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MALONDIALDEHYDE LEVELS AND GLUTATHIONE PEROXIDASE ACTIVITIES IN THE TISSUES OF FRESHWATER CRAYFISH (*ASTACUS LEPTODACTYLUS* ESCH. 1823) IN CULTURE CONDITION

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ABSTRACT

The objective of the study was to characterize the effects of sex on lipid peroxidation (as malondialdehyde, MDA), antioxidant defence system (as glutathione peroxidase, GSH-Px) in the hepatopancreas, gonad and muscle tissues of freshwater crayfish, *Astacus leptodactylus*, in culture condition. The crayfish used in the present study was provided from Keban Dam Lake population of *A. leptodactylus*. 34 males and 30 females crayfish in different size were hold in stock ponds (6 x 6 x 0.5 m). This study was carried out between June 18, 2004 and December 1, 2004 at the crayfish reproduction unit of Aquaculture Faculty of Firat University, Elazig, Turkey. Plastic pipes (20 cm in length and 7 cm in diameter) were provided as shelters for the crayfish. Crayfish were fed 2 % of their total wet weight daily with prepared ration. Crayfish were exposed to natural photoperiod. During the study, the mean water temperature, dissolved oxygen and mean pH were 4ºC, 6.28±0.32 mg L^{-1} and 7.82±0.17, respectively. MDA and GSH-Px levels according to sex and tissue-species were determined. MDA level in the hepatopancreas and gonad of females were higher than males, except muscle tissue ($P<0.001$). However, the GSH-Px activities in the hepatopancreas and gonad of males were higher than females, except muscle tissue ($P<0.001$). As a result, MDA and GSH-Px levels in the hepatopancreas, gonad and muscle tissues of freshwater crayfish, *Astacus leptodactylus*, in culture condition changed both tissues and sex.

Key words: *Astacus leptodactylus*; Malondialdehyde; Glutathione peroxidase; Culture condition.

INTRODUCTION

Astacus leptodactylus is one of the most popular species of European freshwater crayfish. It has many biological advantages such as higher fecundity, growth rate, and better utilization of food (Koksal, 1988). The culture of *A. leptodactylus* in captivity is not carried out in Turkey. However, there has been an increase in the wild catch in recent years (Mazlum, 2007).

Reactive oxygen species (ROS) are produced constantly by the aquatic organism's normal oxygen metabolism. Aerobic metabolism converts oxygen to water by the addition of four electrons with an efficiency of 95-99%. Oxygen is only partially reduced (i.e. addition of only 1, 2 or 3 electrons) forming ROS, including superoxide and the hydroxyl radical as well as hydrogen peroxide (Palace and Werner, 2006). ROS can initiate lipid peroxidation (LPO). It is acknowledged as being highly deleterious, resulting in damage to cellular biomembranes, particularly those of subcellular organelles, which contain relatively large amounts of polyunsaturated fatty acid lipids (PUFA). Tissue PUFA content and composition are critical factors in lipid peroxidation, and as fish tissues and eggs contain large quantities of PUFA. To cope with the continuous generation of ROS from normal aerobic metabolism, cells and tissues contain a series of enzymatic (such as glutathioe reductase (GR), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)) and non-enzymatic (such as vitamins A, C and E) cellular antioxidants (Palace and Werner, 2006; Winston and Di Guilio, 1991; Mourente *et al.*, 1999; Yildirim *et al.,* 2010). Glutathione peroxidase is responsible for the neutralization of both organic and inorganic hydroperoxides (Correla et al., 2003; Cay and King, 1980). It is well established that the GSH-Px 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).

A. leptodactylus is a cold water species like the other European crayfish. Mating takes place during October and November when the water temperature is 7-12 °C (Koksal, 1988). However, in the east of Turkey mating starts at the end of December and in the beginning of January (Harlioglu and Barim, 2004). The biochemical and physiological systems during mating period are influenced by key exogenous/endogenous factors such as developmental stage, age and sex. The relationship of the oxidant (MDA) and antioxidant (GSH-Px) status in hepatopancreas, gonad and muscle tissues of sexually

maturating *A. leptodactylus* in the culture condition was investigated during the mating period in December.

MATERIALS AND METHODS

The crayfish used in the present study was provided from Keban Dam Lake population of *A. leptodactylus*. This study was carried out between June 18, 2004 and December 1, 2004 at the crayfish reproduction unit of Aquaculture Faculty of Firat University, Elazig, Turkey.

34 males and 30 females crayfish in different size were hold in stock ponds (6 x 6 x 0.5 m). Plastic pipes (20 cm in length and 7 cm in diameter) were provided as shelters for the crayfish. The carapace length (mm) and weight (g) were recorded for each crayfish. Crayfish were fed 2 % of their total wet weight daily with prepared ration (Barim, 2005; Ackefors *et al*., 1992) (Table 1).

Table 1: Composition of diet (Barim, 2005; Ackefors et al., 1992)

Ingredient	Percent of 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	37.4
Crude fat	7.6
Crudefibre	4.0
Crude ash	15.0
Nitrogen free extract	29.6
Moisture	6.4
Gross energy (kcal/g)	3.25
Protein/Energy (mg/kcal)	115

(1) Kavilamycine(2) Antioxidant (mg/kg dry diet): butylated hydroxytoluene 12.5.(3) Vitamin premix (IU or mg/kg): 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 dry diet): Mn 80, Fe 35, Zn 50, Cu 5, I 2, Co 0,4, Se 0,15.

Crayfish were exposed to natural photoperiod. The range in photoperiod during the study was approximately 9 dark hours (D)– 15 light hours (L) in June, 9 D – 15 L in July, 10 D – 14 L in August, 12 D – 12 L in September, 12 D – 12 L in October, 13 D – 11 L in November. During the study, the mean water temperature, dissolved oxygen and mean pH were 4ºC, 6.28±0.32 mg L^{-1} and 7.82±0.17, respectively.

Assay of lipid peroxidation: MDA in tissue was estimated by the method of Ohkawa *et al*., (1979). Briefly, 0.1 ml of the tissue sample was added to 0.2 ml 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution (pH 3.5) and 1.5 ml of 0.8% TBA. The mixture was made up to 4.0 ml with distilled water and heated in a water bath at 90 ^{o}C for 60 min. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of n-butanol were added and shaken vigorously and centrifuged at 4000 x g for 10 min. The upper butanol layer was taken and its absorbance at 532 nm was read spectrophotometrically against authentic standard.

Assay of glutathione peroxidase activity: GSH-Px activity was measured by the method of Beutler, (1975). The procedure of analysis performed was based on the oxidation of reduced glutathione coupled to the disappearance of NADPH by glutathione reductase. The results were expressed as U/g protein. The assay mixture consisted of 0.1 ml of 1 M Tris-HCl buffer (pH 8.0), 20 µl 0.1 M glutathione, 0.1 ml of 10 U/ml glutathione reductase, 0.1 ml of 2 mM NADPH and 10 µl of tissue supernatant and 0.66 ml of distilled water. The mixture was preincubated in a water bath at 37 ^{o}C for 10 min. The reaction was started by the addition of 0.01 ml t-buthyl hydroperoxide. The rate of change of absorbance during the conversion of NADPH to NADP+ was recorded spectrophotometrically at 340 nm for 2.5 min.

Statistical analysis: All statistical analyses were performed with Analysis of Variance (ANOVA) followed by Duncan's test (to compare according to both sex and total, for the tissues) and Independent-Sample T Test (to compare according to sex) by using SPSS version 15 computer program. Differences were considered as significant when P values were less than 0.05. Results were expressed as mean ± SE.

RESULTS AND DISCUSSION

The mean concentrations of the antioxidants (GSH-Px) and oxidants (MDA) in the hepatopancreas, gonad and muscle tissues of *A. leptodactylu*s are showed according to tissues in Table 2 and according to the sex in Table 3.

The MDA level in the hepatopancreas and gonad of females were higher than males, except muscle tissue ($P<0.001$). The concentrations of the MDA was the lowest in the muscle (34.48%) tissues, highest in hepatopancreas (238.92%) and gonad (1360.46%) tissues of female crayfish when compared with male crayfish

(P<0.001). GSH-Px activities in the hepatopancreas and gonad of males were higher than females, except muscle tissue (P<0.001). GSH-Px activity was the lowest in hepatopancreas (23.41%) and gonad (42.84%) tissues, highest in the muscle (19.42%) tissues of female crayfish when compared with male crayfish (P<0.001) (Table 3).

Changes in biochemical levels occur as a result of maturation stage. Accumulation of biochemical components, especially lipids, in the maturing ovary has been reported in several shrimp species (Palace and Werner 2006; Izquierdo *et al*., 2001; Palacious *et al*., 2000). Palacious *et al*. (2002), determined that the level of total lipid, acylglycerides, cholesterol, and total protein in mature ovaries of *Penaeus vannamei* increased and acylglycerides represents the bulk of lipids in the hepatopancreas, whereas in the ovary, this lipid class represents a lower fraction because phospholipids are reported to be predominant. Several authors have reported a decrease in total or specific lipids in the hepatopancreas during sexual maturation, and assume they are transferred to the ovary (Palacious *et al*., 2000; Millamena and Pascula, 1990) which indicates these compounds as the major sources of energy during embryonic and early larval development (Rosa and Nunes, 2003; Rosa *et al*., 2005). In the parallel of these research results, the high MDA level in the ovarian tissue of female crayfish may due to transfer of lipids to the ovary during ovarian maturation. Rosa *et al.* (2005), determined that sexual maturation had a significant effect upon the energy content of the gonads of *Illex coindetii* and *Todaropsis eblanea*, because it is associated with an increase in the products of biosynthesis and the biochemical composition of digestive gland and muscle may be influenced not by sexual maturation. In current study, it was observed that the highest levels of the MDA and GSH-Px were found in the hepatopancreas and gonad tissues when compared with muscle (P<0.001). The main cause for these differences could be the different rates of free radical generation and different antioxidant potentials in the tissues.

The incidence of lipid peroxidation may depend upon both the level of antioxidant enzymes and the composition of fatty acids in the organisms, the latter of which may also change with aspects of animal's physiology, including development, age and sex (Correla *et al*., 2003). Also in current study, MDA and GSH-Px levels changed both tissues and sex (Table 2 and 3). Compared to male group, the female group showed significantly higher levels of the MDA in the hepatopancreas and gonad tissues, but the muscle tissues was lower (P<0.001).

Table 2: The relationship of the MDA levels (nmol g^{-1} tissue) and GSH-Px activities (U g^{-1} protein) among hepatopancreas (H), gonad (G) and muscle (M) tissues of the *Astacus leptodactylus*

Sex	Parameters	H	G	M	P values
♂	**MDA**	1.85±0.20[a]	0.43±0.03[b]	0.58±0.04[b]	P<0.001
	GSH-Px	140.21±8.44[a]	101.65±4.46[b]	54.52±2.99[c]	P<0.001
♀	**MDA**	6.27±0.37[a]	6.28±0.28[a]	0.38±0.01[b]	P<0.001
	GSH-Px	107.39±6.58[a]	58.10±4.10[b]	65.11±3.27[b]	P<0.001
♂+ ♀	**MDA**	3.92±0.34[a]	3.17±0.39[a]	0.49±0.02[b]	P<0.001
	GSH-Px	124.83±5.78[a]	81.24±4.08[b]	59.48±2.29[c]	P<0.001

Values with different superscripts within the same line statistically significant (P<0.05).

Table 3: The relationship of the MDA levels and GSH-Px activities according to sex in the tissues of the *Astacus leptodactylus*

Parameters	♂ n=34	♀ n=30	P values
Carapace length (mm)	43.55±0.79	44.67±0.88	P>0.05
Weight (g)	21.98±1.00	24.01±1.37	P>0.05
MDA (nmol g^{-1} tissue)			
Hepatopancreas	1.85±0.20	6.27±0.37	P<0.001*
Gonad	0.43±0.03	6.28±0.28	P<0.001*
Muscle	0.58±0.04	0.38±0.01	P<0.001*
GSH-Px (U g^{-1}protein)			
Hepatopancreas	140.21±8.44	107.39±6.58	P<0.01*
Gonad	101.65±4.46	58.10±4.10	P<0.001*
Muscle	54.52±2.99	65.11±3.27	P<0.05*

* Statistically significant according to the independent sample t-test (P<0.05)

The considerably higher concentration of MDA in the hepatopancreas and gonad of female *A. leptodactylus* was probably related to the relatively low level of antioxidative (GSH-Px) protection and the high metabolic rate associated with gonadal maturation.

The result of the present study showed that MDA level and GSH-Px activity in the hepatopancreas (3.92±0.34 and 124.83±5.78 nmol g^{-1} tissue) of *A. leptodactylus* in the total was lower than that of many aquatic species (Kolaylı *et al.,* (1997), Marcon and Filho (1999),Yilmaz *et al.,* (2006). These differences may due to variation between species, condition, periods, or different technique used.

In the present study, GSH-PX activity was found highest in hepatopancreas tissue in both males, females and total. (Table 2). Our results support with the findings of Wdzieczak *et al*., (1982) and Jagneshwar *et al.,* (2000),

who suggested that GSH-Px plays an important role against the autooxidation of fish lipids in lipid rich organs. The hepatopancreas is metabolically more active and the oxyradical generating enzymes display comparatively higher levels of activity than other tissues (Malik *et al*., 1987).

The results demonstrated noticeable tissue-specific distributions of the investigated the antioxidant and oxidant. This was most likely a reflection of different metabolic activities according to sex during gonadal maturation of the examined tissues.

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