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Original Article

Effects of short-term starvation and re-feeding on antioxidant defense status in *Mesopotamichthys sharpeyi* (Günther, 1874) fingerlings

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Abstract: The objective of this study was to evaluate the impact of short-term starvation and re-feeding on oxidative stress in *Mesopotamichthys sharpeyi* (Günther, 1874). After two weeks adaptation to new conditions, a total number of 270 fingerlings were distributed into nine 300-L fiberglass tanks, equipped with aeration system in three treatments including 4, 8 and 16 days starvation (each in 3 replicates). After starvation, all groups were fed for 32 days. At the beginning of trial and at the end of starvation and re-feeding periods, blood samples were collected, plasma was separated and activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) content were assayed. Based on the results, no significant difference was observed in SOD activity at the end of starvation and also after re-feeding between the different treatments. At the end of starvation the activity of the CAT and GPX increased significantly in 8 and 16 days of starvation groups compared to the base. Moreover, a significant increase in MDA levels of plasma was found during food deprivation. These findings showed that short-time starvation increased antioxidant enzymes activity in plasma of *M. sharpeyi* but short-term food deprivation and re-feeding periods in this species does not cause disturbances in the antioxidant defense status.

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Introduction

Study of oxidative stress indices can show the physiological and health status of fish in starvation and re-feeding. Thus, the complete information provides regarding the optimal duration of these changes and return to the initial situation for any species (Furne et al., 2009). Most of investigations on oxidative stress in fish has been done on the aspects of toxicology, such as the influence of various xenobiotics and heavy metals on antioxidant defense system (Bayir et al., 2011), the comparison of biological transformation processes, on the intensity of lipid peroxidation and other biomarkers of oxidative stress (Trenzado et al., 2006). Also, other studies have been evaluated the effects of season or another stimulus on the activity of antioxidant enzymes (Trenzado et al., 2006). On the other hand, most of researches on fasting and re-feeding in fish are also concentrated on the muscular growth and metabolic and hormonal responses. Newly, the issues related to the impact of food restriction on hematological, biochemical, immune parameters and indices of antioxidant defenses system has been investigated (Pascual et al., 2003; Morales et al., 2004; Rios et al., 2005; Feng et al., 2009; Caruso et al., 2011). Starvation as a stressor activates the axis of hypothalamus-pituitary-interrenal, leading to increased release of glucocorticoid hormones and subsequently cause changes in fluid balance, function of cardiovascular and respiratory system and balance of peroxidation–antioxidant (Vázquez-Medina et al., 2010). Furthermore, nutritional restriction augments poisoning of chemicals and disease outbreaks in

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certain pathological conditions. These effects could be essentially related to the increment of ROS that are created by food shortage (Pascual et al., 2003). Oxidative stress occurs as a result of increased rate of ROS generation or decreased level of antioxidant defense, or both situations (Langseth, 1995). The poor food supply and starvation could cause the enhancement of oxidative damage of lipids, oxidative stress (Welker and Congleton, 2005) and activation of antioxidant defense in fish (Bayir et al., 2011). Many of fish increase antioxidant enzymes activity, when encountered with unendurable starvation for adaptation to new circumstances (Wilhelm Filho, 2007). It is well known that most important antioxidant enzymes include superoxide dismutase (SOD) which converts superoxide radical (O_2•–) to hydrogen peroxide (H_2O_2); catalase (CAT), which detoxifies H_2O_2, glutathione peroxidase (GPX) which detoxifies both H_2O_2 and organic hydroperoxides and glutathione reductase (GR), which accelerate the transformation of oxidized glutathione (GSSG) to reduced glutathione (GSH) (Morales et al., 2004). These enzymes destroy ROS or convert it into metabolites that have less toxicity (Romero et al., 2011). While the evidence suggests that oxidative stress may be involved in causing various diseases in fish, and since few studies have been conducted in the field of the effects of short-term periods starvation and re-feeding on the antioxidant defense in fish. In this study the influence of the short-term food deprivation and re-feeding on oxidative stress indices in *Mesopotamichthys sharpeyi* (Günther, 1874) has been studied.

Material and methods

Fish and rearing condition: The present experiment was carried out on *M. sharpeyi* fingerlings with mean weight of 6.13 ± 0.40 g. Before the beginning of the experiment, the fish were disinfected with brackish water, and were maintained for 2 weeks in 300-L tanks for acclimatization to laboratory conditions. During this period fish were fed with a commercial diet (Bayza, Shiraz, Iran) containing 41.04% crude protein, 5.83% crude fat and 11.45% ash, two times per day (9:00 am and 17:00 pm). Following the adaptation period, fish were weighed individually and distributed randomly to nine 300-L fiberglass tanks (40 fish/per tank) that equipped to aeration system.

Experimental design: To investigate the effects of starvation and re-feeding on blood parameters, three treatments including 4, 8 and 16 days starvation were considered with 3 replications. After starvation all groups were fed for 32 days. The duration of experiment was 48 days. The experimental design was performed such that re-feeding period was started in all treatments at same time. Tanks were placed in an indoor system to protect from the direct solar radiation. During re-feeding period, fish were fed to apparent satiation twice a day. Water temperature (25.6 ± 0.1ºC), dissolved oxygen (7.61 ± 0.05 mg/l) and pH (8.05 ± 0.03) were measured and recorded daily during experiment.

Sampling: Fish were deprived one day before sampling procedure. Fifteen specimens from each treatment (5 fish from each replicate) were randomly captured and were anesthetized with 2-phenoxy-ethanol (2%). The blood samples were taken from caudal vein at the beginning of the trial (as basal sample) and at the end of the starvation and re-feeding periods. Blood samples were centrifuged immediately at room temperature, plasma was separated and stored at -80ºC until analysis.

Analysis: SOD activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (RANSOD kit, Randox Com, United Kingdom). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4- nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction. One of INT under the condition of the assay unit of SOD was considered a 50% inhibition of reduction.

GPX was measured based on Paglia and Valentine (1967) (RANSEL kit, Randox Com, United

Kingdom). GPX catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was measured at 340 nm.

Catalase activity was measured according to Aebi (1984). Briefly, tissue sections were homogenized in triton X-100 1% (Merck, Darmstadt, Germany) and the homogenates were diluted with phosphate buffer (pH 7.0). The reaction was initiated by addition of hydrogen peroxide to the reaction mixture and the level of enzyme activity was quantitated according to the ability of the tissue catalase to decompensate hydrogen peroxide by monitoring the decrease in absorbance at 240 nm against a blank contains phosphate buffer instead of substrate. The value of log A1/A2 for a measured interval was used for unit definition owing to the first-order reaction of enzyme. One unit of CAT is the amount of enzyme that decomposes 1.0 mmole of hydrogen peroxide per minute at pH 7.0 and 25ºC.

The total amount of plasma lipid peroxidation was indicated by the content of MDA as described by Buege and Aust (1978). Briefly, one volume of plasma was mixed with two volumes of a stock solution of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid and 0.25 mol) hydrochloric acid thoroughly. The solution heated for 15 min in boiling water bath. After cooling, the precipitate is removed by centrifugation at 1000 g for 10 min. The absorbance of the clear supernatant determined at 535 nm.

Data were expressed as mean ± SE (Standard Error). Means were analyzed by one way analysis of variance (ANOVA) test using SPSS 16, and where significant difference were indicated, means were tested using Tukey's post hoc to compare the means of treated groups against that of the corresponding control. $P<0.05$ was the accepted significance level.

Results

The specific activity of the antioxidant enzymes is

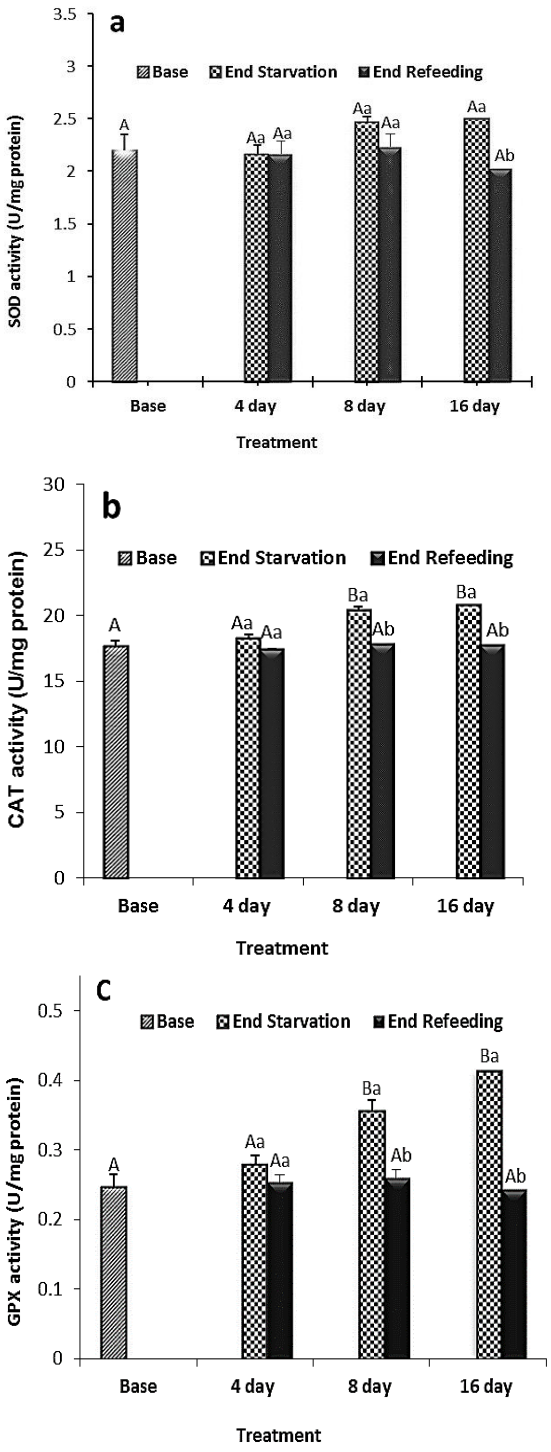

Figure 1. Effects of starvation and Re-feeding on SOD activity (a), CAT activity. (b) and GPX activity (c) in *Mesopotamichthys sharpeyi* (Günther, 1874). Similar capital letters represents no significant differences between the base sample and other experimental groups at the end of starvation and after re-feeding ($P>0.05$). Small letters indicate changes in the activities of these enzymes in each treatment. Data are presented as mean ± SE. Differences were considered statistically significant at the 0.05 probability level

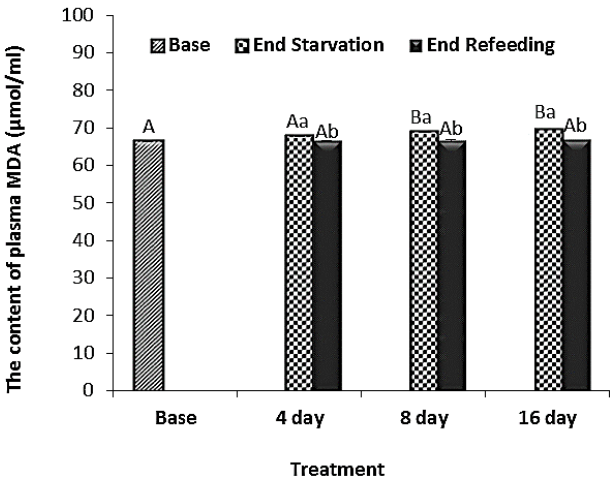

Figure 2. The content of plasma MDA *in Mesopotamichthys sharpeyi* (Günther, 1874) fingerlings during the short-time starvation and Re-feeding. Similar capital letters represents no significant differences between the base sample and other experimental groups at the end of starvation and after re-feeding (P>0.05). Small letters indicate changes of the plasma MDA in each treatment. Data are presented as mean ± SE. Differences were considered statistically significant at the 0.05 probability level.

shown in Figure 1(a-c). No significant difference was found in the activity SOD at the end of the fasting and re-feeding period compare to base (P>0.05) (Fig. 1a). The comparison of SOD activity in each treatment represents a significant decline only in the 16 days starvation after re-feeding (P<0.05). The CAT activity significantly increased in 8 and 16 days starvation groups compared to the base and returned to initial values after re-feeding (P<0.05) (Figs. 1b and 1c, respectively). Moreover, comparison of variations in the activity of CAT in each treatment, indicates significant differences in the food-deprived groups of 8 and 16 days, although in 4-days starvation group no significant difference were recorded at the end of fasting and re-feeding period (P>0.05). Similar results were recorded for GPX, as its activity increased in 8 and 16 days starvation groups compare to base. GPX activity was decreased in these groups after re-feeding period.

As presented in Figure 2, the plasma content of MDA significantly increased as starvation time increased (P<0.05). Whereas, no significant differences were detected between the different starved groups and the base level after re-feeding (P>0.05).The statistical analysis of changes of plasma MDA in each treatment represents significant decrement of this index in the three experimental groups (P<0.05).

Discussion

In the present study, the GPX and CAT activities increased along with the extension of the starvation period. Increased activity of CAT and GPX simultaneously, represents increased production of H_2O_2 throughout the cell (Ritola et al., 2002). Bayir et al. (2011) reported that total or partial food deprivation enhances the production of ROS in liver and gills of brown trout (*Salmo trutta*). Moreover, enhanced ROS rates have been reported as a consequence of starvation in *Sparus macrocephalus* (Zhang et al., 2007) and yellow croaker (*Pseudosciaena crocea*) (Zhang et al., 2008). Like *M. sharpeyi*, limited food availability was caused an augmented production of H_2O_2 in Wistar and Goto-Kakizaki (GK) rats (Santos et al., 2009). Parihar et al. (1997) concluded that oxidative agents in cells may lead to enhancement of antioxidant enzymes as a defense mechanism. Stimulation of antioxidant enzymes have been recognized as an important defensive line against oxidative stress in biological systems (Velisek et al., 2011). In fact, when an organism is exposed to conditions that enhances the ROS generation rates, will produce and activate more antioxidant enzymes to cope with additional reactive oxygen species. When the aerobic organisms were faced with energetic restrictions such as food shortage and/or severe hypoxia, maintain relatively high levels of their antioxidant components to deal with oxidative stress (Ansaldo et al., 2007). Pascual et al. (2003) observed a significant increase in the SOD and GPX activities after food deprivation in Gilthead seabream (*Sparus aurata*). In Common dentex (*Dentex dentex*), Morales et al. (2004) found a marked increase in activities of SOD, CAT and GPX after 5 weeks food restriction. A similar increase in the activity of SOD, CAT and GPX was found in the liver and gills of brown trout (*Salmo trutta*) (Bayir et al., 2011).

Guderley et al. (2003) also detected that fasting leads to increase GPX and GST activities in the liver of the starved Atlantic cod (*Gadus morhua*). Increased GPX activity had been reported in Wistar and Goto-Kakizaki (GK) rats following food deprivation, whereas no observed any significant in the Mn-SOD activity in the both strains. SOD, CAT, GPX and GR are key enzymes in the antioxidant defense system (Furné et al., 2009). SOD converts superoxide anion ($O_{2.-}$) to water (H_2O) and hydrogen peroxide (H_2O_2). Hence, increased SOD activity in the starved fish represents the production the high levels of $O_{2.-}$.On the other hand, increased SOD activity is led to an incensement in the production of H_2O_2, which could be the reason for the increase in CAT and GPX activities (Pascual et al., 2003; Morales et al., 2004; Zhang et al., 2007, 2008; Bayir et al., 2011). CAT is a key antioxidant enzyme to remove H_2O_2 and also a basic mechanism to restrict the formation of hydroxyl radicals highly reactive (Regoli et al., 2000b). Increment in CAT and GPX activities in the current study may be not only due to increased H_2O_2, but also caused by diminish in lipid peroxidation that has led to oxidative stress. Inal et al. (2001) expressed that the increase in GPX activity could be related to lipid peroxidation. In addition, the high activities of CAT and GPX have been reported to reduce lipid peroxidation in mammals and fish (Barja de Quiroga et al., 1989; Rodriguez-Ariza et al., 1993; Regoli et al., 2005). Valko et al. (2006) reported that peroxisomal fatty acid oxidation is a potentially important source of H_2O_2 production during prolonged starvation. Hence, according to the results, the increase in CAT activity and GPX, and no significant changes in the activity of SOD could be due to the formation the excess H_2O_2 by the metabolism of peroxisomal long chain fatty acids.

Oxidative damage often assessed through the determination of lipid peroxidation in the form of MDA (Huang et al., 2010). MDA is one of the compounds resulting from lipid peroxidation that is formed as a result of the decomposition of lipid peroxides in the presence of iron or copper during the Fenton reaction (Rumely and Paterson, 1998). Based on the results, the plasma MDA content increased in parallel with food deprivation. Increased plasma MDA content in the present study represents increased lipid peroxidation and oxidative stress which correspond with the observed increase in CAT activity and GPX. Similar findings previously have been obtained in rainbow trout (*O. mykiss*) and sea bream (*S. aurata*) deprived of food that starvation caused an increase in MDA levels in the liver (Hidalgo et al., 2002; Pascual et al., 2003). Also, Morales et al. (2004) found that long-term food deprivation increases the MDA level in the liver of starved *D. dentex* compared to the control group. Furthermore, Bayir et al. (2011) in a study on Brown trout (*S. trutta*) observed that MDA level in the gill and liver rises with increasing time of starvation. A remarkable increase in the lipid peroxidation indices can show high sensitivity of lipid molecules into ROS attack and determine the amount of oxidative damage imposed to these molecules (Velisek et al., 2011).

In the present study, a positive relationship was recorded between enzyme activities and time of starvation. Increase in the activity of these enzymes could be due to increased use of lipid reserves that followed by augmented production of ROS. Activity of evaluated enzymes returned to normal after re-feeding showing compensatory capacity of *M. sharpeyi*.

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