Paper ID E76

Effects of acute water pH stress on the stress indicators in zebrafish (*Danio rerio*)

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Abstract

The aim of this study was to know the acute stress effects of water pH on the stress responses of zebrafish (*Danio rerio*). Tests were performed in a wide pH range from 4.3-10.7. Results showed that as a secondary stress response blood glucose levels were elevated within a short period from 2.53 mmol/l to 7.23 mmol/l in acidic (pH 5.0) and 2.43 mmol/l to 8.23 mmol/l in basic (pH 10.0) treatments. RBC counts showed a significant decrease in acidic and basic treatments while WBC showed a gradual increase compared to control. Fish also showed a distinct behavior in swimming, feeding and egg laying at different pH treatments. The results revealed that these parameters are indicative of pH toxicity to fish and may be used as early indicators in toxicity of aquatic animals.

INTRODUCTION

Aquatic systems are highly vulnerable due to their tendency to accumulate relatively high concentrations of chemicals entering from a variety of point and non-point to its water bodies such as rivers, streams, marine ecosystem and groundwater [1]. pH is one of the main factor affecting water quality. Extreme pH negatively affects fish growth and reproduction. Acid and bases of pH and other hazardous chemicals in all aquatic water bodies are a common phenomenon due to release of pollutants from the industries in the present day life. The aquatic environment is particularly sensitive to toxic contaminants since a considerable amount of chemicals used in industry, urbanization and Agriculture that enters in the aquatic environment. At present rapidly growing industrial activities have lead to continuous release of acidic and basic pollutants which may cause damages the physiology of aquatic organisms. It also affects the general body physiology and physiology of reproduction. In order to investigate the effects of pH on general physiology and physiology, it is necessary to clarify the biological response of fish. Zebrafish (*Danio rerio)*, a freshwater fish, has become the most widely used standard model to study developmental genetics. Therefore, we have investigated the effects of pH exposure on physiological and ecological process on Zebrafish.

MATERIALS AND METHODS

This experiment was conducted in the wet laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

A. Experimental species

Zebrafish was collected from the field laboratory complex of the Faculty of Fisheries, Bangladesh Agricultural University and fed with commercial feed (Krishibid Feed Ltd.) and zooplankton and constantly aerated for 7 days for acclimation.

B. Experimental Procedure

A total of thirty adult sized (5±1 cm) Zebrafish were stocked in each aquarium (45×30×30 cm^3) having 30 liter water capacity and dividing into three groups at pH 5.0-6.0 (Acidic), 6.5-8.2 (Control) and 9.0-10.0 (Basic) treatments. Acetic acid (CH_3COOH) and Sodium hydroxide (NaOH) were used for adjusting pH in aquaria throughout the study period.

C. Blood component analysis

Blood samples were collected by cutting the tail and glucose level was estimated by using a digital glucometer (Glucolab, Korea). Some blood samples were taken in 2% EDTA solution to quantify RBC and WBC.

D. Statistical analysis

The data were subjected to an analysis of variance (ANOVA), followed by comparison of means using Duncan's multiple range test to determine significance of each data treatment. All statistical analyses were performed using SPSS v16.

RESULTS AND DISCUSSION

Our study revealed that experimental fish can tolerate a wide range of pH from 4.3-10.7. Below or above this range it became lethal effects in this species.

A. Blood glucose level

Blood glucose levels in fishes increase during stress probably as a result of catecholamine action on stored glycogen in liver and other tissues [2]. In the present study blood glucose levels were changed due to acute stress of pH in fish. Glucose levels were elevated from 2.53 mmol/l to 7.23 mmol/l in acidic treatments and 2.43 mmol/l to 8.23 mmol/l in basic treatments with a highest peak at 6 hr in both medium whereas in the control medium it remained same throughout the period (Fig. 1). Significant difference ($P<0.05$) among the values were observed at certain hours (1hr, 6hr, 12hr and 24hr) in different treatments. These results are in agreement with on Chub (*Leuciscus cephalus*) [3], *on Clarias gariepinus* [4].

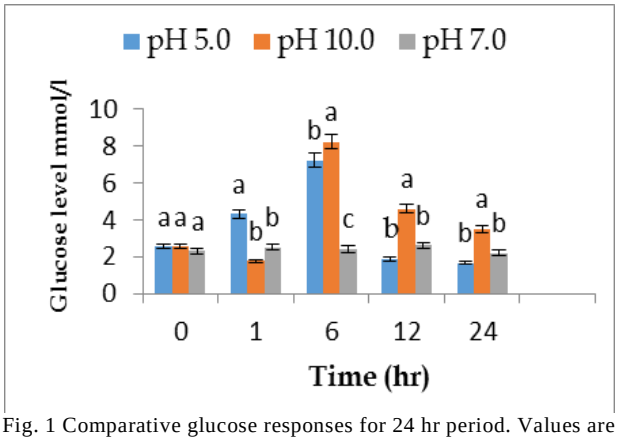

Fig. 1 Comparative glucose responses for 24 hr period. Values are presented as (mean ± SE). Different superscript alphabets in each treatment group are significantly different at $P<0.05$.

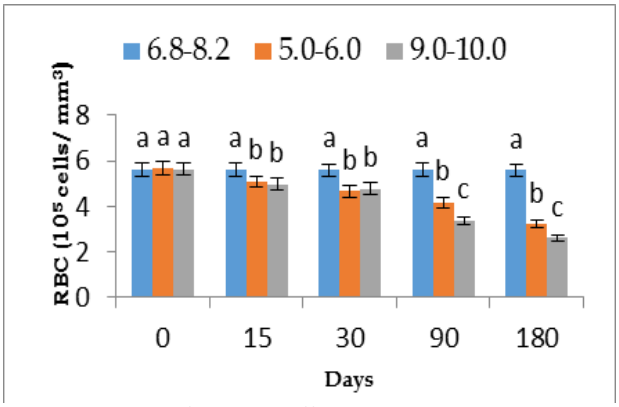

Fig. 2 Number of RBCs at different treatments during study period. Values are presented as (mean ± SE). Different superscript alphabets in each treatment group are significantly different at $P<0.05$.

B. Counting of red blood cell (RBC)

Red blood cell decreased from 5.68×10^5 cells/mm^3 to 3.21×10^5 cells/mm^3 in acidic treatment and 5.63×10^5 cells/mm^3 to 2.61×10^5 cells/mm^3 in basic treatments and had significant difference at 15, 30, 90 and 180 days with control ($P<0.01$) (Fig. 2). The reduction observed may be as a result of stress which affects the metabolism and normal functioning of the fish physiology. Similar reduction was also observed in *Tilapia guineensis* [5] and in common carp [6].

C. Counting of white blood cell (WBC)

WBC by acting as first line of defense against any type of infection/pathogen makes an organism immune enough to fight any possible stress by increasing their number. WBC was also varied from experimental to control. WBC increased from 4.39×10^3 cells/mm^3 to 7.84×10^3 cells/mm^3 in acidic treatments and 4.34×10^3 cells/mm^3 to 8.53×10^3 cells/mm^3 in basic treatments. While in control treatments it remained more or less similar throughout the experimental period. Counting of WBC experiment of fish between treatments studied, had a statistically significant difference ($P<0.05$) (Fig. 3). This finding is in line with *Clarias gariepinus* [7] due to lead effect and in *Tilapia guineensis* [5] due to salinity stress.

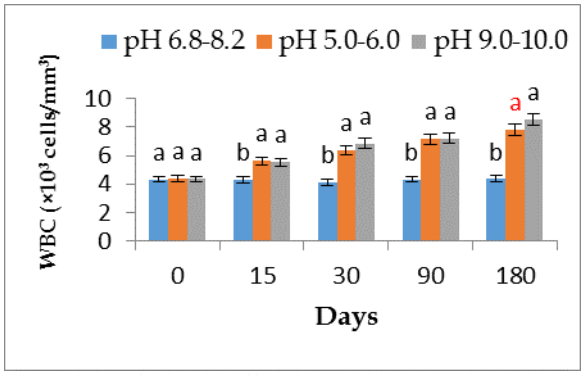

Fig. 3 Number of WBC at different treatments during study period. Values are presented as mean ± SE. Different superscript alphabets in each treatment group are significantly different at $P<0.05$.

D. Behavioral Responses in Acutely Stressed pH

Behavioral responses in acutely stressed condition were much more severe than any other responses. When fishes were in acute stressed condition (sudden changes of pH more than 10.0 or less than 5.0) they did not take any of feed, movements were increased and sometimes they tried to escape by jumping over the aquarium. Failure of acclimatization turned them into exhausted and finally fishes were died. Similar findings were also observed in Banded killfish (*Fundulus diaphanus*), Blugill (*Lepomis macrochirus*) and Stripped bass (*Morone sexatilis)* [8]. They showed that significant avoidance of the highest pH level in a gradual increase of pH by all three species which was very much similar with the findings of the present study.

ACKNOWLEDGEMENT

The authors wish to thank MoST BD for financial support to MS Islam for making it possible to carry out this research.

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