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Stress Response and Amino Enzymes Catabolism of Nile Tilapia *Oreochromis niloticus* Exposed to Hyperosmotic Culture Conditions

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ABSTRACT

In archipelagic countries, aquaculture of tilapia is commonly affected by variable and fluctuating salinities associated with tidal flow, salt water intrusions, rainfall and season which can cause stress to the species and affects the overall production. In the present study, the effect of salinity exposure on stress response and amino catabolic enzymes of Nile tilapia *Oreochromis niloticus* was investigated. Seventy two Nile tilapia *Oreochromis niloticus* (size: 44.57±0.48 g) were distributed to 6 units of 60 L plastic tanks to constitute the two salinity exposure treatments run in triplicates. The experimental fish were exposed to two salinities at 15 and 30 ppt for a period of 7 days. Results indicated that after 24 h exposure stressors plasma cortisol and blood glucose were found to be significantly elevated ($p<0.01$) at 30 ppt as compared to those exposed at 15 ppt. This elevated cortisol and glucose levels persist at the higher treatment until the 7th day of exposure. Enzymes involved in the amino acid catabolism for energy production were found elevated in response to salinity stress. Serum ALT and AST were significantly higher ($p<0.01$) at 30 ppt as compared to the control. Survival was negatively affected by higher salinity level ($p<0.01$). This study demonstrates that exposure to higher salinity could result in elevated stress responses and amino enzymes catabolism of Nile tilapia. The present results add to the understanding of the physiological responses of tilapia on salinity stress and would be helpful in formulating strategies to optimize the aquaculture of this species in environments with fluctuating patterns of salinity.

Key words: *Oreochromis niloticus*, stress response, tilapia, enzymes

INTRODUCTION

The culture of tilapia is an important global industry and this species has become an important food fish in the world. Due to the favorable attributes of this species, tilapia is known to be extremely tolerant to wide range of environmental conditions and exhibiting fast growth rates making this species a top choice for aquaculture production (El-Sayed, 2006). However, in an archipelagic country like the Philippines, the fresh water resource is basically scarce and aquaculture of tilapia is commonly done in areas affected by variable and fluctuating salinities associated with tidal flow, salt water intrusions, rainfall and season which can cause stress to the species and affects the overall production.

Stress in farmed fish is of considerable significance to both welfare and productivity, as it has been linked to reduction in growth, abnormal behavior and immune depression (Ashley, 2007). In tilapia production, salinity has been suggested as one key factor to influence various levels of stress

responses. Stress response is the overall physiological reaction of an organism to stress agents. It represents a level of tolerance that is primarily dependent on the fish species, nature and intensity of the stress agent and the period of exposure to stressors (Wedemeyer, 1996). The physiologic response includes biological processes which are coordinated by the hypothalamic-pituitary-adrenal axis culminating in the release of cortisol to the circulation (Barton, 2002), followed by the release of glucose (from liver and muscle) to the blood circulation and eventually the utilization of glucose into cells through the insulin action (Martinez-Porchas *et al*., 2009).

In fish trans-deamination of Non-Essential Amino Acids (NEAAs), like aspartate and alanine, and the further conversion of these amino acids to glutamate by amino-transverses are important biochemical pathways in fish to generate energy needed by the cells during the stress period, osmoregulation and during seawater acclimation (Tseng and Hwang, 2008).

Tilapia has been known to adapt and tolerate a wide range of salinities but the physiological responses influenced by salinity stressors and their relation to the overall performance of this fish are not fully evaluated to date. In environment, where salinity fluctuations are commonly experienced by the fish, information on stress response and energy production to compensate for this adaptation has been scarce. Thus, the aim of this study is to determine the effect of hypersaline exposure on the stress response and amino acid catabolism enzymes of Nile tilapia *Oreochromis niloticus*.

MATERIALS AND METHODS

The experiment was conducted at the Institute of Aquaculture Hatchery, College of Fisheries and Ocean Sciences, University of the Philippines, Visayas, Philippines. This study was carried out for a period of 7 days starting from September 12, 2014 until September 19, 2014. Seventy two Nile tilapia *Oreochromis niloticus* with a total length of 14.67±0.12 cm and weighing 44.57±0.48 g were used in the study. Fish were conditioned in 200 L fiberglass tank at a density of 1 fish 10 L^{-1} and fed with Tateh® commercial floating feed twice a day at 1% ABW for a period of 1 week. The desired salinity level was obtained by mixing tap water and seawater (30 ppt) from the reservoir.

Two experimental treatments run in triplicates following a complete randomized design. The first treatment was consisted of a fish group exposed to 15 ppt, while the second consisted of fish group exposed to 30 ppt. The experimental fish were maintained in a 60 L rectangular plastic tank with 12 fish per tank. Experimental tanks received continuous aeration, 70% of water was changed twice daily and uneaten feeds and wastes were removed daily to maintain good water quality. The fish were fed twice daily at 3% ABW with Tateh® commercial feeds. Blood sampling was done twice in the study. Water parameters including dissolved oxygen, pH and ammonia were maintained to optimum levels throughout the experimental duration.

Survival performance: Every sampling period, percentage survival was recorded and calculated according to the quotient of the number of fish stock and harvest (Kucuk *et al*., 2013).

$$\text{Survival (\%)} = \frac{\text{Nf}}{\text{Ni}} \times 100$$

where, Nf and Ni are the numbers of harvested and stocked fish.

Stress response indicator parameters: Three fish anesthetized from each treatment were anesthetized with 1 mL L^{-1} phenoxyethanol as described by Morgan *et al*. (1997), blood withdrawn through caudal vein puncture using heparinized 1 mL tuberculin syringe. Collected blood was centrifuge at 3000 rpm for 10 min using -4°C refrigerated centrifuge (HETTICH 4903-02-0) and blood plasma was stored at -80°C in ultra-low freezer (ILSHIN NKH10579) until assayed.

Quantitative analysis of plasma cortisol and blood glucose: Cortisol was analyzed using enzyme-link immunosorbent assay (ELISA) kit (SunLong Biotech Co., LTD) and read using the microtiter plate reader (Ledetect 96 ELISA).

Blood glucose was quantified following the method of Mustafa *et al.* (2013) where in a drop of fresh blood from caudal severed fish was touched with glucose strip inserted in a standard glucometer (Apex Biotechnology Corp.) and result was obtained *in situ*.

Enzyme assays: Plasma aspartate transaminase (AST) and alanine transaminase (ALT) were analyzed using enzyme link immunosorbent assay (ELISA) kit (SunLong Biotech Co., LTD).

Data analyses: Data on plasma cortisol, blood glucose, AST and ALT was presented in Means±SE, of triplicate groups. Analysis of data was carried out using independent sample t-test in SPSS 20.

RESULTS

Following the 24 h exposure to salinity, stress response indicators such as plasma cortisol and blood glucose were found higher at ($p<0.01$) 30 ppt as compared to those obtained from the treatment exposed to 15 ppt. Similar pattern of response is observable in the amino acid catabolism indices such as; plasma AST and ALT, which were found significantly higher at elevated salinity level ($p<0.01$).

After 7 days of salinity exposure, levels of plasma cortisol and blood glucose were significantly higher than that obtained at day 1 at both 15 and 30 ppt. However, at the 7th day of exposure significantly higher levels of cortisol and glucose were manifested at the higher salinity treatment at 30 ppt (Table 1).

In terms of amino acid catabolic enzymes, after 7th day of salinity exposure, plasma ALT at 30 ppt was significantly elevated as compared to the levels observed after 24 h of salinity exposure. However, there was a slight decline in plasma AST level aft`er 7th day of exposure as compared to the earlier sampling. Similar to the stress indices, both AST and ALT were found significantly higher ($p<0.01$) at 30 ppt than 15 ppt. Survival was found to be lower in treatment exposed to higher salinity levels (Fig. 1 and 2).

Table 1: Effect of chronic salinity exposure on plasma cortisol, blood glucose and survival of Nile tilapia *Oreochromis niloticus*

Sampling and salinity (‰)	Cortisol (ng mL^{-1})	Glucose (ng mL^{-1})	Survival (%)
Day 0			
15	64.89±0.69^{b}	137.67±2.08^{b}	97.33±4.62^{a}
30	78.52±4.54^{a}	208.50±4.50^{a}	69.67±4.62^{b}
Day 7			
15	72.23±5.06^{b}	214.33±18.72^{b}	82.00±15.59^{a}
30	93.37±3.57^{a}	275.17±24.70^{a}	35.67±10.26^{b}

Data is Means±SE, n = 3, Means having the same superscript letter on the same column are not significant ($p>0.05$)

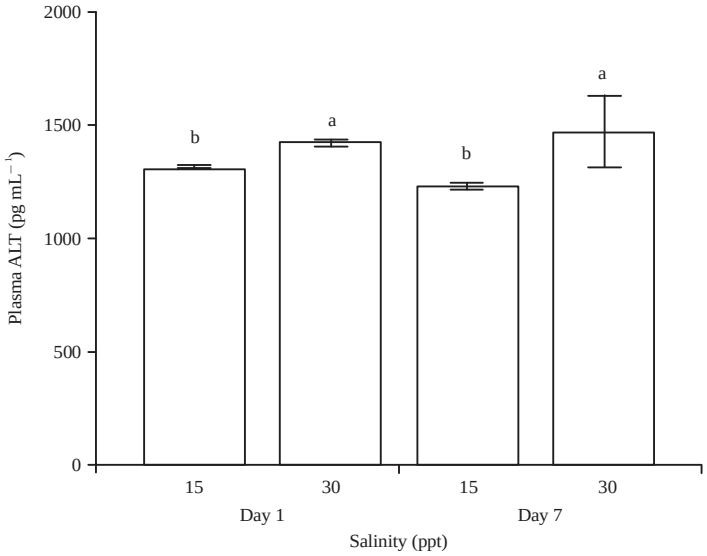

Fig. 1: Effect of chronic salinity stress on plasma alanine transaminase (ALT) within 24 h and 7 days of salinity exposure

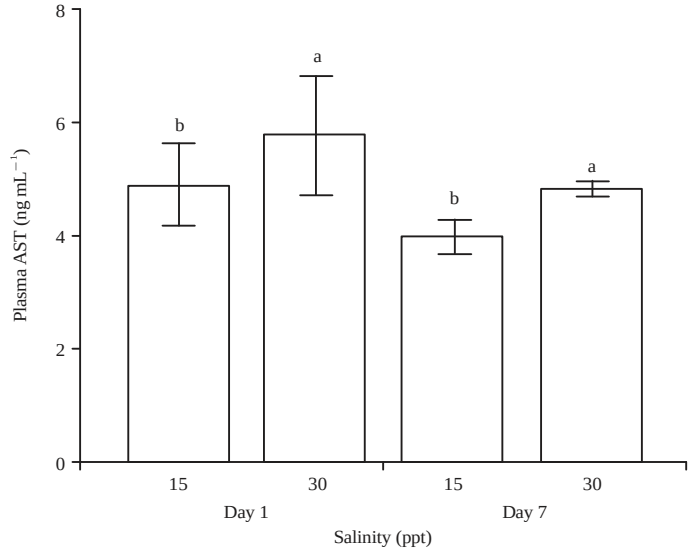

Fig. 2: Effect of chronic salinity stress on plasma aspartate transaminase (AST) within 24 h and 7 days of salinity exposure

DISCUSSION

In tropical archipelagic environment, the exposure of tilapia to fluctuating and higher levels of salinity is commonly observed. This stress condition could influence the growth pattern of the farmed fish and the overall profitability of the venture. The present study investigated the influence of salinity to the stress response and amino acid catabolism enzymes of tilapia exposed to higher salinity conditions. The present results indicated the rapid linear increase in plasma cortisol levels as influenced by the level of salinity and the time period of exposure. Although, it is known that tilapia has a wide range of salinity tolerance however it is a factor that limits the

overall performance of this species. Rapid rise in the level of cortisol as an effect of acute and chronic stress is a response commonly observed in teleost (Martinez-Porchas *et al*., 2009). Stress response is manifested in fish through a systematic biochemical signaling resulting in the production of molecules needed for tissue damage and repair. Synthesis of cortisol is a primary response triggered by neuro-hormonal signaling that can result in glycogenolysis and eventually elevates the production of glucose to be used for energy. Similar to the present findings, significant rise in blood cortisol was also documented in the goldfish *Carassius auratus* exposed to increasing levels of salinity (Al-Khashali and Al-Shawi, 2013). Also, in pejerrey *Odontesthes bonariensis* it was shown that cortisol in blood rises in response to hyperosmotic salinity exposure magnitude and period (Tsuzuki *et al*., 2007). In the present study, the increased level of cortisol in treatment exposed to higher salinity is in agreement with the findings of several authors (Kammerer *et al*., 2010; Lim *et al*., 2005; Al-Khashali and Al-Shawi, 2013; Mancera *et al*., 1993) showing that the rise of cortisol level is a part of the overall physiological adaptation of fish exposed to saltwater.

In the present study, the prolonged elevated levels of cortisol in response of tilapia to a higher salinity exposure could be attributed to the direct role of this hormone in osmoregulation. Dange (1986) has documented that cortisol has a direct stimulatory influence on branchial Na^+/K^+-ATPase activity in tilapia exposed to hyperosmotic conditions. This hormone has also been implicated in increasing the number and sizes of gill chloride cells involved in ion exchanges with Na^+/K^+-ATPase activity and are also noted to increase the size and number of chloride cells mitochondria (Uchida *et al*., 1997).

The elevated level of blood glucose in response to higher salinity exposure in the present study is in agreement with previous findings indicating that stress is accompanied with hyperglycemia (Martinez-Porchas *et al*., 2009). Glucose is considered a secondary stress response and the rise is usually mediated with the rise of cortisol. The persistent elevated levels of glucose in the higher salinity as observed in the present study are observed to correlate with the increased cortisol levels. The rise of glucose in plasma is not as rapid as for cortisol and it has been documented that an increase of glucose manifests in hours or even days as long as the stressor is present (Martinez-Porchas *et al*., 2009). Rise in blood glucose is primarily generated by cortisol-mediated gluconeogenesis that also inhibits cellular uptake of circulating glucose thus increasing the levels in blood circulation. The rise in glucose is a manifestation for the higher needs of tissues to fuel the metabolic needs of osmoregulation and an important source of energy for maintaining homeostasis in fish during chronic stress (Braun *et al*., 2013; Pankhurst, 2011). Lim *et al*. (2005) suggested that following the transfer to seawater, tilapia requires higher blood glucose level as an energy source for reorganization of osmoregulatory mechanisms. Increase energy demands for the maintenance of hydromineral balance in environment of higher salinity has been considered a factor in the rise of blood glucose in response to osmotic stress (Fiess *et al*., 2007; Vonck *et al*., 1998).

Increasing blood glucose in higher salinity level and a higher osmotic environment has been reported in previous studies involving tilapia (Lim *et al*., 2005; Karsi and Yildiz, 2005; Fiess *et al*., 2007) as well as in other fish species (Tarkhani and Imanpoor, 2012; Yang *et al*., 2009). Further, evidence suggests that increased osmotic stress at elevated salinities resulted in the activation of the glucocorticoid and elevation of blood glucose levels (Lim *et al*., 2005; Karsi and Yildiz, 2005). These earlier findings are in agreement with the results of the present study indicating the rise of blood glucose in tilapia as a response to a higher salinity exposure.

Catabolism of fish protein for energy production manifests, when carbohydrate levels are almost depleted. In the present study, enzymes involved in protein and amino acid metabolism that included plasma AST and ALT were consistently elevated in group exposed to a higher salinity at 30 ppt. Although the role of amino acid as osmoregulatory molecule in fish has not been fully

elucidated but the rise in the levels of AST and ALT indicate the importance of amino acids as metabolic fuel for tissues during osmotic acclimation. Amino acids may be needed for metabolic reallocation of energy resources, could be used as energy source or a possible cellular osmolyte to balance the hyperosmotic stress (Aragao *et al*., 2010).

In other fish species, it is suggested that elevation of ALT and AST is generally due to stress. Salaei (2006) reported that levels of plasma AST and ALT in *Cyprinus carpio* were found elevated during the process of catching in earthen pond. The work of Tseng and Hwang (2008) suggested that transaminases, which include ALT and AST are elevated in response in fish exposure to hyperosmotic culture conditions. The findings of the present study corroborates with the results of Al-Khashali and Al-Shawi (2013), elucidating the influence of salinity exposure to elevation of serum AST and ALT in goldfish *Carassius auratus*. Exposure of seabass *Dicentrarchus labrax* to salinity stress was also observed to increase the level of serum AST and ALT (Roche *et al*., 1989). Further, Sultan (2007) reported that plasma ALT and AST concentration of yellowfin seabream *Acanthopagrus latus* juveniles were all elevated when the fish were exposed to different salinities. Collectively, these earlier works agree with the findings of the present study.

Significant decrease of percentage survival was observed in higher salinity for the rest of the sampling periods. Nile tilapia has been reported as less salt-tolerant among other species of tilapia and 30 ppt is beyond their optimum salinity requirements (El-Sayed, 2006; Mjoun *et al*., 2010). The stress caused by exposure to hyperosmotic condition in the present study is the main factor involved in the gradual death leading to a lower survival of the experimental animals. Further, it has been documented that ionoregulatory failure is the major cause of mortalities among stenohaline teleost species exposed to salinity stressors (Amiri *et al*., 2009; Fashina-Bombata and Busari, 2003; Velasco-Santamaria and Cruz-Casallas, 2008). The findings of the present study are in agreement with earlier findings indicating that teleost exposed to hyperosmotic conditions above their optimum tolerance level could suffer from osmo-regulatory challenges (Fridman *et al*., 2012; Kamal and Mair, 2005; Lemarie *et al*., 2004). Ionic imbalance cause impairment of health and death as reported by Enayati *et al*. (2013) in grass carp *Ctenopharyngodon idella* reared above 8 g L^{-1}. Wang *et al*. (2009) found higher plasma Na^+ and Cl^- in *O. mossambicus* exposed to salinity stress prior to death. The low survival of treatments exposed to higher salinities in the present could have been attributed to iono-osmoregulatory failures similar to those observed in previous studies.

CONCLUSION

Findings of the present study suggest that hyperosmotic salinity exposure affects the physiological function of Nile tilapia *O. niloticus* by increasing the cortisol level, blood glucose and levels of amino acid catabolizing enzymes that includes ALT and AST. These results add to the present knowledge on the stress response and coping mechanism of tilapia to hyperosmotic environment.

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