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Different Levels of Dietary DL-α Tocopheryl Acetate Modulate the Antioxidant Defence System in the Hepatopancreas, Gills and Muscles of the Freshwater Crayfish, *Astacus leptodactylus* (Eschscholtz, 1823)

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Abstract: This experiment was conducted to study the effects of different levels of dietary DL-α tocopheryl acetate on Lipid Peroxidation (LPO) as Malondialdehyde (MDA) and on the antioxidant defence system in the gills, hepatopancreas and muscles of the freshwater crayfish, *Astacus leptodactylus*. Crayfish were fed 2% of their total wet weight with vitamin E supplemented diets or a control diet, daily for 122 days. The vitamin E contents of the control diet and of the experiment 1 (E_{100}), 2 (E_{600}) and 3 (E_{1200}) diets, were 66, 100, 600 and 1200 mg kg^{-1}, respectively, on a dry weight basis. The activities of Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-P_x) and levels of reduced Glutathione (GSH) and MDA were measured in the hepatopancreas, gills and muscles of the freshwater crayfish, *Astacus leptodactylus*. The results of the experiment indicated that vitamin E inhibited LPO at the hepatopancreas in a comparatively lower dose than in the gills and muscles. SOD activity decreased significantly in the gills and muscles of the crayfish fed with supplemented diets, but in the hepatopancreas a decrease was observed only in response to the higher doses of vitamin E (600 and 1200 mg kg^{-1} feed). CAT activity was reduced significantly in the gills and muscles but not in the hepatopancreas. While GSH-P_x activity was significantly elevated in the hepatopancreas by vitamin E, its activity remained unaltered in the gills and muscles. The GSH content of the gills, hepatopancreas and muscles was substantially elevated in the vitamin E supplemented crayfish. Thus, the findings of the present investigation suggest that dietary vitamin E is capable of reducing LPO level and modulating the antioxidant defence system in gills, hepatopancreas and muscles. Nevertheless, the response is highly tissue specific. Moreover, the highest doses of vitamin E (600 and 1200 mg kg^{-1} feed) did not provide much additional protection in any of the tissues.

Key words: Crayfish, vitamin E, antioxidant enzyme, lipid peroxidation, gills, hepatopancreas, muscle

INTRODUCTION

Oxygen radicals and Hydrogen peroxide (H_2O_2) are continually produced as byproducts of aerobic metabolism and may cause oxidative damage (Kang *et al*., 1994). This oxidative damage includes Lipid Peroxidation (LPO), enzyme inactivation and oxidative tissue damage (Borg and Schaich, 1984; Kappus and Sies, 1981). Under normal physiological conditions, the harmful effects of Reactive Oxygen Species (ROS) are effectively neutralized by the antioxidant defence system of organisms, which in general comprises antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GSH-P_x) and free radical scavengers (vitamins C and E, carotenoids, Glutathione (GSH)), whose function is to remove ROS, thus, protecting organisms from oxidative stress. Of particular concern are the processes by which environmental contaminants may enhance oxidative stress in aquatic organisms. In addition, it is recognised that the antioxidant defences play a significant role in protecting aquatic animals from oxidative stress (Chow, 1991; Henrique *et al*.,1998). Among the various antioxidants, vitamin E is unique and deserves special attention for its important role in maintaining the quality of aquaculture products (NRC, 1993). By its ability to inhibit LPO vitamin E protects biomembranes against oxidative stress. DL-α tocopherol achieves the highest level of vitamin E activity in animals (United States Pharmacopeia, 1993). Vitamin E is usually supplemented in feed as DL-α tocopheryl acetate, which is stable with respect to oxidation during feed processing and storage. He *et al*. (1993)

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demonstrated that vitamin E deficiency in shrimps made the mitochondrial and microsomal fractions of the hepatopancreas and muscles more susceptible to ascorbic acid-stimulated LPO, which can be prevented by dietary supplementation with vitamin E. Similarly, Jagneshwar *et al*. (2000) reported that a reduction of LPO in the hepatopancreas and gills of freshwater prawn occurred when the diet was supplemented with vitamin E. Although, some information is available on the antioxidant role of vitamin E in invertebrates (Kanazawa, 1985; He *et al*., 1992, 1993), there is relatively little concerning crustaceans, particularly *A. leptodactylus*. Moreover, relatively little concerning reports on crustaceans related to vitamin-E are mainly confined to LPO, with no information available on other components of the antioxidant defence system in general, on regarding and *A. leptodactylus* in particular. Thus, the aim of the present study was, to investigate whether dietary supplementation with vitamin E would decrease oxidative stress (in terms of LPO), in *A. leptodactylus*, an important species in freshwater aquaculture and whether any changes would be accompanied by alterations in its antioxidant defence system, with a view to improving to health and therefore, the production quality of the species through dietary manipulation. Three important organs, the gills, muscles and hepatopancreas, were chosen for the study because the gills are the respiratory organs and exposed to ambient oxygen, while the hepatopancreas, the main digestive gland rich in lipids, is responsible for regulating the overall metabolism of the body (Muriana *et al*., 1993).

MATERIALS AND METHODS

Leptodactylus sampling: This study was carried out over 122 days at the Crayfish Reproduction Unit of the Aquaculture Faculty of Firat University, Elazig. Turkey. The crayfish used in the study were provided from the Cip Fish Reproduction Foundation Population of *A. leptodactylus*.

Experimental animals: The practical control diet used in this study (Table 1) was modified after Reiqh *et al*. (1990). The control diet was formulated to contain approximately 37% crude protein on a dry-weight basis and 3.25 kcal g^{-1} gross energy. The levels of dietary vitamin E (supplied as DL α-tocopheryl acetate) were 100, 600 and 1200 mg kg^{-1} diet for diets 1-3, respectively. No vitamin E was added to the control diet, except that supplied by the vitamin premix and feed ingredients. DL-α-tocopheryl acetate was donated by Roch. The ingredients for each diet were thoroughly mixed in a commercial food mixer, before the addition of water, the cold pelleted by force through 3 mm holes using a laboratory pellet mili, air-dried at 55°C for up to 24 h and stored in a refrigerator at 4°C until further use.

Table 1: Composition and proximate analysis of the control diet (Reight *et al*., 1990)

Ingredient	Dry weight (%)
Fish (anchovy) meal	35.78
Soybean meal	38.64
Wheat flour	19.30
Sunflower oil	4.00
Dicalcium phosphate	1.00
Sodium phosphate	0.40
Avilamycine[1]	0.10
Antioxidant[2]	0.10
Vitamin premix[3]	0.50
Mineral premix[4]	0.18
Proximate composition	
Crude protein	37.40
Crude fat	7.60
Crudefibre	4.00
Crude ash	15.00
Nitrogen free extract	29.60
Moisture	6.40
Gross energy (kcal g^{-1})	3.25
Protein/energy (mg $kcal^{-1}$)	115.00

[1]Kavilamycine; [2]Antioxidant (mg kg^{-1} dry diet): Butylated hydroxytoluene 12.5; [3]Vitamin premix (IU or mg kg^{-1}): Vitamin A 2.000,000 IU, vitamin D_3 200.000 IU, vitamin E 20.000 IU, vitamin K 3.000 mg, vitamin B_1 1.000 mg, vitamin B_2 3.000 mg, Niacin 30.000 mg, Calcium D-Pantothenate 10.000 mg, vitamin B_6 2.000 mg, vitamin B_{12} 4 mg, Folic Acid 600 mg, D-Biotin 200 mg, Choline Chloride 100.000 mg and vitamin C 60.000 mg; [4]Mineral premix (mg kg^{-1} dry diet): Mn 80, Fe 35, Zn 50, Cu 5.I 2, Co 0.4, Se 0.15

Experimental design: The vitamin E contents of the control diet, diet 1-3 were 65.83, 100, 600 and 1200 mg kg^{-1}, respectively, on a dry weight basis. The vitamin E level of the control diet was analysed by high performance liquid chromatography (Miller *et al*., 1994). The crude protein content was analysed by Kjeldahl's method. Gross energy was calculated based on physiological fuel values of 9 kcal g^{-1} for lipid and 4 kcal g^{-1} for protein and carbohydrate; dry matter was calculated after the sample was dried at 105°C for 6 h; ash content after 24 h in a furnace at 550°C. Lipid was analysed by an ether extraction method (AOAC, 1990).

The experiment was carried out with 3 replicates for each dietary treatment. Forty four crayfish were used in each replicate (144 crayfish in total). The crayfish were housed in 12 concrete tanks (25×25×110 cm). Plastic pipes (15 cm in length and 7 cm in diameter) were provided as shelters for the crayfish. Adequate aeration was provided for each tank by a simple air pump. The crayfish were weighed and vere fed 2% of their total wet weight daily, divided between 3 separate feedings (Ackefors *et al*.,1992).

During the trial, dissolved oxygen, pH and water temperature were measured daily. Ammonia, iron, copper, alkalinity, hardness, calcium and water flow were measured twice a week. Mean dissolved oxygen was

7.07±0.50 mg L^{-1}; mean ammonia, iron and copper content were <0.001 mg L^{-1} (for each parameter); mean calcium was 83.94±2.85 mg L^{-1}; mean alkalinity was 351.36±9.72 mg $CaCO_3$; mean hardness was 38.00±1.50 FS°; mean pH was 7.19±0.39 (APHA, 1985) and mean water temperature was 20.04±1°C.

Chemicals: Thiobarbituric Acid (TBA), Bovine Serum Albumin (BSA), Glutathione Reductase (GR), cumene hydroperoxide and 5, 5-Dithio-bis (2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. USA. All other chemicals were of analar grade.

Tissue preparation and biochemical assay: Following the experimental period, crayfish in the intermoult stage were decapitated; the gills and hepatopancreas were rapidly removed, washed thoroughly in ice-cold physiological saline, wiped dry, weighed an electronic balance and processed immediately for biochemical analysis.

Lipid peroxidation assay: For the study of LPO, tissue homogenates (10%, w v^{-1} homogenate in 1.15% KCI) were centrifuged at 1000×g for 10 min at 4°C. The resulting postnuclear supernatant was used for the estimation of LPO by monitoring the formation of Malondialdehyde (MDA) by the method of Placer *et al.* (1966). MDA formation was calculated from the extinction coefficient of 1.56×10^5 M cm^{-1} (Wills, 1969) and was expressed as nmol MDA mg^{-1} protein. The protein content of the samples was measured according to the method of Lowry *et al.* (1951), using BSA as standard.

Assay of antioxidant enzymes: A 10% (w v^{-1}) homogenate was prepared in ice cold 50 mM phosphate buffer (pH 7.4) using a motor driven glass teflon homogeniser and centrifuged at 10 000 g for 20 min at 4°C. The supernatant was used directly for the assay of CAT (EC: 1.11.1.6) activity following the decrease in absorbance of H_2O_2 at 240 nm (Aebi, 1984) and was expressed as µmol/min/mg protein. SOD activity was estimated by the generation of superoxide radical by photoreduction of riboflavin (Beyer and Fridovich, 1987). For the assay of glutathione peroxidase (GSH-P_X, EC 1.11.1.9) activity, the oxidation of GSH by H_2O_2 was coupled to NADPH oxidation in the presence of exogenous Glutathione Reductase (GR) to maintain sub strate concentration (Paglia and Valentine, 1967) and expressed as nmol NADPH oxidised/min/mg protein.

Assay of antioxidant small molecules: A 10% (w v^{-1}) homogenate of the tissue in 5% (w v^{-1}) metaphosphoric acid was centrifuged at 1000 g for 30 min at room temperature and the deproteinized supernatant was used for assay of GSH using DTNB (Sedlak and Lindsay, 1968)

Statistical analysis: Results are presented as means±Standard Error of Means (SEM). Differences among the control and treatment means were analyzed by one way Analysis of Variance (ANOVA), followed by Duncan's new multiple range test. Differences were considered statistically significant when $p<0.05$.

RESULTS

Analysis of lipid peroxidation: Figure 1, respectively, shows the effects of dietary supplementation with vitamin E on LPO in the gills, hepatopancreas and muscles of *A. leptodactylus*. Vitamin E supplementation (100, 600 mg kg^{-1} feed) in the diet significantly prevented LPO in the gills, in comparison to the control crayfish. Clear dose-dependent inhibition of LPO was observed in response to diets E_{100}, E_{600} (39, 52% inhibition of LPO, respectively).

On the other hand, in the hepatopancreas *in vivo* LPO was significantly lower in all 3 dietary groups, in comparison to the control crayfish. Although, complete dose-dependent inhibition of LPO was observed in response to diets E_{100} and E_{600} (29 and 44% inhibition of LPO, respectively), no further inhibition of LPO was observed in the case of diet E_{1200}.

Likewise, dose-dependent inhibition of LPO was observed in the muscles, in response to diets E_{100} and E_{600} (45 and 68% inhibition of LPO, respectively). However, inhibition of LPO was not observed in the case of diet E_{1200}.

It is also evident from the results that the magnitude of LPO inhibition in response to the dietary vitamin E was higher in the hepatopancreas than in the gills and muscles.

Antioxidant enzyme activities in all of the tissues: Supplementary vitamin E in the diet resulted in a decrease in the activity of SOD in the gills, hepatopancreas and muscles of the crayfish. Total SOD activity in gills (Fig. 2) was reduced significantly in all 3 experimental diet groups, with a dose-dependent response in diets E_{100} and E_{600} and no further diminution in diet E_{1200}. On the other hand, diet-E_{100} failed to evoke any significant alteration in total SOD activity in the hepatopancreas, whereas diets E_{600} and E_{1200} reduced total SOD activity significantly, by 42 and 48%, respectively, in comparison to the control group (Fig. 2). The activity of SOD in the hepatopancreas was unaffected by vitamin-E supplementation, except in diets E_{600} and E_{1200}, where the later activity was reduced significantly. Although, a significant decrease in the activity of CAT was observed in the gills and muscles (Fig. 3) following vitamin E supplementation, no significant change in its activity was noted in the hepatopancreas (Fig. 3).

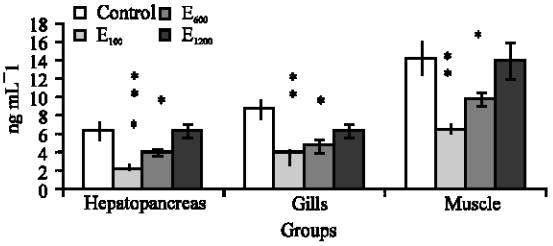

Fig. 1: Effect of different levels of dietery DL-α tocopheryl acetate on LPO in hepatopancreas, gills and muscle of *A. Leptodactylus*. Data are mean±Standard Error of Mean (SEM) of 3 replicates for each dietary treatment. Bars having different superscripts (lowercase letter) are significantly different ($p<0.05$) *$p<0.05$, **$p<0.01$, ***$p<0.001$

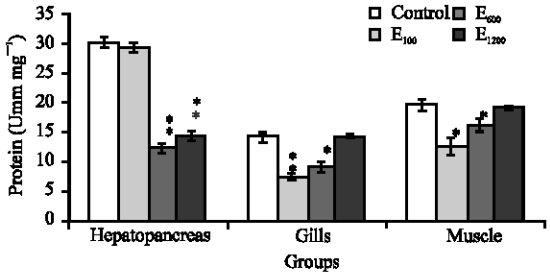

Fig. 2: Effect of different levels of dietary DL-α tocopheryl acetate on SOD activities in hepatopancreas, gills and muscle of *A. Leptodactylus*. Data are mean±Standard Error of Mean (SEM) (n = 12). Bars having different superscripts (lowercase letter) are significantly different ($p<0.05$). *$p<0.05$, **$p<0.01$, ***$p<0.001$

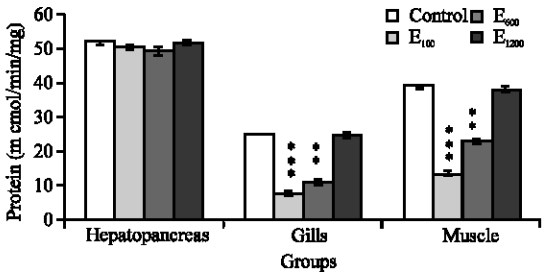

Fig. 3: Effect of different levels of dietary DL-α tocopheryl acetate on CAT activities in hepatopancreas, gills and muscle of *A. Leptodactylus*. Data are mean±Standard Error of Mean (SEM) (n = 12). Bars having different superscripts (lowercase letter) are significantly different ($p<0.05$). *$p<0.05$, **$p<0.01$, ***$p<0.001$

Dietary supplementation of vitamin E to crayfish failed to produce any significant change in the activity of GSH-P_x in the gills (Fig. 4). However, a clear dose-dependent and significant elevation in the activity of GSH-P_x was observed in the hepatopancreas and muscles (Fig. 4).

Glutathione content (non-enzymatic antioxidant): Supplementary vitamin E significantly elevated the level of GSH in the gills, hepatopancreas and muscles, in comparison to the control group.

While the magnitude of the increase in the level of GSH in the gills was 908, 975 and 1010%, in response to diet E_{100}, E_{600} and E_{1200}, respectively; the same schedule of treatment augmented the GSH

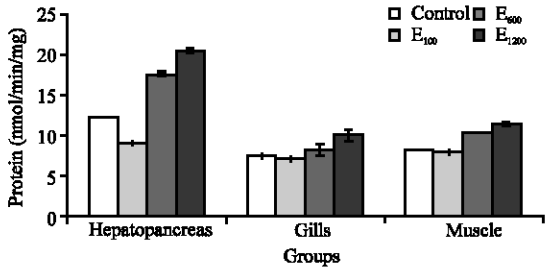

Fig. 4: Effect of different levels of dietery DL-α tocopheryl acetate on GSH-P_x activities in hepatopancreas, gills and muscle of *A. Leptodactylus*. Data are mean±Standard Error of Mean (SEM) (n = 12). Bars having different superscripts (lowercase letter) are significantly different ($p<0.05$). *$p<0.05$, **$p<0.01$, ***$p<0.001$

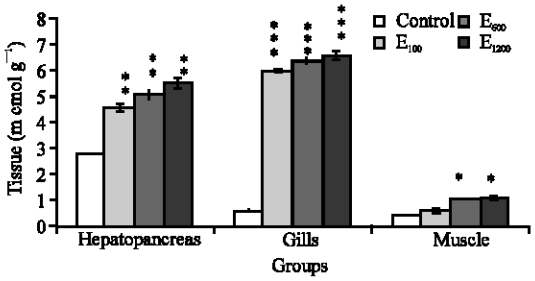

Fig. 5: Effect of different levels of dietery DL-α tocopheryl acetate on GSH activities in hepatopancreas, gills and muscle of *A. Leptodactylus*. Data are mean±Standard Error of Mean (SEM) (n = 12). Bars having different superscripts (lowercase letter) are significantly different ($p<0.05$). *$p<0.05$, **$p<0.01$, ***$p<0.001$

values by only 63, 82 and 97%, respectively, in the hepatopancreas. In addition, the increase in the level of GSH in muscle was 31, 55 and 61%, in response to diets E_{100}, E_{600} and E_{1200}, respectively (Fig. 5).

DISCUSSION

The results of the present study illustrate that vitamin E supplementation has a profoundly inhibitory effect on LPO in the gills, muscles and hepatopancreas of crayfish. Moreover, the hepatopancreas was found to be more sensitive to vitamin E than the gills and muscles. Although, only the 2 higher levels of dietary vitamin E (diets E_{100} and E_{600}) inhibited LPO significantly in the hepatopancreas and muscle, all 3 dietary levels of vitamin E (E_{100}, E_{600}, E_{1200}) were effective in suppressing LPO in the gills, with diets E_{600} and E_{1200} producing similar results. Gatlin *et al.* (1986) demonstrated that the hepatic microsomes of fish fed a vitamin E deficient diet were more susceptible to LPO. The results of our study are in accordance with He *et al.* (1993), who found lowered levels of acid-stimulated mitochondrial and microsomal LPO in the hepatopancreas of shrimps fed diets containing >100 mg of vitamin E/kg feed. They also observed that comparatively less dietary vitamin E is required to suppress LPO in the hepatopancreas. Similarly in our study, it was observed that less vitamin E was required for the suppression of LPO in the hepatopancreas of the crayfish than in the gills and muscles. Such tissue specific differential suppression of LPO by dietary vitamin E may be due to uneven distribution and deposition of vitamin E in the hepatopancreas, which is reported to accumulate vitamin E in many aquatic species (Lowry *et al.*, 1951; Bai and Lee 1998). Bai and Lee (1998) demonstrated that there was a greater accumulation of vitamin E in the liver than in the plasma and muscles of juvenile Korean rock fish fed a vitamin E supplemented diet. It is presumed that vitamin E, being lipid soluble, is stored in lipid rich organs like, the hepatopancreas. The researchers Bai and Lee (1998), also observed that tissue accretion of α-tocopherol in the liver of Korean rock fish increased linearly in response to vitamin E in the diet up to 120 mg, but that no further elevation in the level of tissue α-tocopherol occurred when supplementation was increased to 500 mg kg^{-1} feed. It is possible that the quantitative accumulation of α-tocopherol in the hepatopancreas might have been same for the higher doses (E_{600} and E_{1200}), which could be a reason for the failure of diet E_{1200} to provide any additional protection to the hepatopancreas.

Among the antioxidant enzymes, the activities of SOD and CAT exhibited tissue specific variable responses to dietary vitamin E. Although, both total SOD and CAT activities were diminished significantly in response to all dietary levels of vitamin E in the gills, a significant reduction in SOD activity was observed in the hepatopancreas only in response to diets E_{600} and E_{1200}. Decreased production and availability of the substrate (O_2^-) in response to vitamin E supplementation may be a reason for decreased SOD activity. It has been reported that O_2^- is efficiently scavenged by vitamin E in biological systems (Cay and King, 1980). Vitamin E has been shown to regulate superoxide (O_2^-) generation in human neutrophils (Ando *et al.*, 1996) and monocytes (Cachia *et al.*, 1998). Recently Chow *et al.* (1999), noticed a similar effect of vitamin E on rat liver mitochondria. Place *et al.* (1993) demonstrated an increase in SOD activity in the tissues of rainbow trout fed an α-tocopherol deficient diet, which indicates that reduced dietary tocopherol increased the activity of SOD. Therefore, increased tocopherol in the diet might have elevated tissue tocopherol level, which in turn compensated for the function of SOD. It is well established that dietary tocopherol influences the tissue tocopherol content in fish (Cowey *et al.*, 1981; Stephan *et al.*, 1995; Bai and Lee, 1998). Another significant observation is the vitamin E modulated substantial elevation of GSH content in the 3 tissues. GSH is important as a direct free radical scavenger (Winterbourn, 1995) and it has been demonstrated to scavenge superacid radical in a dose-dependent manner (Hussain *et al.*, 1996). Further, the key feature of this reaction is that it does not produce H_2O_2 in the system (Winterbourn and Metodiewa, 1994). Thus, the elevated GSH level in combination with tocopherol might have spared the function of SOD.

As SOD activity decreased in the 3 tissues in response to vitamin E supplementation, it would be logical to conclude that the formation of H_2O_2 might also have decreased. However, the responses of the 3 tissues to vitamin E supplementation regarding the removal of H_2O_2 differend. H_2O_2 is neutralized by 2 different enzymes present in the cellular system, they are CAT and GSH-P_x. Each differs in its affinity for H_2O_2 and intracellular H_2O_2 concentration is one of the factors in deciding, which of these enzymes will be functional since each has a different Km value for the substrate i.e., CAT: 25 mM (Lehninger *et al.*, 1993) and GSH-P_x: 1-10 μM (Barman, 1974). Furthermore, CAT only removes H_2O_2, while GSH-P_x is responsible for the neutralization of both organic and inorganic hydroperoxides. It is now well established that H_2O_2 plays a critical role in the regulation and expression of antioxidant enzymes in various cellular systems. Nevertheless, the regulation of the gene expression of antioxidant enzymes, particularly CAT, differs from one cell system to other. Rohrdanz and Kahl

(1998) showed that H_2O_2 is able to induce m-RNA of CAT in rat hepatocytes and H411E cells, but that regulation of the gene expression of the enzyme differs in each cell type. Therefore, the observed differential response to vitamin E supplementation of CAT and GSH-P_x in the gills, hepatopancreas and muscles is not surprising. The gills, the level of H_2O_2 may further decrease due to the diffusion of H_2O_2 to the surrounding water. Filho *et al.* (1994) have demonstrated that the elimination of H_2O_2 through gill diffusion is an important physiological mechanism in fish. Thus, the low concentration of H_2O_2 may be responsible for the decrease in CAT activity in the gills and muscle as CAT is an ineffective scavenger of H_2O_2 at low concentrations. Our findings of decreased CAT activity in the gills and muscle in response to dietary vitamin E also corroborates the findings of Saurez *et al.* (1999), who noticed a decrease in CAT activity in the erythrocytes, liver and brain tissues of rats in response to vitamin E supplementation. On the other hand, the amount of H_2O_2 in the hepatopancreas may have a positive effect on the regulation of gene expression of GSH-P_x. Study results indicate that vitamin E supplementation induces GSH-P_x activity in the hepatopancreas. It is well established that the GSH-P_x system is particularly important in preventing free radical initiation in membranes since, it is a very effective scavenger of H_2O_2 (Cay and King, 1980). Our results are in agreement with the findings of Wdzieczak *et al.* (1982) and Jagneshwar *et al.* (2000), who suggested that GSH-P_x plays an important role against the autooxidation of fish lipids in lipid rich organs. Further, the hepatopancreas is metabolically more active and the oxyradical generating enzymes display comparatively higher levels of activity than other tissues (Malik *et al.*, 1987). As the hepatopancreas is lipid rich and has a high metabolic rate, it may undergo spontaneous autooxidation and thus, the generation of $O_2^{\cdot}$ and H_2O_2 may be of a comparatively higher level in this organ than in the gills and muscle. As CAT activity was unaltered in the hepatopancreas the induction of the GSH-P_x system may be a compensatory physiological adaptive response to reduce the oxidative stress. Moreover, the increased Se-dependent GSH-P_x activity may be due to the quantity of H_2O_2 and other organic peroxides in the hepatopancreas as well as to the greater availability of Se in the tissue. The synergistic function of Se and vitamin E to increase GSH-P_x activity has already been well established in the liver of fish (Gatlin *et al.*, 1986; Wise *et al.*, 1993). Therefore, the increased GSH-P_x activity in the hepatopancreas protected the organ from the formation of lipid peroxides by reducing H_2O_2 levels, which in turn attenuated $OH^{\cdot}$ generation.

Among the non-enzymatic antioxidants, the GSH content of the crayfish exhibited a tissue specific response to dietary to vitamin E. GSH content was significantly elevated in all 3 tissues, however, the magnitude of the increase was greater in the gills. GSH is a major non-protein thiol in mammals and is essential for the structural and metabolic integrity of cells (Meister and Anderson, 1983). Evidence indicates that aquatic organisms like teleosts also maintain a high level of GSH in their tissues (Thomas *et al.*, 1982) and its level has been found to be elevated as a protective mechanism (Thomas and Juedes, 1992). The increased GSH content might also be related to low SOD activity, since SOD and GSH act synergistically, as discussed above. However, the elevated GSH content may be the result of an increased availability of cysteine, the amino acid co-substrate for glutathione and of active involvement of α-glutamylcysteine synthetase and glutathione synthetase (Meister, 1989). Earlier findings also suggest that vitamin E acts synergistically with sulphur containing amino acid and can spare the function of cysteine, the chief amino acid present in GSH (Drapper, 1980).

The sharp and higher increase of GSH in the gills compared to the hepatopancreas may be related to the prevention of oxidative challenge in the gill tissue. SOD and CAT activities were found to have decreased in the gills and GSH-P_x activity was unaltered, so the higher reduction in the level of GSH might be a protective mechanism against the cell injury. Earlier findings also suggest that the presence of high GSH level in the red cells (Filho, 1996) and in the gills (Marcon and Filho, 1993) of fish is associated with the attenuation of oxidative stress.

CONCLUSION

Overall, the results of the present investigation clearly indicate that dietary vitamin E reduces the degree of endogenous as well as tissue LPO and acts as an effective antioxidant in the tissue of the freshwater crayfish, *Actacus leptodactylus*. However, the response to vitamin E supplementation is tissue specific. It is further evident that since vitamin E is lipid soluble, it provides protection against oxidative stress in the hepatopancreas at a comparatively lower dose than in the gills and muscle. Dietary supplementation with vitamin E at 100 mg kg^{-1} feed modulated some of the antioxidants, increased the amount of adequate antioxidant function in the gills, hepatopancreas and muscles and reduced *in vivo* LPO. However, the higher doses of vitamin E (E_{600} and E_{1200}) did not provide much additional protection against oxidative stress.

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