

The Role of Antioxidant (Vitamin E) in the Control of Lead (Pb) Pollution and Enhancement of Growth Within Nile Tilapia (*Oreochromis niloticus*).

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ABSTRACT

The present study was undertaken to investigate whether treatment with vitamin E (VE) protects against intoxication induced by lead (Pb) exposure in *Oreochromis niloticus*. Fish were exposed for 60 days to a 10 mg Pb / Kg diet. The oxidative stress affected by Pb in fish was treated by antioxidant (VE) supplementation. Fish were assigned to four dietary groups. The diets differed in their constituents of Pb and VE. Growth hormone (GH) levels were significantly increased ($p < 0.05$) in fish fed the VE supplemented diet and decreased in fish fed the Pb supplemented diet reference to control. The supplementation of VE with Pb reduced the accumulation of that metal in muscle and prevented its negative impact on growth when compared with control. The treatment with dietary VE reduced Pb concentrations in muscles and increased GH levels in serums of Pb treated fishes. While diets devoid of that antioxidant (VE) showed reduced GH levels in serums and increased Pb concentrations in muscles of these fishes.

These observations suggest that VE has a definite role in the prophylaxis of Pb poisoning, and that GH levels may be consid-

ered a sensitive bioindicator of Pb pollution and of antioxidant defence in fish.

INTRODUCTION

Vitamins are organic compounds necessary in the diet for normal fish growth and health. They often are not synthesized by fish, and must be supplied in the diet. Vitamins are divided into two groups: water-soluble and fat-soluble (Steven Craig, 2002). Vitamin E receives the most attention for its importance as an antioxidant (Steven Craig, 2002). Vitamin E is a fat-soluble vitamin that exists in 8 forms. Each form has its own biological activity, which is the measure of potency or functional use in the body (Traber and Packer, 1995). Alpha-tocopherol (α -tocopherol) is the name of the most active form of vitamin E. It is also a powerful biological antioxidant (Farrell & Roberts, 1994 and Trader, 1999). Antioxidants, such as vitamin E, act to protect the cells from the effects of free radicals, which are potentially damaging by-products of energy metabolism, or from environmental exposure like radiation (Farrell & Roberts, 1994). Free radicals may be defined as any molecule that has one or more unpaired electrons. They can damage cells and may contribute to the development of cardiovascular disease, cancer, tissue injury in liver, brain, kidney, lung, nervous system and other organs (Weiss, 1989 and

Halliwell, 1994). The redox cycling of heavy metals, as well as their interaction with organic pollutants is a major contributor to the oxidative stress resulting from aquatic pollution (Iqbal et al., 2005). Small molecule antioxidants such as vitamin E are able to interact with oxidizing radicals directly (Jones et al., 1995). Studies are underway to determine whether vitamin E, through its ability to limit production of free radicals, might help prevent or delay the development of the previous diseases (U.S. Department of Agriculture, 2004).

VE has also been shown to play a role in immune function, in DNA repair, and other metabolic process (U.S. Department of Agriculture, 2004). VE functions to protect membrane lipids from damage (Frei, 1991). Many foods are the source of VE, such as vegetables, oils, nuts, green leafy vegetables and fortified cereals (U.S. Dept. of Agri., 2004). In calves, administration of antioxidants reduced the uptake of lead in tissues and prevented the clinical signs of lead poisoning (Bratton et al., 1981). In addition, the supplementation of antioxidants diminished lead-induced biochemical alterations in the urine, blood, kidney and liver of rats, and reduced accumulation of lead in the tissue of rats (Flora et al., 1984). Dietary supplementation of lead and ascorbic acid prevented lead-induced growth retardation, reduced food consumption, anemia, renal hyper-

trophy, and accumulation of lead in tissues (Suzuki & Yoshida, 1979a). Variation of antioxidant enzymes and the effect of heavy metals on these enzymes were investigated in fresh water fish (Paula et al., 2001). Researchers observed higher hepatic levels of copper in fish captured at the polluted sites. Dietary supplementation of VE and VC is safe for the general population because these nutrients supply antioxidants, support functions for homeostasis, and protection against free radical damage (Hathcock et al., 2005). Antioxidant treatment has also been shown to prevent late memory impairment in animals (Barichello et al., 2007). Antioxidants improve myocardial efficiency in patients with heart failure (Shinke et al., 2007). Fernanda et al., (2008) studied the antioxidant defenses and biochemical changes in fish in response to copper exposure. They found that antioxidants significantly protected fish from heavy metal toxicity (Mourete et al., 2002 and Senug et al., 2007).

GH inhibition has the potential to identify heavy metals in environmental samples, and antioxidants were screened for their ability to protect GH activity from heavy metal inhibition (El-Sheblly, 2002 and Apigall et al., 2003).

The present work aims to evaluate the protective role of antioxidants (VE) in reducing accumulation and toxicity of heavy metal (Pb), and enhancement of growth of *Oreochromis niloticus*.

MATERIALS and METHODS

Eighty adult, mixedsex Nile tilapia (*Oreochromis niloticus*) with an average weight of 82.3 ± 6.3 g and length of 17.9 ± 2.1 cm were acquired from a private farm, acclimatized, and randomly distributed in 8 glass aquaria (70 L each) in dechlorinated tap water at 25 oC under a natural photoperiod (12h light- 12h dark). The aquaria were supplied with continuous aeration. Fish were allowed to acclimatize to their new environment for 7 days prior the initiation of treatment. The fish were assigned to 4 dietary treatment groups (duplicate replicates per treatment) for 60 days. Fish were fed on the basal diet

Table (1): Composition and approximate chemical analysis of the experimental basal diet.

Ingredients	Diet %
Rice bran	34
Yellow corn	26
Fish meal	25
Meat meal	12
Molasses	3
Total	100
Chemical analysis on dry metal basis	%
Moisture	7.02
Crude protein	24.92
Ether extract	8.54
Nitrogen free extract	37.48
Ash	16.21
Crude fibers	5.73

Table (2): The effect on muscle lead (Pb) content and serum growth hormone (GH) levels after treatment with vitamin E (VE) in lead exposed and un-exposed fish (*Oreochromis niloticus*).

Treatments (Dietary groups)	Muscle Pb content (mg/kg wet weight)	Serum GH levels (ng/ml)
- Group A (control) basal diet	1.78	0.69
- Group B (basal diet + VE)	0.53	0.81
- Group C (basal diet +Pb)	4.06	0.34
- Group D (basal diet +VE + Pb)	1.93	0.65

(Table 1). Group A (control) received the basal diet devoid of VE and Pb (- VE - Pb). Group B received the basal diet supplemented with 300 mg α -tocopherol VE/ kg diet (+ Ve - Pb). Group C received the basal diet supplemented with 10 mg Pb as lead acetate/ kg diet (- VE + Pb). Group D received the basal diet supplemented with 300 α - tocopherol VE and 10 mg Pb / kg diet (+ Ve + Pb). The fish were fed daily between 9:00 and 10:00 A.M. at a rate of 5 % of the total body weight. Unconsumed feed and wastes were siphoned daily. The average water quality parameters were within the optimal values: temperature 25 ± 0.2 oC, pH 7.6 ± 0.3 , dissolved oxygen 7.3 ± 0.8 mg/ L, alkalinity 81 ± 11 mg/ L Ca Co₃, unionized ammonia 0.007 ± 0.001 mg/ L and nitrate 0.002 ± 0.001 mg/ L. Water quality parameters were determined according to APHA (American Public Health Association, 1992).

At the end of exposure period, 10 fish from each treatment (5 fish from each aquaria) were netted and blood samples were collected from their caudal veins, centrifuged at 3500 rpm for 15 minutes to obtain serum, and stored at -20 oC until analysis. Growth hormone (GH) levels were determined in the serum by a private medical laboratory.

Parts of the dorsal muscles of each fish were kept at -20 oC prior to heavy metal (Pb) analysis. Lipid free dry tissues were used for determination of heavy metal content using Atomic Absorbtion Spectrophotometry (Perkin Elmer, model 2380) according to Allen et al (1979). Lead concentrations were expressed as mg/ kg wet weight.

RESULTS

The results of the present study are shown in Table 2. Exposing fish to Pb caused elevation in muscle and reduction of GH levels in serum. These two levels are the indicator of growth rate. However, VE may restore the

reduced GH levels and mitigate the effect of Pb. Fish fed diets supplemented with VE (group B) showed the highest GH levels (0.81 ng/ ml) in serum when compared with the control group (0.69 ng/ ml). The lowest levels of GH (0.34 ng/ ml) were recorded in group C, which received a diet supplemented with Pb and devoid of VE. Serum GH levels in fish fed the group D diet (+ VE + Pb) did not differ significantly (0.65 ng/ ml) from those fed the control diet ($p < 0.05$).

Concentrations of Pb in muscle varied among the dietary groups. The Pb content in muscle reflected Pb content in the diets, as well as the presence of VE. The most significant increase of Pb concentrations (4.06 mg/ kg wet weight) was recorded in the muscle of fish fed diet C (- VE + Pb) as compared with the control (1.78 mg/ kg wet weight). The lowest concentrations of Pb (0.53 mg/ kg wet weight) were recorded in the muscle of fish fed diet B (+ VE - Pb). Addition of VE in diet D (+ VE + Pb) resulted in decreased accumulation of Pb (1.93 mg/ kg) in muscle as compared to fish fed diet C, and demonstrated no significant difference ($p < 0.05$) when compared with the control.

DISCUSSION

The results reported here show that antioxidants have the ability to prevent oxidative stress induced by Pb heavy metal. Exposing fish to Pb for 60 days significantly inhibited the activity of serum GH. This inhibition was reduced in fish supplemented with VE. Adding the vitamin caused an elevation in serum GH level in Pb-treated fish, compared with the Pb-treated diet only. The results of the present work indicate that fish fed the group B diet had significantly ($p < 0.05$)

higher GH levels than the remaining groups. The significantly lower GH levels of fish in group C, that received Pb only, are probably a result of toxicity related to Pb. Whereas there were no significant differences in GH levels among group D and the control (group A), reflecting the positive role of VE. Mourente et al., (2002) recorded that growth and survival of fish were relatively unaffected by heavy metals in a VE supplemented diet. Also, Steven (2002) recorded growth depression in fish fed a Pb-supplemented diet, a decrease that was prevented with the supplementation of antioxidants.

The treatment of Pb-exposed fish with VE caused a significant decrease in Pb in the tissue. The present results agree with Tandon et al., (1987) who recorded that supplementation of vitamin B complex reduced Pb intoxication in rats. Bralton et al (1981) also recorded the preventative effect of antioxidants in Pb poisoning. They attributed the preventive effect of antioxidants to the in vivo formation of readily excretable complex(es) between Pb and the vitamin or its metabolite(s). The fact that supplementation of a Pb-rich diet with antioxidants was more effective than the treatment of Pb-exposed fish indicates that the effects were due to the inhibition of Pb absorption, and the availability of chelatable Pb in the former situation (Flora et al., 1984). Ping et al (1998) recorded that Pb exposure might decrease the defense capacity of an animal, and ascorbic acid greatly enhanced the prophylactic potential for removal of Pb from tissue. Guluzer et al., (2006) recorded that vitamins have the ability to mobilize Pb into the urine. Seung et al., (2007) recorded that VE appears to provide protection against oxidative stress caused by Pb toxicity. The present results agree with these previous observations.

The data of the present work indicates that the intake of VE in Pb-exposed fish prevents the accumulation of Pb in tissue, and enhances the growth factor of fish.

Finally, GH levels can be used as a tool to rapidly screen for and identify heavy

metals in environmental samples. Pb toxicity exerts a negative effect on fish growth, thus, fish displayed reduced growth when fed experimental diets deficient in VE. Additionally, antioxidants have a growth stimulating effect on fish and may be used as an additive in food to produce that effect.

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