *et al*., 2001).

Finally, the present study suggests that the level of ascorbic acid (500 mg/ kg diet) used in this investigation enhances fish tolerance to environmental stress and reduces ochratoxin toxicity.

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Table 1. The erythrocyte count (C/mm3), haemoglobin content (g /100 ml) and haematocrit value (%) of the *O. niloticus* fed on a diet containing 400 and 600µg ochratoxin (OA)/ kg with or without ascorbic acid (Vitamin C).

| **Dose Parameter** | **Control** | **Low dose** | | **High dose** | |
|---|---|---|---|---|---|
| | | ***400 OA*** | ***400 OA + Vit C*** | ***600 OA*** | ***600 OA + Vit C*** |
| Erythrocyte count (C/mm3) | 1.70 ± 0.034 a | 1.471 ± 0.042 b** | 1.473 ± 0.102 ab | 1.388 ±0.04b ** | 1.473 ± 0.101 a |
| Haemoglobin content (g/100 mL) | 6.78 ± 0.147 a | 5.42 ± 0.174 b ** | 6.15 ± 0.246 a | 5.05 ± 0.125 bc* | 5.93 ± 0.229 b* |
| Haematocrit value (%) | 17.75 ± 0.54 a | 15.00 ± 0.447 b*** | 16.73 ± 0.302 a | 12.33 ± 0.494 c** | 16.00 ± 0.365 ab |

Data are represented as mean ± S.E, n=5
Means with a common superscript letters in the same row are not significantly (P>0.05).
Asterisks indicate significance from the corresponding control
*Significant at P< 0.05 **Significant at P<0.01 ***Significant at P<0.001

Table 2. The mean corpuscular volume (MCV) mean corpuscular haemoglobin, (MCH) and mean corpuscular haemoglobin concentration (MCHC) of the O*reochromis niloticus* blood fed on a diet containing ochratoxin with or without ascorbic acid (vitamin C).

| **Dose Parameter** | **Control** | **Low dose** | | ***High dose*** | |
|---|---|---|---|---|---|
| | | ***400 OA*** | ***400 OA + Vit C*** | ***600 OA*** | ***600 OA + vit C*** |
| MCV (Um3) | 104.41 ± 4.10 ac | 101.83 ± 2.846 a | 111.01 ± 1.773 c | 88.83 ± 2.95b*** | 115.41 ± 2.789 c |
| MCH (Pg) | 39.88 ± 0.381 a | 36.79 ± 0.936 b* | 41.84 ± 2.66 ab | 36.38 ± 0.69 b* | 40.25 ± 1.206 ac |
| MCHC (%) | 38.197 ± 1.43 a | 36.133 ± 0.47 a | 37.66 ± 2.05 a | 32.98 ± 0.814 b* | 37.06 ± 1.406 a |

Data are represented as mean ± S.E, n=5
Means with a common superscript letters in the same row are not significantly (P>0.05).
Asterisks indicate significance from the corresponding control
*Significant at P< 0.05 **Significant at P<0.01 ***Significant at P<0.001

Table 3. Plasma glucose , total lipids, total proteins and lactate dehydrogenase of *O. niloticus* fed on a diet containing ochratoxin with or without ascorbic acid (vitamin C)

| **Dose Parameter** | **Control** | **Low dose** | | **High dose** | |
|---|---|---|---|---|---|
| | | ***400 OA*** | ***400 OA +vit C*** | ***600 OA*** | ***600 OA +vit C*** |
| Glucose (mg/L) | 98.85 ± 2.221 a | 157.50 ± 1.89 b** | 106.34 ± 2.648 a | 178.46 ± 4.48 c*** | 104.1 ± 1.252 a |
| Total lipids (g/L) | 11.175 ± 0.538 a | 9.110 ± 0.294 b** | 11.135 ± 0.476 a | 9.995 ± 0.269 ab | 10.86 ± 0.372 a |
| LDH (IU/L) | 177.30 ± 6.72 a | 149.5 ± 6.27 bc* | 162.5 ± 6.8 ac | 133.50 ± 3.14 b*** | 165.0 ± 6.15 ac |
| Total protein (g/100ml) | 2.91 ± 0.03 a | 1.75 ± 0.01 b*** | 2.36 ± 1.08 abc | 1.33 ± 0.04 c*** | 2.24 ± 0.04 abc** |

Data are represented as mean ± S.E, n=5
Means with a common superscript letters in the same row are not significantly (P>0.05).
Asterisks indicate significance from the corresponding control
*Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001

Table 4. Liver total protein, aspartate amino-transferase (AST) and alanine amino-transferase (ALT) (IU/g) in the liver of *O. niloticus* fed a diet containing ochratoxin with or without ascorbic acid (vitamin C).

| **Dose Parameter** | **Control** | **Low dose** | | **High dose** | |
|---|---|---|---|---|---|
| | | ***400 OA*** | ***400 OA + vit C*** | ***600 OA*** | ***600 OA + vit C*** |
| Total protein (mg/g) | 25.55 ± 1.858 ab | 22.206 ± 1.429 a | 27.77 ± 1.51 b | 17.76 ± 0.928 c** | 26.66 ± 1.217 ab |
| Aspartate amino-transferase (IU/g) | 183.33 ± 2.288 a | 162.16 ± 1.75 b** | 177.46 ± 4.113 ac | 164.46 ± 3.06 b** | 175.73 ± 2.26 c* |
| Alanine amino-transferase (IU/g) | 22.866 ± 1.033a | 30.20 ± 1.138 b** | 19.53 ± 1.183 a | 36.36 ± 1.55 c*** | 26.16 ± 1.319 a |

Data are represented as mean ± S.E, n=5
Means with a common superscript letters in the same row are not significantly (P>0.05).
Asterisks indicate significance from the corresponding control
*Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001

Table 5. Muscle total protein, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (IU/g) in *O. niloticus* fed a diet containing ochratoxin with or without ascorbic acid (vitamin C).

| **Dose Parameter** | **Control** | ***Low dose*** | | ***High dose*** | |
|---|---|---|---|---|---|
| | | ***400 OA*** | ***400 OA +vit C*** | ***600 OA*** | ***600 OA +vit C*** |
| Total protein (mg/g) | 16.643 ± 0.084 a | 14.44 ± 0.702 b* | 18.88 ± 0.428 c** | 12.22 ± 0.702 b** | 16.66 ± 0.730 a |
| Aspartat amino-transferase (IU/g) | 131.33 ± 2.493 ab | 117.43 ± 2.94c** | 138.83 ± 2.373 a | 95.99 ± 2.212 d*** | 127.08 ± 2.806 b |
| Alanine amino-transferase (IU/g) | 38.86 ± 1.049 a | 40.60 ± 0.822 a | 39.06 ± 1.117 a | 121.2 ± 2.40 b*** | 43.80 ± 1.06 c* |

Data are represented as mean ± S.E, n=5

Means with a common superscript letters in the same row are not significantly (P>0.05).

Asterisks indicate significance from the corresponding control

*Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001