EFFECTS OF SALINITY STRESS ON SOME HEMATOLOGICAL PARAMETERS OF *TILAPIA MOSSAMBICUS* (PETERS, 1852)

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ABSTRACT

Fluctuations in environmental salinity impose stress on the physiology of aquatic organisms. The effect of 3, 6, 9, 12 and 15 ppt of salinity on the hematology of juvenile *Tilapia mossambicus* was studied after 14 days of exposure. All fish died after two days exposure to 15 ppt salinity. After 14 days of exposure fish exposed to 12 ppt salinity showed a significant decrease in red blood cell count, hematocrit, hemoglobin and mean corpuscular hemoglobin ($P<0.05$) . WBC count of fish exposed to 9 ppt salinity increased significantly ($P<0.05$) and returned back to the level of control group at 12 ppt of salinity. Compared to the control fish, mean corpuscular volume increased significantly ($P<0.05$) at 12 ppt salinity whereas no significant difference was observed in the values of mean corpuscular hemoglobin concentration in fish exposed to different salinities. The data clearly suggest significant effect of salinity induced stress on the hematology of the test fish.

Keywords: Salinity stress, hematology, *Tilapia mossambicus*

INTRODUCTION

Natural and human induced environmental changes (temperature, pH, salinity, photoperiod and pollutants) in water bodies could affect physiological response of aquatic animals including fish. Hematological variables are considered good physiological indicators and frequently used to assess stress level and healthy state of fish exposed to toxicants (Affonso *et al*., 2002). Environmental changes are always associated with the physiological changes in organisms. Hematological parameters of fish are closely related to the response of fish to environmental stress including water quality parameters (Valenzuela, 2008). There have been numerous studies regarding effect of different water quality parameters on hematological parameters of fish. It regards, effect of thermal stress on the hematology of gilthead sea bream, *Sparus aurata* (Sala-Rabanal *et al*., 2003), photoperiod on rainbow trout, *Oncorhynchus mykiss*, (Valenzuela, 2008) and common carp *Cyprinus carpio* (Ruchin, 2006), water pH on the hematology of *Tilapia guineensis* (Akinrotimi *et al*., 2012) and *Tilapia mossambicus* (Arain and Rauf, 2013). Salinity is an important water quality parameter which plays a significant role to maintain homeostasis in fish. Changes in the salinity of water can disturb ion regulation and osmotic pressure causing severe physiological stress in fish which may lead to death (Tsuzuki *et al*., 2001). The Mozambique tilapia, *Tilapia mossambicus*, is an exotic fish introduced in Pakistan from Malaya, in 1951, but due to its omnivorous food habit and adaptability to adverse environmental conditions it has become abundant in local fresh water reservoirs (Naik, 1973). Tilapia farming has witnessed vast expansion in developed and developing countries, because of its robust growth rate and hardy characteristics. It can be commercially farmed in ponds, in cages in lakes or rivers, or in water tanks or raceways. In this context, the present study was carried out to examine effects of different water salinities on the hematological parameters of *Tilapia mossambicus.* Such information would provide a better understanding of the physiological mechanisms of this fish.

MATERIALS AND METHODS

A group of juvenile tilapia (*Tilapia mossambicus*) mean length 10.5 ± 1.2 cm; mean weight 76.18 ± 6.33 g were purchased from Masha-Allah fish hatchery Thatta, Karachi, Pakistan, and transported to the laboratory in aerated plastic containers. Fish were kept in a 300 L fiber glass tank for one week to acclimatize to the laboratory conditions. Water in the tank was aerated continuously and changed daily. Fish were fed twice a day with a commercial fish food twice a day (2% of body weight). After acclimation, fish were exposed to 3, 6, 9, 12 and 15 ppt salinities for 14 days to investigate their hematological response. Required salinity levels were obtained by dissolving chemical grade Sodium chloride (NaCl) in dechlorinated tap water. Water salinity in the aquaria was measured using a digital salinity meter (TDS-EZ, HM Digital, Inc. USA). Ten fish were transferred to 80 L glass aquaria and three replicates were used for each salinity range. Two control aquaria containing same number of fish

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and same quantity of dechlorinated tap water were also run in parallel as controls. The water quality parameters of experimental water include pH 7.5 ± 0.2, temperature 24 ± 3 °C, dissolved oxygen 6.8 ± 0.4 mg/L, total hardness 116.5 ± 1.2 mg/L as $CaCO_3$ and were determined using the standard methods (APHA, 2005). Fish were fed twice a day, but feeding was stopped 24 h prior to the blood sampling. After 14 days of salinity treatment, five fish from each replicate of different salinity treated groups and control group were caught using a small dip net causing minimum disturbance in the aquarium. Blood was collected using 18 G needle attached to a plastic syringe by cardiac puncture and transferred into plastic vials containing EDTA as an anticoagulant and used for hematological examinations. Hematological parameters examined included, erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leucocyte count (WBC) and were determined according to the unified methods for hematological examination of fish (Svobodova *et al*., 1991). Data was analyzed by one way analysis of variance (ANOVA) to test the significant difference among different hematological parameters in experiment fish groups and controls. All statistical analyses were carried out using SPSS 17.0 computer programme (SPSS Inc. Chicago, USA) and $P<0.05$ was considered statistically significant.

RESULTS

All the fish exposed to 15 ppt salinity died after two days of exposure while no mortalities were recorded in other experimental fish groups. The increase in salinity significantly affected some of the measured hematological parameters. Compared to the control and fish exposed to 3 ppt of salinity there was a significant decrease ($P<0.05$) in RBC count, Hct, Hb, MCH values and a significant increase in MCV values ($P<0.05$) of fish exposed to 12 ppt of salinity, but no statistically significant difference was observe in comparison to fish exposed to other levels of salinity (Table 1). On the other hand a different pattern was observed for WBC count for experimental fish groups. The WBC of fish exposed to 3 ppt and 6 ppt increased gradually and reached to statistically significant level in fish exposed to 9 ppt ($P<0.05$) and subsequently returned to the level of control group. MCHC values of all treated groups were not significantly different when compared with control group ($P>0.05$).

Table 1. Hematological variations in juvenile tilapia (*Tilapia mossambicus*) after 14 days exposure to different salinities

Parameters	Control	Experimental groups			
		3 ppt	6 ppt	9 ppt	12 ppt
RBC (10^6/mm^3)	1.70 ± 0.21^a	1.68 ± 0.19^a	1.64 ± 0.35^a	1.52 ± 0.41^a	1.18 ± 0.28^b
Hct (%)	12.48 ± 1.98^a	12.31 ± 1.14^a	11.88 ± 0.48^a	11.32 ± 1.28^a	8.32 ± 0.95^b
Hb (g/dl)	6.98 ± 0.21^a	6.81 ± 0.18^a	5.92 ± 0.37^a	5.45 ± 0.11^a	4.88 ± 0.35^b
MCV (fl)	140.12 ± 10.14^a	138.41 ± 11.98^a	155.44 ± 12.48^a	162.18 ± 13.25^a	180.41 ± 10.4^b
MCH (pg)	28.42 ± 2.32^a	27.81 ± 1.41^a	26.55 ± 2.56^a	25.13 ± 1.90^a	20.13 ± 1.82^b
MCHC (%)	25.48 ± 1.09^a	24.30 ± 1.43^a	23.12 ± 1.94^a	24.97 ± 2.01^a	22.85 ± 1.15^a
WBC (10^3/mm^3)	28.58 ± 2.42^a	33.44 ± 1.98^a	36.21 ± 2.90^a	45.24 ± 0.78^b	30.74 ± 1.42^a

DISCUSSION

Mozambique tilapia, *Tilapia mossambicus* is a fresh water fish and small changes in salinity could have large effects on the physiology of this fish. In the present study the hematological changes in juvenile *T. mossambicus* after 14 days exposure to different salinity levels included a significant decrease in RBC count, Hct, Hb, MCH values and increase in MCV and WBC counts. Changes in environmental salinity lead to significant alterations in hematological parameters and indicate suppressed hemopoeitic system or compensatory response to environmental stress (Schreck, 1990). There are some reports that suggested variations in hematological parameters in different fish after exposure to different salinities. Salati *et al*. (2010) reported an increase in RBC count and hematocrit values in adult common carp, *Cyprinus carpio* following two weeks exposure to 12 g/L of salinity. While, no difference was

observed in these parameters of long term fresh water salt water acclimatized juvenile *Acipenser naccarii* (Martinenz-Alvarez *et al*., 2002) and *Accipenser oxyrhynchus* (Baker *et al*., 2005). On the other hand, in consistence with the results of our study, decreased RBC count and hematocrit have previously been reported in common carp, *C. carpio* (Hafez and Oryan, 2002) and in juvenile great sturgeon (*Huso huso*) after 20 days exposure to 12 ppt salinity (Zarejabad *et al*., 2009). The difference in the RBC count and Hct level in these studies may be attributed to the difference in size, age of fish and acclimation period. In the present study, like RBC count and hematocrit, the hemoglobin level also decreased in fish exposed to higher salinity indicating a positive correlation between hematocrit percentage and hemoglobin level. Similar to the findings of present study a significant decrease in hematocrit and hemoglobin has been reported in fingerling rainbow trout (*Onchorhynchus mykiss*) following 10 days exposure to 7 ppt and 11 ppt of salinity (Hosseini *et al*., 2011). Tavares-Dias *et al*. (2000) also reported a positive correlation between hemoglobin and hematocrit in teleost fish. In contrast to the results of present study, an increase in RBC count, Hct and Hb have been reported in grass carp, *Ctenopharyngodon idella* (Yildiz and Uzbilek, 2001) and common carp, *Cyprinus carpio* (Salati *et al*., 2010) in response to increased environmental salinity and authors have attributed this response to an increase release of RBC from spleen and changes in water content of blood in fish.

WBCs are key component of innate immune system and are involved in regulation of immunological function in fish as in mammals (Balarian *et al*., 2004). In the present study, WBC count increased significantly in fish exposed to 9 ppt salinity and returned to the level of control fish values in fish exposed to 12 ppt salinity. These findings are in consistence with the results reported in *Channus punctatus* (Dheer *et al*., 1986) and *C. carpio* exposed to higher salinities (Salati *et al*., 2010). On the other hand no marked changes in WBC count were observed in *C. idella* (Yildiz and Uzbilek, 2001) and *Huso huso* (Zarejabad *et al*., 2009). It seems that exposure to increased environmental salinity can increase production of leucocytes through stimulation of leukopoeitic process in fish. However, these changes in WBC count vary among different fish species depending upon life history, genetic makeup and nutritional status of fish. In conclusion, alterations in hematological parameters of *T. mossambicus* exposed to different high salinities induced severe physiological stress in fish which may affect the healthy state of fish and can ultimately lead to death.

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