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Effects of Reproduction on Oxidative Stress and Some Antioxidant Levels in tissues of *Capoeta umbla* (Heckel. 1843)

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In the present study was investigated the effect of reproduction on malondialdehyde (MDA). superoxide dismutase (SOD), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) levels in tissues (liver, muscle, kidney, spleen and gonad) of sexually matured *Capoeta umbla.* For this study was determined three different period; before reproduction (X) period (February), reproductive (Y) period (May) and after reproduction (Z) period (August). Especially, statistically significant changes were detected in the analysed oxidant and antioxidant parameters of liver and gonad tissues in the X and Y periods compared with the Z period. Generally, the MDA value of all tissues increased during the reproductive period. It was determined that levels of SOD changed in both sexes in all periods. The GSH-Px and GSH activity of kidney and muscle tissues were not effected by the reproduction periods.

Keywords: Capoeta umbla, Oxidative stress, Antioxidants.

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1. Introduction

The reactive oxygen species (ROS) are naturally produced during the survival of organism. But, ROS is highly deleterious because of cytotoxic oxidants at pathological levels. To cope with the continuous generation of ROS, there are antioxidants that consist of enzymatic antioxidants and non-enzymatic antioxidants. Especially, the key antioxidant players in this antioxidant defense system include superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.16), glutathione peroxidase (GSH-Px, EC 1.11.1.9) and glutathione reductase (GR, EC 1.6.4.2). In a healthy body, ROS and antioxidants remain in balance. When the balance is disrupted towards an overabundance of ROS, oxidative stress (OS) occurs [1-4]. Lipid peroxidation (LPO), the biggest indicator of OS, is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of ROS. It leads to the creation of lipid peroxides and other intermediates. These intermediates may influence the properties of cell membranes and their physiological functions. The most common of these intermediates are malondialdehyde (MDA) and 4-hydroxynonenal [3,5,6]. Aquatic organism are more susceptible to the attack of ROS according to other aerobic organisms, because they have rich source of polyunsaturated fatty acid lipids [7]. Studies have shown that the antioxidant defences and oxidative stress in these organism can be affected by several stressors, including intrinsic (age and phylogenetic

position, reproduction, feeding habits, etc) or extrinsic (salinity and temperature changes, pathogens, starvation, etc.) factors [8-10]. The induction of antioxidant defence enzymes may provide sensitive early-warning signals of incipient oxidative stress conditions. These enzymes can be induced by various environmental pro-oxidant conditions (such as pollution), endogenous/exogenous factors (such as age, reproductive, diet and temperature variations) [4,5,11,12]. Reproductive success is vital for sustaining an aquaculture system. One main factor affecting maturation, fecundity and survival of larvae in aquaculture organism is the fish condition. Fish condition is largely affected by their nutrition and environmental conditions [7,13].4It is very important to investigate the biochemical changes specific to the species in this period in order to create the conditions suitable for the natural environment of the fish during the breeding period. Therefore, *C. umbla*, a carp species living in the Keban Dam Lake, was examined in this study. Keban Dam Lake is one of the largest man-made lake in Turkey and is used for electrical energy production and irrigation. Its area and volume are 687. 31 km^2 and 30.6 million m^3. respectively [14].

In this study aimed to determine the oxidant (MDA) and the antioxidant (SOD, CAT, GSH and GSH-Px) levels in liver, Muscle, spleen, kidney and gonad tissues of *C.umbla* collected in three different periods before reproductive period (X) (February), reproductive period (Y) (May) and after reproduction period (Z) August) from Keban Dam Lake.

2. Experimental

C. umbla used in the research was caught between February and August from Keban Dam Lake. By determining the age from the scales of these fish. individuals reaching sexual maturity were preferred [15]. In the study, the development of fish in 3 different periods was investigated.

A- Before the reproductive (X) period; Samples were taken in February.

B- Reproductive (Y) period; Samples were taken in May.

C- After reproductive (Z) period; Samples were taken in August.

The caught fish were brought to Firat University Faculty in ice and autopsied in the laboratory. Liver, muscle, kidney, spleen and gonads were removed. wrapped in foils. and stored in a deep freezer at -20 °C until analysis.

MDA level assay: The level of MDA as a marker of lipid peroxidation was measured according to the method of Ohkawa, Ohishi and Yagi [16] on the basis of the reaction with thiobarbituric acid (TBA). The formed MDA created a pink complex with TBA and the absorbance was read at 532 nm. The MDA level of tissues was expressed as nmol g^{-1} tissue.

SOD activity assay: The SOD activity was determined according to the Sun, Oberley and Li [17] method. that is based on the principle that xanthine reacts with xanthineoxidase to generate superoxide radicals that react with nitroblue tetrazolium to form a coloured formazan dye. To analyse the SOD activity. 600 µL of the SOD reaction mixture containing 0.1 mM EDTA. 0.1 mM xanthine. 25 µmol L^{-1} of nitroblue tetrazolium and 50 mg of bovine serum albumin was added to 125 µL of the supernatant. Then. 25 µL of 9.9 nM xanthine oxidase solution was added to each tube. The amount of formazan found by measuring the absorbance at 560 nm using a spectrophotometer. The results of SOD activity are provided as U mL^{-1}.

GSH-Px activity assay: The level of GSH-Px was determined using the procedure described by Beutler [18]. which records the disappearance of NADPH through its absorbance at 340 nm. The procedure of analysis performed was based on the oxidation of GSH by GSH-Px coupled with the disappearance of NADPH by glutathione reductase measured at 37°C. The absorbance at 340 nm was placed on record over a period of 5 min. The activity was then calculated from the slope of the lines as µM of NADPH oxidized per minute. The GSH-Px activity was provided as U mL^{-1}.

GSH level assay: Glutathione concentration was determined by a kinetic assay using a dithionitrobenzoic acid (DTNB) recycling method described by Ellman [19]

and were expressed as µmol mL^{-1}. One millilitre of the sample was deproteinated by the addition of a solution containing 0.2 g of Na_2EDTA. 1.67 g of metaphosphoric acid and 30 g of NaCl in distilled water. DTNB and Na_2HPO_4 were added to the supernatants and cleared by centrifugation (10 min. 3000 g/min). The GSH level was measured based on its reaction with DTNB to yield a yellow chromophore. which was measured spectrophotometrically at 412 nm.

Statistical procedures: All results are expressed as mean ± S.E. The data were analyzed with an Independent-Sample T Test and Duncan Test. SPSS 12.0 for Windows was utilized for statistical analysis. The level of significance was set at $p < 0.05$.

3. Results and Discussion

In this study, the data obtained as a result of the analyzes were statistically evaluated and arranged in tables (Table 1). The changes in biochemical levels occur as aresult of maturation stage, so this was considered as an additional variable because of its effect on the mobilization and accumulation of reserves in several tissues. Accumulation of biochemical components, especially lipids, in the maturing ovary has been reported in fish and crustaceans [7,20,21]. For example, Palacious et al. [20] found that the level of total lipid, acylglycerides, cholesterol and total protein in mature ovaries of Penaeus vannamei increased, and acylglycerides represent 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. Wilhelm Filho et al. [22] determined that in *Perna perna*, the TBA-reactive substance content observed in May (reproductive period) were approximately double than those found in the rest of the year. Their gonads have a higher lipid and carbohydrate mobilization and protein synthesis. Bell et al. [23] and Cavalli et al. [24] demonstrated that highly unsaturated fatty acids, which are vital components of cellular membranes, are particularly susceptible to attack by reactive oxygen radicals. Uncontrolled damage to membrane fatty acids and the accumulation of their oxidized breakdown products can have deleterious consequences for cell and organ function and may increase the requirement for antioxidants [23,24]. This study has determined that MDA levels especially in the liver (table 1A) and ovarian (Table 1D) tissues were significantly higher in the X and Y periods compared with the Z period. The increase in MDA levels may be linked to the accumulation of lipids in tissues and the increased metabolic activity during the reproductive period.

Table 1. Comparison of the mean concentration of the tested malondialdehyde (MDA (nmol g^{-1} tissue)), superoxide dismutase (SOD (U ml^{-1}), glutathione peroxidase (GSH-Px (U g^{-1})), glutathione reductase (GR (U g^{-1} protein)) in tissues (liver (Tablo 1A), spleen (Tablo 1B), kidney (Tablo 1C), gonad (Tablo 1D) ve muscle (Tablo 1E)) of *C. umbla* in three different periods (X: before reproduction. Y: reproduction. Z: after reproduction) according to sex (S). P_1: Comparison of male and female in X. P_2: Comparison of male and female in Y. P_3: Comparison of male and female in Z. P_M: Comparison (x, y, z) of male in X, Y and Z. P_F: Comparison (a, b, c) of female in X, Y and Z. Significance between groups was shown as asterisk (*p < .05, **p < .01, ***p < .001).

Tablo 1A

Period	S	Parameters			
		MDA	SOD	GSH-PX	GSH
X	♂	31.72 ± 2.31 ^{y}	13.27 ± 1.59 ^{x}	1.12 ± 0.11 ^{xy}	0.46 ± 0.04
	♀	55.16 ± 3.25 ^{b}	8.12 ± 1.05 ^{b}	1.59 ± 0.29 ^{b}	0.33 ± 0.03 ^{b}
	P_1	***	**	**	*
Y	♂	32.95 ± 3.76 ^{x}	12.28 ± 1.30 ^{x}	1.18 ± 0.27^{x}	0.47 ± 0.04
	♀	80.19 ± 3.76 ^{a}	17.82 ± 2.01 ^{a}	2.18 ± 0.35 ^{a}	0.20 ± 0.01 ^{c}
	P_2	***	**	***	**
Z	♂	25.28 ± 2.03 ^{y}	7.16 ± 0.90 ^{y}	0.49 ± 0.22 ^{z}	0.48 ± 0.03
	♀	42.16 ± 3.27 ^{c}	8.09 ± 2.11 ^{b}	0.55 ± 0.12 ^{c}	0.47 ± 0.04 ^{a}
	P_3	***	-	-	-
	P_M	**	**	***	-
	P_F	***	***	***	***

Tablo 1B

Period	S	Parameters			
		MDA	SOD	GSH-PX	GSH
X	♂	27.18 ± 2.56^{x}	13.19 ± 2.66	1.40 ± 0.29	0.24 ± 0.05
	♀	43.07 ± 2.19 ^{a}	9.96 ± 1.95 ^{b}	1.02 ± 0.11 ^{b}	0.23 ± 0.03
	P_1	***	*	*	-
Y	♂	29.18 ± 2.21^{x}	12.98 ± 1.85	1.42 ± 0.29	0.26 ± 0.11
	♀	42.09 ± 3.06 ^{a}	15.06 ± 1.94^{a}	1.45 ± 0.21 ^{a}	0.25 ± 0.01
	P_2	***	*	-	-
Z	♂	20.19 ± 2.03^{y}	14.26± 2.23	1.38 ± 0.33	0.24 ± 0.01
	♀	21.16± 3.27 ^{b}	7.28 ± 1.87^{b}	1.40 ± 0.29 ^{a}	0.24 ± 0.03
	P_3	-	***	-	-
	P_M	**	-	-	-
	P_F	***	***	**	-

Tablo 1C

Period	S	Parameters			
		MDA	SOD	GSH-PX	GSH
X	♂	27.18 ± 2.56 ^{x}	10.87± 1.39	1.24 ± 0.25	0.19 ± 0.02
	♀	43.07 ± 2.19 ^{a}	10.03 ± 1.14 ^{b}	1.27± 0.38	0.18 ± 0.04
	P_1	***	**	-	-
Y	♂	29.18 ± 3.21 ^{x}	9.86 ± 2.06	1.25 ± 0.13	0.18 ± 0.03
	♀	42.09 ± 3.06 ^{a}	16.25 ± 3.02 ^{a}	1.27 ± 0.36 ^{a}	0.18 ± 0.06
	P_2	**	*	-	-
Z	♂	20.19+ ± 2.03 ^{y}	9.24 ± 2.13	1.26 ± 0.24	0.19 ± 0.01
	♀	21.16 ± 3.27 ^{b}	8.03 ± 1.73^{c}	1.28 ± 0.19	0.17 ± 0.04
	P_3	-	-	-	-
	P_M	**	-	-	-
	P_F	***	***	-	-

Tablo 1D

Period	S	Parameters			
		MDA	SOD	GSH-PX	GSH
X	♂	27.81 ± 1.20 ^{x}	11.13± 1.29 ^{y}	1.54± 0.39 ^{y}	0.21± 0.01
	♀	32.68 ± 1.95 ^{b}	15.62± 1.75 ^{b}	2.21 ± 0.76 ^{b}	0.19 ± 0.01 ^{b}
	P_1	**	**	**	-
Y	♂	29.68 ± 3.01 ^{x}	16.18 ± 1.87 ^{x}	3.16 ± 0.74 ^{x}	0.23 ± 0.02
	♀	44.28 ± 3.27 ^{a}	30.26 ± 1.08 ^{a}	5.13 ± 0.82 ^{a}	0.15 ± 0.02 ^{c}
	P_2	***	***	***	**
Z	♂	16.91 ± 1.96 ^{y}	5,32 ± 1.19 ^{z}	1.66 ± 0.21 ^{y}	0.23 ± 0.02
	♀	18.26 ± 2.06 ^{c}	4.86 ± 1.02^{c}	1.18 ± 0.32 ^{c}	0.28 ± 0.04 ^{a}
	P_3	-	-	*	*
	P_M	***	***	***	-
	P_F	***	***	***	***

Tablo 1E

Period	S	Parameters			
		MDA	SOD	GSH-PX	GSH
X	♂	20.06 ± 1.86 ^{x}	27.18 ± 2.94	3.28 ± 0.37	0.26 ± 0.03
	♀	17.86 ± 2.42 ^{b}	29.16 ± 3.51 ^{b}	4.29 ± 0.45	0.25 ± 0.02
	P	*	-	*	-
Y	♂	19.51 ± 2.37 ^{x}	25.14± 2.54	3.32 ± 0.26	0.25 ± 0.04
	♀	22.18 ± 2.61 ^{a}	30.96 ± 2.47 ^{b}	4.66 ± 0.88	0.24 ± 0.03
	P	*	*	*	-
Z	♂	13.21 ± 2.14 ^{y}	24.98 ± 3.27	3.05 ± 0.20	0.26 ± 0.02
	♀	11.26 ± 1.12 ^{c}	22.16± 3.32 ^{c}	4.82 ± 0.37	0.26 ± 0.07
	P	-	-	**	-
	P_M	**	-	-	-
	P_F	***	**	-	-

It was found in many studies that liver is metabolically more active and the oxyradical generating enzymes display comparatively higher activities than other tissues. Additionally, liver being lipid rich and for its high metabolic rate. it may undergo spontaneous autooxidation and thus the generation of O_2^{-*} and H_2O_2 may be comparatively more in this organ than other organs [25-27]. In this study, statistically significant changes in MDA levels according to sex were observed. These level were higher in liver, muscle, spleen and gonad tissues of males and females in kidney tissues of females. In addition, statistically significant changes were observed in the MDA level in the gonad tissue of both males and females in this study (Table 1D).

In many studies have been shown that OS plays a role in multiple physiological processes from oocyte maturation to fertilization and embryo development. Especially, in the initial stages of oogenesis increases the number of cell in mitochondria and with metabolism and incomplete reduction of oxygen during cell respiration. more O_2^{*-} is unavoidably synthesized from the liberation of electrons [28]. The aquatic organism were determined that OS observed in reproduction period were approximately double those found in the rest of the year and their gonads have a higher protein synthesis lipid acylglycerides and carbohydrate mobilization [20,22]. Similarly, Barim Oz and Yılmaz [4] determined that level of MDA increased approximately 100 % in the hepatopancreas and ovarian tissues of *A. leptodactylus* during reproduction. It was reported that highly unsaturated fatty acids are particularly susceptible to attack by reactive oxygen radicals. and uncontrolled damage to membrane fatty acids and the

accumulation of their oxidized breakdown products can have deleterious consequences for cell function [3,6]. The main cause for increase of MDA level in gonad may be the inability of the antioxidant mechanism as the results of excessive production of O_2^{*-}generation because of the accumulation of lipids and protein [4,29]. Moreover, the changes in tissue MDA level were interestingly highest in female compared with male. In immunocytochemical identifications of Lee and Chang [30] was found that the amount of the incorporated vitellogenin was high in the vitellogenin stage (stage III-V) according to the early stage (stage I-II) of ovarian development in *M. rosenbergi*. For this reason. the rise in MDA can be associated to peroxidation increasing because maximal gamet formation with high in precursor protein of egg yolk.

SOD, GSH-Px and GSH enzymes, used as a biomarker of ROS production are the first line of defence against oxidative stress. SOD catalyses the transformation of O_2^{-*} to H_2O_2 and water [31]. In the present study, the SOD activity in liver and gonad increased in the R periods compared to the BR and AR periods. Furthermore, this level in gonad of female was higher. Increased SOD level during high temperature, gonadal development and breeding period has also been determined in *P. perna* by Wilhelm Filho et al. [22], in *P. viridis* by Verlecar et al. [32] and in *S. glanis* by Bayir et al. [33]. High level of H_2O_2, change cell physiology through the production of OH^* by Fenton reaction in these periods were also observed [32]. For this reason, increase of SOD may be associated to neutralise the overproduction of O_2^- anions and H_2O_2 due to peroxidation. However, the activity of SOD was higher in spleen tissues in Y period according to X period. It may indicates an increasing need to destroy O_2^- in tissues during metabolic activity [34]. In the present study, the SOD activity in liver and gonad was significantly increased in the Y periods compared to the Y and Z periods. Furthermore, this level in gonad of female was higher. Increased SOD level during high temperature, gonadal development and breeding period has also been determined in *P. perna* by Wilhelm Filho et al. [22], in *P. viridis* by Verlecar et al. [32] and in *S. glanis* by Bayir et al. [33]. High level of H_2O_2, change cell physiology through the production of OH^* by Fenton reaction in these periods were also observed [32]. For this reason, increase of SOD may be associated to neutralise the overproduction of O_2^- anions and H_2O_2 due to peroxidation. However, the activity of SOD was higher in spleen tissues in Y period according to X period. It may indicates an increasing need to destroy O_2^- in tissues during metabolic activity [34].

GSH-Px is mainly involved in the removal of organic peroxides. Hence, GSH-Px is considered to play an very important role in protecting membranes from damage due to LPO [3,6,14]. The present study illustrates that the GSH-Px activity in gonad and liver of female and male, the spleen of female increased in the Y and X periods compared to the Z period. But, this level was not change muscle and kidney. This observation is in good agremeent with an earlier report [22,32,6,29] which the production of O_2^{-*} radicals increase during metabolic activities. The increased GSH-Px activity in the liver protected the organ from the formation of lipid peroxides by reducing H_2O_2 levels, which in turn attenuated OH^{-*} generation. It was reported that H_2O_2 is neutralized by two different enzymes present in the cellular system, they are GSH-P_x and CAT. 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 [12]. Furthermore, GSH-P_x is responsible for the neutralization of both inorganic and organic hydroperoxides. As paralel of our study, Nahrgang et al. [35] also determined that in *M. edulis*, during gonadal development and spawning season, the level of GSH-Px was higher those found in the rest of the year. As mentioned earlier, high level of GSH-Px might not be sufficient to reduce OS in reproduction period as evidenced by high level of MDA.

GSH, the non-enzymatic antioxidants, is a primary reductant and is the most abundant thiol-containing substance of low molecular weight in the cells. In this way, it serves multiple functions in protecting tissues from oxidative damage and keeping the intracellular environment in the reduced state. In addition, this enzyme

reduces hydrogen- and organic-peroxides *via* a reaction catalyzed by GSH-Px; it serves as a scavenger of OH^* and $^1O_2$ [3,6]. Our study found that the GSH activity in liver and gonad of female was generally lower in X period and Y period. Additionally, this activity was not changed in muscle, kidney and spleen. This idea was corroborated by the observations of Wilhelh Filho et al. [22] who described that the concentration of the GSH in *P. perna* decreased during reproduction period The decrease in this enzyme activities were likely responses to an increased utilization of GSH. Furthermore, severe oxidative stress may suppress GSH levels due to the impairment of reproduction mechanisms in *A. leptodactylus* [14,29,36]. Earlier findings also suggest that the presence of high GSH level is associated with the attenuation of oxidative stres [3,6].

4. Conclusions

As a result of the analysis, the MDA activity of all tissues increased with the reproductive period. Especially, statistically significant changes were detected in the analysed oxidant and antioxidant parameters of liver and gonad tissues in the X and Y periods compared with the Z period. The levels of SOD changed in both female and male tissues in all periods. The GSH-Px and GSH activity of kidney and muscle tissues were not effected by the reproduction period. For this reason, antioxidant substances should be added to the diets of these fish during the reproduction period in production studies to be carried out under culture conditions.

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