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Physiological Effects of Thermal Stress on Red Hybrid Tilapia

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Abstract: Exposure to any type of stressors either environmental, chemical or perceived stressors, results in a series of physiological responses in animals. The purpose of this study was to investigate the effects of thermal stress on physiological responses in red hybrid tilapia. Adult red hybrid tilapia were previously exposed to gradual increment of water temperature in thermoregulated recirculating tanks at a rate of 1°C/8 h from 28 to 31°C and kept for 1, 7 and 14 days. Cortisol, HMG-CoA reductase, total protein and osmolality were determined. Plasma cortisol and HMG-CoA levels were significantly increased in the heat-stressed groups compared to non-stressed groups. Osmolality was also significantly higher ($p<0.05$) at day 14 in heat-stressed groups compared to non-stressed groups. Our study demonstrated that exposure to elevated water temperature affect physiological parameters in red hybrid tilapia, thus suggesting a potential threat to its biological performance.

Key words: *Red hybrid tilapia, thermal stress, physiology*

INTRODUCTION

The rise of water temperature due to climate change puts additional stress on the freshwater ecosystems [1]. Aquatic biophysical factors such as temperature, salinity and dissolved oxygen are among the major factors influencing the aquatic environment [2]. For instance, in aquatic ectothermic animals, the rates of their many physiological processes are influenced by the water temperature which directly affects their biological performance. The performance of aquatic animal could be affected by any changes of the environment due to the role of water temperature as a major metabolic modifier [3]. Water temperature affect the oxygen solubility in the water thus affect metabolism of aquatic organism [2]. This condition will lead to a spatial shift in animal distribution [4] as water temperature limits their physiological functions. For economically important freshwater fishes, thermal changes would pose threats to aquaculture yield and productivity. Changes in the water temperature can induce either detrimental or adaptive alteration in the performances of aquatic animals. Tilapia is highly cultured in Asian countries as important food resources due to its delicious meat that contains high amount of protein [5]. A number of studies have been conducted on the impacts of global climate change on the aquatic ecosystems particularly the marine ecosystem[6],[7],[8],[9], with less studies focusing on the freshwater ecosystem.

To date, the effect of temperature on freshwater tropical fish has only been examined in a few commercially important fish species such as common carp, *Cyprinus carpio* and rohu, *Labeo rohita* [10], as well as one species of tilapia, i.e. *Tilapia mossambica* [11]. HMG-CoA reductase is an enzyme that involve in the production of cholesterol, which also plays an important role during stress with respect to internal homeostasis [12]. In addition, cholesterol is an important precursor for steroid biosynthesis (cortisol) in the mitochondrial inner membrane [13][14][15]. However, limited research has addressed the importance of this enzyme in relation to cortisol synthesis.

Studies have shown that the effect of increased global water temperature has been dispersing throughout the

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aquaculture industry around the world [16], possibly causing a negative socio-economic impact in regards to global food production [17]. Thus this study was developed to determine the effect of elevated water temperature on physiological responses in red hybrid tilapia, one of the most globally cultured freshwater fish species.

RESEARCH DESIGN AND DATA GATHERING PROCEDURES

Healthy adult male red hybrid tilapia, *Oreochromis sp.* were purchased from a local supplier (n=168; mean total length of 37.0 $\pm$ 3.2 cm and mean body weight of 500.0 $\pm$ 15.0 g). Prior to the experiments, the fish were acclimated for two weeks in thermoregulated recirculating tanks. A preliminary study was carried out to determine the 24-hour thermal tolerance endpoint, i.e., the lethal temperature (LT_{50}), and the loss of equilibrium temperature ($24hLET_{50}$). Briefly, a total of 24 healthy adult male fish were randomly selected and exposed to gradually elevated temperature at a minimum rate of 1°C/8 h [18] from 28 to 30°C, 28 to 32°C, 28 to 34°C and 28 to 36°C in duplicate in 1,000L aerated fibreglass tanks equipped with thermoregulator of ±0.1°C accuracy. 24h LT_{50} and $24hLET_{50}$ for tilapia were identified to be 33.6°C and 31.6°C, respectively [19].

Fish were euthanized by anaesthetic (MS-222) overdose within 1 min after capture, and blood sample was collected within 10 min via cardiac puncture using heparinised 1 ml syringe with 21G 1" needle, and kept in heparinised vacutainer tube on ice. Blood samples were spun down at 6000 g for 5 min to separate the plasma from the blood cells. The supernatant (plasma) was transferred into a clean 1.5 ml tube in aliquots and stored at -20°C until further analyses.

Cortisol

Plasma cortisol level was determined using Enzoscience Cortisol EIA Kit (Farmingdale, New York, USA). Thawed plasma and standards were pipetted into a 96-well microplate. All solutions were prepared according to the manufacturer`s instruction. The assay validity for fish cortisol has been reported to be comparable to Cayman Cortisol Assay Kit [20]. Cortisol standards were used to generate the standard curve. Plasma cortisol levels were measured at 405 nm using Halo MPR-96 Visible Microplate Reader (Dynamica GmBH, UK) by comparing with a range of standard concentrations.

HMG-CoA reductase

HMG-CoA reductase activity in plasma sample was measured using HMG-CoA Reductase Assay Kit (Sigma-Aldrich CS1090) according to the manufacturer's instructions using HMG-CoA as substrate and NADPH as cofactor. The resulting absorbance was measured at 340 nm in 96-well plate reader (Halo MPR-96, Dynamica GmBH, UK). The concentration of HMG-CoA reductase was expressed as mg/dL.

Osmolality and total protein

Plasma osmolality (mOsm/kg) was determined using a freezing point depression osmometer model 3320 (Advanced Instruments, USA). The total plasma protein was determined by Bradford assay [21]. Approximately, 200 µL of the respective blood plasma were diluted with 0.8 mL of distilled water and gently mixed with 5 mL of ready-made Bradford Reagent (Sigma B6916). After the incubation at room temperature for 5 min, the absorbance of solution was measured using a Shimadzu UVmini-1240 spectrophotometer at 595 nm (Shimadzu, USA). Bovine serum albumin (BSA) (Thermo scientific, USA) was used as standard for standard curve preparation. The protein concentration was calculated based on the plotted linear graph of standard curve and expressed as g/dL

DATA ANALYSIS

Data obtained were compared with the control. The differences between experimental groups were analyzed by Student's t-test using SPSS 20.0 (IBM Corp., USA). A value of $P<0.05$ was considered to be statistically significant. Data was presented as mean $\pm$ standard error of the mean (S.E.M).

RESEARCH FINDINGS

Results

Plasma cortisol levels for days 1, 7 and 14 in heat-stressed groups were 92.0 $\pm$ 0.00, 97.76 $\pm$ 0.03 and 91.04 $\pm$ 0.08 pg/mL, respectively. The cortisol levels increased steadily with the duration of exposure for days 1 and 7 but slightly reduced on day 14. The cortisol levels increased significantly ($p<0.05$) in heat-stressed group compared to control (Figure 1).

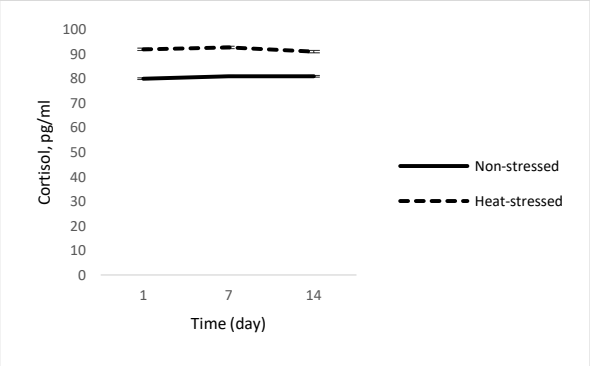

Figure 1 Cortisol levels in non-stressed and heat-stressed groups of red hybrid tilapia exposed to 31ºC

The mean HMG-CoA reductase levels in the plasma samples from male red hybrid tilapia *Oreochromis* sp. for days 1, 7 and 14 are presented in Figure 2. The mean HMG-CoA reductase levels (mg/dL) in the plasma on days 1, 7 and 14 were 77.49 ± 11.24, 115.76 ± 14.78 and 126.20 ± 8.51, respectively. Our results showed gradual increase in HMG-CoA reductase level as the duration of exposure was increased. There was a significant difference (p<0.05) in the HMG-CoA reductase levels among the days of treatment, in which day 14 was significantly higher (p< 0.05) followed by day 7 and 1.

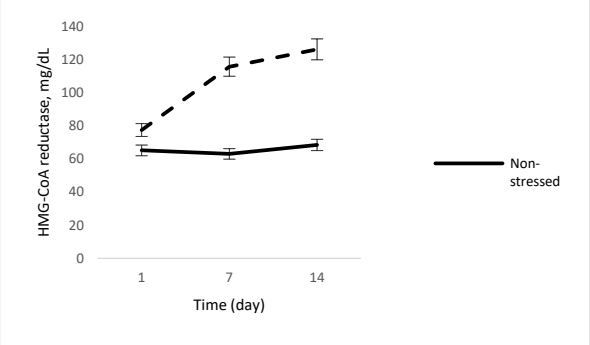

Figure 2 HMG-CoA reductase levels in non-stressed and heat-stressed groups of red hybrid tilapia exposed to 31ºC

Plasma osmolality in the stressed groups on days 1, 7 and 14 were 309.5 ± 5.7 , 312.9 ± 5.6 and 333.8 ± 3.3 mOsm/kg, respectively. The plasma osmolality was significantly higher on day 14 (p<0.05, Figure 3).

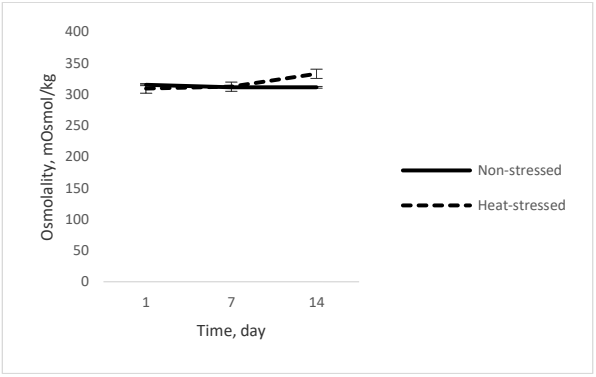

Figure 3 Osmolality levels in non-stressed and heat-stressed groups of red hybrid tilapia exposed to 31ºC

Total plasma protein for the control and stressed groups on days 1, 7 and 14 are presented in Figure 4. The mean protein concentration for the stressed group on days 1, 7 and 14 were 35.47 ± 0.03, 34.21 ± 0.03 and 33.18 ± 0.04 g/100mL, respectively. However, the mean total protein did not differ significantly from the control (p>0.05).

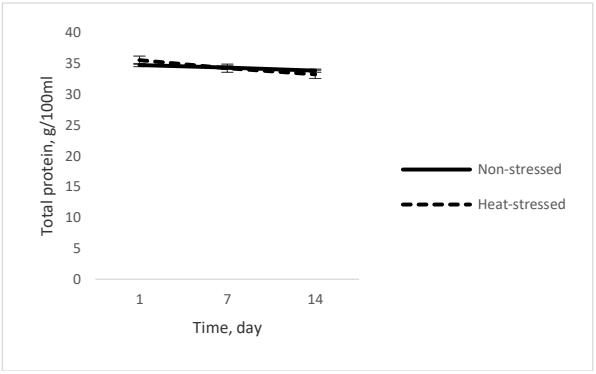

Figure 4 Total protein levels in non-stressed and heat-stressed groups of red hybrid tilapia exposed to 31ºC

Discussion

Thermal tolerance of an organism is a critical aspect in thermobiological studies to assess the effect of thermal phenomena such as climate changes on animal population dynamics [22][23]. High water temperature also affects the behavioural responses and morphological changes of fish [24][25][26]. In the present study when the fish was subjected to high temperature, they were first swam aggressively and later became passive. Similar results were also demonstrated in seabass (*Dicentrarchus labrax*) [27] possibly due to the need to maintain their energy consumption during stressful conditions.

Cortisol is a corticosteroid hormone which is produced by interrenal tissue, in fish. This stress hormone regulates many physiological processes [28][29][30]. The present study found significant cortisol responses to thermal stress, similar to those reported by Lermen et al [31] in other fish species. Similarly, high cortisol levels were also reported in *Oreochromis niloticus* acclimated to 38 and 40 °C during 10-min short term exposure as observed by Delaney et al [32]. These facts suggested that thermal stress may increase the secretion of this hormone, thus risking the fish to increase susceptibility to infection [33] due to its immunosuppressive effect [34]. In addition, prolonged cortisol elevation may also depress animal growth rates and reproductions [35].

Cortisol is also associated with protein synthesis and catabolism [36]. For instance, Vijayan et al [37] reported that cortisol suppression increased the heat-shock mRNA expression and protein level in rainbow trout hepatocyte culture. As such, it is assumed that at higher temperature, an increased of cortisol may trigger the degradation of the damaged proteins to prevent the increase in protein aggregation which is detrimental to cell survival [38][39] thus reduced the number of circulating total protein. The decreased of total protein has been also demonstrated in silver catfish exposed to high temperature [47]. At the cellular level, high temperature affects protein conformation of many protein-associated enzymes, thus inducing protein damage [48 [49][50].

HMG-CoA reductase plays an important precursor role during stress exposure in respect to internal homeostasis [12]. This enzyme is involved in the production of cholesterol which requires 30 rate-limiting step reactions with the first series of reactions is the conversion of acetyl CoA to HMG-CoA reductase prior to conversion of mevalonic acid [40]. During stressful conditions, fish needs to generate enough energy to cope with the condition, mainly by producing cholesterol [41]. Therefore it is expected in fish subjected to high temperature, the activity of this enzyme is increased. The present study has successfully demonstrated a significant increase of HMG-CoA reductase by 50% on day 14. Similar patterns were also found in other fish species such as sturgeon, (*Acipenser baerii*) [42] and sea bass (*Dicentrarchus labrax*) after short-term exposure to stress [43].

The present study showed that highest plasma osmolality was observed on day 14. The changes in plasma osmolality was consistent with the findings of Sardella et al [44] who reported a slightly higher osmolality in the juvenile Mozambique tilapia exposed to 25 and 35 ºC compared with those exposed to 15 ºC. The percentage of increment (~ 7.0%) was also comparable with notothenioid fish as reported by Franklin et al [45]. This increase in plasma osmolality may related to hemoconcentration due to cortisol actions which also enhances the permeability of the blood vessels to water [46].

CONCLUSION

The research confirmed that that exposure to 31 °C for 14 days resulted in some physiological responses in male red hybrid tilapia possibly affect its production performance and aquaculture yield. In this context, it is suggested that the production of genetically selected or hybrid fish species with higher temperature threshold tolerances to aquatic temperature will be beneficial to aquaculture sector in coping with the global warming.

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