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Oxidative Stress Responses Subjected to Heat-Stressed Tilapia Fish

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ABSTRACT

Warming water temperatures due to climate change pose a significant threat to numerous aquatic species. This study was conducted to examine the oxidative stress level on enzyme activities of tilapia blood plasma subjected to heat stressed. Thermal exposure was conducted based on preliminary study at 31 °C and subjected to gradual acclimation (1˚C/8h) from 28 to 31˚ for 14 days. Result showed that all the antioxidant enzymes level of superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) on blood plasma were significantly ($p<0.05$) increased with the duration of exposure. The increasing of antioxidant levels indicating possible negative effect on physiological performance and fitness, which in turn implicates potential threat of increased global temperature to aquaculture production.

1. Introduction

Freshwater fishes are known as ectothermic species that are sensitive to extreme environments. Freshwater fish react directly and indirectly towards any disruptive change in water quality parameters. Exposure to prolonged low water quality result in the alteration of internal substances, cell structure properties as well as metabolisms. Studies have shown that the effects of increased global water temperature have been dispersing throughout the aquaculture industry around the world [1], causing a negative socio-economic impact regarding global food production [2]. Oxidative stress is described as a stress mechanism in fish biology involving reaction components called reactive oxygen species (ROS), several reactive molecules and free radicals derived from molecular oxygen[3].

The occurrence of oxidative stress due to inefficient and insufficient of antioxidant defence system associated with the imbalance production of ROS during stress condition leads to energy utilisation in fish to cope with or tolerate environmental stress [4].

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To date, the effect of temperature on freshwater tropical fish has only been experimented in a few commercial fish such as goldfish (*Carassius auratus*) [5] and grass carp (*Ctenopharyngodon idellus*) [6]. Studies on oxidative stress have been focused more on marine fish such as gilt head bream (*Sparus aurata*) [7], greater amberjack (*Seriola dumerili*) [8] and seabass, (*Dicentrarchus labrax*) [9]. Other studies focused on the effects of toxic chemical such as glyphosate in silver catfish (*Rhamdia quelen*) [10], endosulfan in tilapia Mosambica (*Oreochromis mossambica*) [11], malathion in rohu (*Labeo rohita*) [12] and atrazine in grass carp (*Ctenopharyngodon idella*) [13].

According to Parihar *et al.,* [14], superoxide dismutase is the first defence system against oxidative stress by dismutating the superoxide radical. The defence reaction produces a nonreactive hydrogen peroxide in aqueous form that reacts actively with other superoxide radicals and non-radicals. This uncontrolled reaction is a precursor to generate more reactive ROS radicals. Hence, a catalase will neutralise the hydrogen peroxide to generate water and oxygen molecules. Furthermore, glutathione-S-transferase (GST) helps in discharging organic anions and other hydrophobic compounds from xenobiotics. The multitalent GST was suggested to be a biomarker for detoxification during fish stress [15].

Therefore, any study on the thermal stress effect on physiological changes of fish through oxidative stress mechanism towards its surrounding ecosystem would provide a more valuable information for industrial uses. The evaluation of oxidative stress involves the determination of regulation activity of antioxidant enzymes i.e., superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST). Findings on these oxidative parameters in *Oreochromis* sp. will provide some useful information for biomarker use in line with the current climate change issue. It is hypothesized that thermal stress will affect activity thus influences the interaction of antioxidant enzymes to counteract ROS over time.

2. Methodology

This study was conducted at the Aquaculture Research Centre, under Department of Agrotechnology and Bio-Industry, Polytechnic Sandakan Sabah. Tilapia, *Oreochromis* sp. with body weight of 450.0 ± 15 g and 25.0 ± 3.3 cm total length were sampled from the pond culture of Polytechnic Sandakan Sabah. At the sampling time, the water quality parameters of the water at the pond culture were recorded by using YSI 556 MPS (USA) with range between 26–28 °C, 3.5–5 ppm, 6.8–7.3 pH, 6.5–7.0 ppt for temperature, dissolved oxygen, pH, and salinity, respectively. Healthy fish were brought back and acclimatized in the recirculating fiberglass aquaculture system (RAS). Fish were fed at 3.0% of their body weight (BW) twice daily with commercial pelleted feed (Star Feedmills Sdn Bhd).

2.1 Experimental Design: Fish Sampling and Plasma Extraction

The lethal temperature (LT_{50}) and loss of equilibrium temperature, also known as thermal tolerance limits (24h) or critical thermal maximum (CTmax) were previously experimented to be 33.6 °C and 31.6 °C [16] determined from Beitinger *et al.,* [17], Ospina and Mora [18] and Souchon and Tissot [19] respectively. Six random fish samplings were conducted for control group and at day 1, 7 and 14.

Water quality parameters during the treatment were measured and recorded using multiparameter YSI 556 MPS (USA). Individual fish was anesthetized with NIKA Transmore (0.1 ml/L) prior to blood collection. Approximately 2 mL of blood was collected from the gills by using 23 G needle and allocated into 2 vacutainer tubes. Plasma samples were obtained by centrifuging the blood in heparinised tube (2,000 rpm) at 4 °C for 20 min [20]. The obtained supernatant was stored and frozen at -20 °C until analysis. Blood plasma was analysed for total protein [21] content before being subjected to an antioxidant assay.

2.2 Superoxide Dismutase

Superoxide dismutase (SOD) was assayed using McCord and Fridovich [22]. Twenty µL plasma was added to 220 µL freshly prepared buffer solution containing 2-(4-iodophenyl)-3-4(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H- tetrazolium, monosodium salt (pH 7.0) and 10.7 mM EDTA (Sigma, USA). The reduction of oxygen reaction was related to xanthine oxide (XO) (Sigma, USA) that inhibited by SOD. Four vials (blank 1 contained 20 µL double-distilled water (ddH20) and 20 µL enzyme; blank 2 contained 20 µL sample and 20 µL dilution buffer; blank 3 contained 20 µL dilution buffer and 20 µL ddH2O; blank 4 contained 20 µL sample, 20 µL dilution buffer and 20 µL enzyme) were prepared according to the method provided. Then, 200 µL of buffer solution was added to all the vials and incubated at 37 °C for 20 min. Absorbance of water soluble formazon in blue colour resulting from the XO reaction and reduction of SOD was measured using a microplate spectrophotometer at 450 nm. Levels of SOD activity were calibrated against SOD standard curve and expressed as $U.min^{-1}$ of total protein. The calculation of SOD activity is as follows:

$$\text{SOD activity} = \frac{(\text{Absorbance blank 1} - \text{Absorbance blank 3}) - (\text{Absorbance of sample} - \text{Absorbance of blank 2})}{(\text{Absorbance of blank 1} - \text{Absorbance of blank 3})} \times 100$$

2.3 Catalase

Catalase activity was assayed using spectrophotometric method from Beers and Sizer [23] using 4 mL quartz cuvette. The reaction was measured based on the reduction of hydrogen peroxides to water and hydrogen. Eighty µL tissue extract was added to 2.92 mL of 0.030% $v.w^{-1}$ hydrogen peroxide, and absorbance was measured at 240 nm at 1.5 min interval. Bovine catalase (Sigma, USA) diluted in 100 mL of 50 mM phosphate buffer was used as standard. The activity was calculated using the molar extinction coefficient for hydrogen peroxide of 0.040 $nmol.L^{-1}$. The calculation for catalase activity was expressed as $U.mg^{-1}.min^{-1}$ of total protein as follows:

$$\text{Catalase activity} = \frac{(\text{Absorbance/min})}{(0.040 \text{ x mL protein})}$$

2.4 Glutathione-S-Transferase

Glutathione-S-transferase (GST) was assayed as described by Habig *et al.,* [24] using a microplate reader. The reaction was measured based on the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma, USA) with reduced glutathione (GSH) (Sigma, USA) catalyzed by GST. The reaction master mix was freshly prepared by mixing 9.8 mL of Buffer Dulbecco, 0.1 mL of 200 mM reduced glutathione solution and 0.1 mL of 100 mM CDNB (1-chloro-2,4-dinitrobenzene) in 95% ethanol solution.

A total of 180 µL of reaction master mix was allocated to each well in microplate and 20 µL of sample was added directly to the microplate and mixed well. The absorbance at 340 nm can be determined using the extinction coefficient of 0.0053 nm over a period of 6 min after a lag time of 1 min. Levels of GST activity were calibrated against GST standard curve and expressed as nmol.mg^{-1}min^{-1} of total protein.

The calculation for GST activity is as follows:

$$\text{GST activity} = \frac{(\text{Absorbance/min})}{(0.040 \text{ x mL protein}} \text{ x } \frac{0.2\text{mL}}{0.02\text{mL}} \div \text{total protein}$$

2.5 Data Analyses

The data collected were analysed as mean ± SE (standard error), sample performances were checked primarily using Shapiro-Wilk Test as well as homogeneity using Levene Test for normality satisfaction. One-way analysis of variance (ANOVA) was performed to determine the effects of the time period (day exposure) and their interactions on oxidative stress responses (lipid peroxidation, superoxide dismutase, catalase, glutathione-S-transferase) in muscle, liver and kidney parameters. A post-hoc least significant difference (LSD) test and Tukey-Kramer HSD post hoc test was used to separate all means which differ among groups. Relationship between the variables were analysed through a Person Correlation test. Statistical analyses were performed using IBM SPSS Statistics version 27.0, and the level of significance for all tests was set at $p < 0.05$.

3. Results

3.1 Antioxidant Activity

The mean SOD, CAT and GST activities in the blood plasma of tilapia *Oreochromis* sp. for control, days 1, 7 and 14 is shown in Figure 1. The mean SOD in the blood plasma on control, days 1, 7 and 14 were 29.97 ± 4.90, 38.59 ± 4.45, 44.78 ± 6.35 and 56.22 ± 5.31 Umin^{-1} of total protein, respectively, indicating gradually increased activities with increased duration of exposure. The SOD level was significantly higher ($p < 0.05$) on day 14 compared with control, days 1 and 7. However, there was no significant difference ($p > 0.05$) between control, days 1 and 7.

The mean CAT in the blood plasma on control, days 1, 7 and 14 were 24.66 ± 1.10, 29.50 ± 1.36, 28.38 ± 1.67 and 33.92 ± 1.29 U.min^{-1} of total protein, respectively, indicating gradually increased activities with increased duration of exposure. The results showed significantly higher ($p < 0.05$) CAT activity in blood plasma on day 14 compared with control, days 1 and 7. The mean GST in the blood plasma on control, days 1, 7 and 14 were 14.61 ± 0.80, 16.76 ± 2.12, 17.46 ± 1.40 and 21.40 ± 1.95 nmol.min^{-1} of total protein, respectively, indicating gradually increased activities with increased duration of exposure. The GST activity levels on days 7 and 14 were significantly higher ($p < 0.05$) compared with day 1 and control. However, the GST activity levels on days 7 and 14 did not differ significantly ($p>0.05$). There was a significant positive correlation between GST level with SOD and GST, with r coefficient 0.305 and 0.455, respectively.

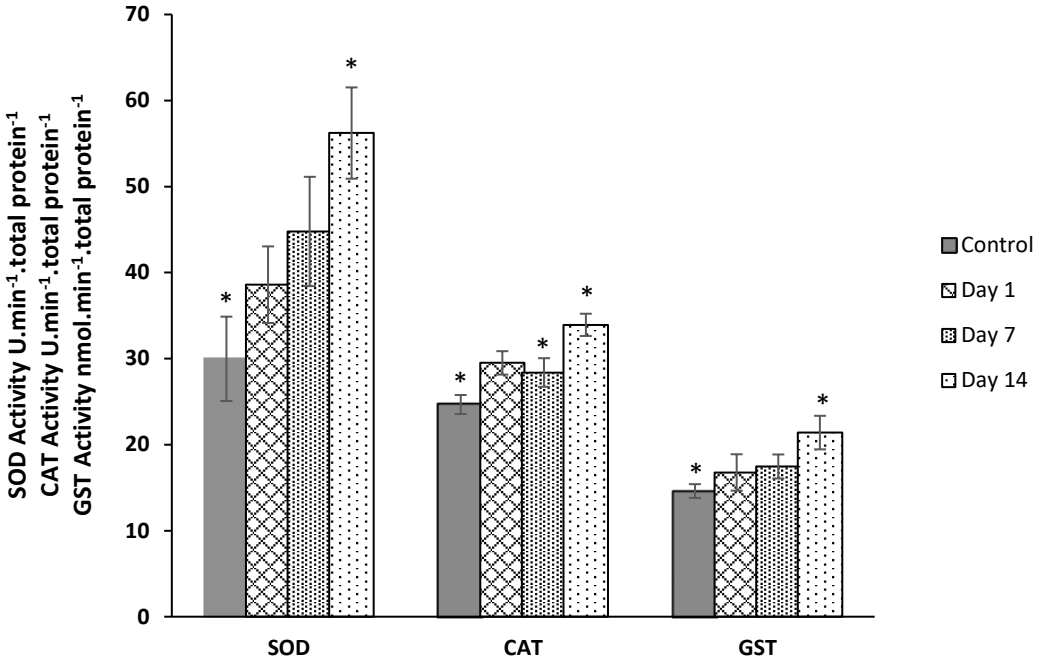

Fig. 1. Superoxide dismutase (SOD, U.min-1 of total protein), catalase (CAT, U.min-1 of total protein) and glutathione-S-transferase activities (GST, nmol.min-1 of total protein) of tilapia exposed to heat stressed with the duration of exposure (means ± SE);* denotes ($p<0.05$) between groups with similar enzyme

Figure 2 shows the relationship between the duration of exposure and antioxidant enzyme activities (SOD, CAT, GST) in blood plasma of tilapia *Oreochromis* sp. The enzyme SOD (R2 = 0.467), CAT (R2 = 0.334) GST (R2 = 0.305) activity showed a positive relationship with the duration of exposure. As the duration of exposure increased, all the enzymes increased in activity. The CAT (= 29 $U.min^{-1}$ of total protein) and GST (= 18 $nmol.min^{-1}$ of total protein) activities were almost at the same level, and it was about half of that of SOD (= 42 $U.min^{-1}$ of total protein) activity.

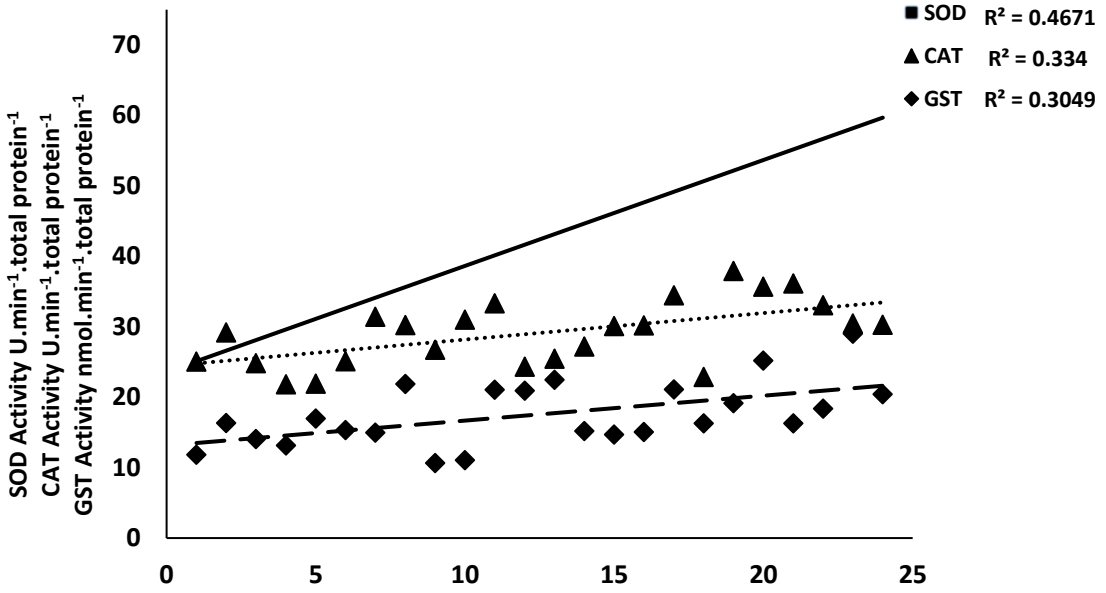

Fig. 2. Plot activities of superoxide dismutase activities (SOD, U.min^{-1} of total protein) (solid rectangle), catalase activity (CAT, U.min^{-1} of total protein) (solid triangle) and glutathione-S-transferase (solid diamond) (GST, nmol.min^{-1} of total protein) of blood plasma tilapia *Oreochromis* sp.

Fish may be threatened by the rising temperatures as a result of global warming. Many organisms adapt to climate variability through alterations of internal body system including the regulation of physiological defence system and integration of oxidative stress mechanism [4].

Heat stress influences the cellular body to produce reactive oxygen species (ROS), thus the prolonged stress consequently increases the amount of ROS thus showing a significant activity of antioxidant defence system in the biological functions of organisms. Excessive ROS accumulation in membrane systems could cause a membrane disturbance that led to membrane leakage and consequently disturbed all the regulation cycles and induced physiological disturbances [25,26].

Antioxidant enzymes can be found in almost all physiological fish systems especially in blood and organ tissues [27]. In our study, we found that these enzymes (SOD, CAT and GST) production tend to increase significantly with increased temperature. The results were in alignment with Vinagre *et al.,* [28] which reported higher activities of SOD and CAT of rock goby (*Gobius paganellus*). The significant increase of antioxidant defence activities may indicate that this tissue is highly sensitive to thermal stress, in which its activities in all tissues can be used as bioindicator of oxidative damage.

The increase of SOD activity was probably associated with the activation of SOD or the ability of the cells to scavenge free radicals. Generally, most of fish locomotory system possess on anaerobic metabolism, which use high amount of oxygen consumption [29]. Therefore, high amount of SOD activity in blood is probably associated with high reduction of oxygen to superoxide radicals [30]. High activity of SOD will in turn accumulate some amount of hydrogen peroxide over a short period, thus induce the antioxidant activity in fish blood [31]. From our observations, we found that, at high temperature, fish were passive. This suggested that metabolic system was able to function continuously and probably develop a new adaptation system [31].

From our experiment, catalase activity also was slightly increased in activity. A study by Madeira *et al.,* [7] on *Sparus aurata* showed increased catalase with the increasing water temperature, which suggested that it could be the main scavenger tissue to counteract ROS in the physiological system. Typically, the peroxidase activity of catalase reacts at relatively low concentrations of hydrogen peroxide [32] into oxygen and water. Besides superoxide dismutase (SOD) and catalase (CAT), glutathione-S-transferase (GST) are also important components in the oxidative stress defence system of fish [33].

Our results showed slightly increased GST activity in blood plasma. This enzyme catalysis the terminal steps in oxidative defence pathway. It is possible that at increasing water temperature, the increase in GST activity is probably attributed to the functional defence system. Similarly, an increase in GST levels was also observed in *Carassius auratus* [25] exposed to high temperature. Similarly, a study carried out by Madeira et *al.,* [7] demonstrated that temperature had a direct effect on the GST activity in blood tissue. The differences of antioxidant activity enhanced by enzyme instability may explain heterogeneity among the various tissues supporting the current data [31]. A 2-fold increase in GST activity in blood plasma suggested a higher accumulation of free radical production in blood plasma. These results also indicate that blood is an effective tool in elimination and detoxification of ROS. High antioxidant activity is very important for the improvement of counterbalance activities in stress tolerance [15].

An increase in SOD, CAT and GST levels in blood plasma, which is considered as metabolites that derived from various part of regulation system included from the organ that needs high oxygen consumption rate and high level of antioxidant defence system [34,35]. As all the antioxidant activities in blood showed the cell injury from various cell, the level may further increase as the increased of the degree injury of the cells with further increased of day exposure.

4. Conclusions

In this context, these results are significant in the context of climate change and its impacts on fisheries and aquaculture because over induction of oxidative stress due to water warming can induce health problems, mortality and shortened lifespan of fishes. Future studies on the mechanisms of stress response such as cell signalling and gene expression, are needed to investigate the adaptation mechanism in tilapia exposed to high water temperature. Besides, investigating the physiological responses of fish exposed to a longer experimental duration will provide us information on the impacts of global warming on fish production.

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