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Effect of Altered pH on Haematological Parameters in an Air Breathing Singhi Catfish, *Heteropneustesfossilis* (Bloch 1794)

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Abstract—*Anthropogenic activities of day to day life create a harmful effect in an environment. Thus, the changes in pH in an environment due to an anthropogenic activity can create physiological changes in organisms. The effect of exposure to an acidic and alkaline pH shows changes in hematological parameters in Heteropneustesfossilis. Prior to study, the catfish were exposed to altered pH of 6.4 and 8.4 for 96 hours, 5 days and 10 days, which include significant decrease in red blood cell (RBC), hemoglobin, haematocrit and increased in white blood cell (WBC). The parameters of present study can be used as indicator of environmental pollution related to pH stress.*

Keywords*: WBC, RBC, Hemoglobin, PCV.*

Introduction

The best indicators of land-based pollution are the aquatic animals, mainly the fishes, as the pollulated water is directly exposed to their tissue through diet and respiration. The circulatory system of fish has close association with the external environment (Wendelaar Bonga, 1997), water pollution that occurs due to anthropogenic activities can alternate the level of pH by frequent or continuous introduction pollutant and some aquatic animals could adapt to tolerate the changes of environmental pH range. Both fresh and ocean water contaminated bacteria, organic pollution and oil pollution by different surface water vehicle.

As aquatic environment has greatest food chain, the aquatic animals largely depends on physiochemical features like pH, dissolved oxygen, nitrites, nitrates, phosphate, CO_2 and temperature etc in water (Svobodova *et al.,* 1993), so the aquatic organisms' major fishes shows great response to any changes in water quality (Borkovic *et al.,* 2008). Therefore, the fishes are referred as good indicator to monitor water pollution (Kemaji *et al.,* 2018), because the xenobiotics gets direct physical contacts with fish body causes physiological dysfunctions like changes in enzymatic activities and haematological changes (Qayoom*et al.,* 2016).

Heteropneustesfossilis (Bloch, 1794) belong to the order Siluriformes. *Heteropneustesfossilis* is recorded from South and Southeast Asia: Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar, Thailand and Laos (Eschmeyer and Fricke 2009). It is introduced in Iran and Iraq. Records from India include the Andaman Island and Uttar Pradesh (Dehra Dun, Nainital). It is locally known as "Shing" or "Shingi" under the order Siluriformes and family Clariidae. Among the air breathing catfishes, H. fossilis is most preferred, highly nourishing and palatable because of its low fat, less spine and high digestibility. Having high medicinal value, it is often recommended for patients after recovery from malaria due to its energizing qualities (Bhuiyan, 1964). The fishes are useful indicator of environmental water pollution because they are sensitive to change in water quality. Due to the constantly exposed to chemical contamination by different anthropogenic activities that increases the toxicity with overall deleterious effect (Correia *et al.,* 2003). In freshwater ecosystem, the level of pH fluctuates within daily and seasonal framework and the aquatic organisms become tolerant to environmental pH. But according to Tucker and Abramo, 2008; the sudden change in pH can cause damage or even can kill the animals.

Methodology

Animal sample

Air breathing singhi catfish (*H. fossilis*, weighing 35-50 g body mass) were purchased from a local fish market of Guwahati, Assam. The fishes were acclimatized for 4-6 weeks in Aquaculture and Biodiversity Centre, Gauhati University, in the cemented tanks (1.5 m length × 1 m wide × 1m height) containing tap water of pH 7.4± 0.1, maintaining the temperature at about 30 ± 2^0C. As a feed, the sun dried silk

worms and dried fish were mixed with corn flour and given on alternate days. On regular and daily basis, the cemented tank was cleaned and water was changed. After proper acclimatization, where the mortality rates were zero and consumption of food was normal then the fishes were used for experimentation. Food was withdrawn 24 hours prior to the grouping for experimentation.

Sample collection

After the acclimatization, three experimental groups were designed for categorizing the experimental fishes. The first group of fishes was control batch kept in water with normal pH of 7.4. Other two groups of fishes were exposed to pH 6.4 and 8.4. The acidity and alkalinity of the water was induced by the addition of 1N hydrochloric acid (HCl) and sodium hydroxide (NaOH) respectively. The fishes were exposed to 96 hours, 5 and 10 days at the pH of 6.4 and 8.4. The water temperature was maintained at 30 ± 2^0C and on a daily basis water of the tank was changed. After exposure the fishes were immediately sacrificed at 96 hours, day 5 and day 10. Blood from the Control and altered pH treated fishes were obtained by cutting of caudal peduncle and collected in Eppendorf tubes containing 1 % of Ethylene diamine tetra acetic acid (EDTA), which act as an anticoagulant (Mgbenka*et al.,* 2003).

Red Blood Corpuscular (RBC) Count

The total count of Red Blood Corpuscles (RBC) was done with the Neubauer chamber as described by Sohn and Henry (1969). The red glass bead pipette was used and all were repeatedly counted in triplicate. Blood was drawn up to 0.5 marks in RBC pipette and immediately, the diluting fluid (Hayem's solution) was drawn up to the 101 mark (thus the dilution is 1:200). Pipette was shaken thoroughly and diluted blood was charged into the counting chamber, after discarding two drops. The solution was allowed to settle for few seconds and the number of RBCs was counted in five small squares of the RBC column under high power microscope and the number of RBCs per cubic mm was calculated.

White Blood Cell Count (WBC):

The blood was sucked up to the mark 0.5 in the pipette. The pipette was held in horizontal position and by placing the tip of the pipette against the blood kept in tube and blowing gently on the rubber tube, adjusted the blood so that it was exactly on the 0.5 mark. Dipped the pipette in white blood cell diluting fluid and sucked up to 11 mark. The pipette was held horizontally and shaken for 10 s for thorough mixing. The pipette containing diluted blood was thoroughly shaken and the first 4-5 drops was discarded. Then 2 drops of the diluted blood from the pipette was placed between the cover slip and ruled areas without any air bubbles. The four corner squares of the area 1 square mm, which was composed of 16 small squares, each (1mm x 1mm), were used for white blood cell count. Counting of WBC was done by following the same rules as in RBC counting.

Estimation of Hemoglobin

Anticoagolated blood is added to the 0.1 N HCl and kept for 5-7 minutes to form acid haematin. The color of this acid haematin should be matched with the solution, present in the calibration tube. Distilled water is added to the acid haematin until the color matches and the final reading is directly noted from the graduation in the calibration tube using Sahli' shaemoglobinometer. N/10 HCL was placed in diluting tube up to the mark 2. Blood was sucked in the haemoglobin pipette up to 20 cubic mm and blown it into diluting tube and rinse well. After 10 minute distilled water was added in drops and mixed the tube until it has exactly the same color as comparison standards.

Estimation of Packed Cell Volume (PCV)

PCV was estimated by employing the microhaematocrit method (Snieszko, 1960).

Results

The hematological parameter shows significantly decrease in red blood cell (RBC), hemoglobin, haematocrit (PCV) and greatly increase in white blood cell (WBC) count in both alkaline (pH 8.4) and acidic pH (6.4) compare to the control group i.e, pH 7.4 (table 1).

Table 1: Effect of altered pH on RBC, WBC and hemoglobin in *H. fossilis* at 96 hours, 5 days and 10 days. Values are expressed in Mean±SD and singnificant difference at P<0.05.

Group	Duration	RBC (X 10^6 /ml)	WBC (X 10^3 /ml)	Hb (g/dl)	PCV(%)
control	96h7.4	5.32±0.59	18.33±1.5	7.3±1.01	34.33±1.52
	5days7.4	5.12±0.82	18.9±1.01	7±1	33±1
	10days7.4	4.32±1.12	19.98±1.9	6.6±1.58	34.16±1.04
Treated 1	96h6.4	4.49±1.33	19.843±1.7	6.1±0.85	30.66±1.52
	5days6.4	4±1	20.55±1.8	5.4±1.44	29.33±0.57
	10days6.4	2.89±0.57	22.55±2.3	4±1	29±1
Treated 2	96h8.4	4.35±1.5	19±1	5.4±1.63	30±0.40
	5days8.4	4.15±0.78	20.12±1.8	4.5±1.5	29.33±0.57
	10days8.4	2.9±0.55	21.99±1.7	4±1	28.36±1.26

*Each value is represented as mean ± SD (n=3); Values are significant at p<0.05 (ANOVA). Treated 1=Acidic pH (6.4) Treated 2= Alkaline pH (8.4).

Discussion

The results of present experimental study show the hematological parameters of fish treated with altered pH to monitor aquatic pollution. The outcomes of different comparative studies of hematological parameters are very much useful to access the quality of water (Kopp *et al.,* 2013; Khan *et al.,* 2015; Osman *et al.,* 2018). The experiment, the total count of red blood cell (RBC) decreases in both acidic and basic pH treated group of *H. fossilis*, which shows similarity with cadmium treated fish (Gill and Epple, 1993; Al-Asgah*et al.,* 2015), also supported by Svobodova*et al.,* 2003. According to Das et al., 2006, the reduction in the RBC count of zebrafish following exposure to both acidic and basic pH indicated a reduced blood oxygen carrying capacity. The present study on the WBC count shows elevation in treated group comparative with control group.Xenobiotics causes tissue damage could stimulate immune response by increasing the count of WBC (Moraes 2007).

The hemoglobin content in *H. fossilis* shows significantly decrease in altered pH treated group, which is similar to Soundararajan*et al.,* 2014, in zinc exposed *Heteropenustesfossilis* reported the reduction in Hb content. The significant decrease in RBC count and haemoglobin in *H. fossilis* may be due hemolysis and shrinkage of blood cell due to toxic effect of acidic and alkaline pH.In fishes the hemoglobin content gets greatly reduced which are exposed to toxicant could be due to the enzyme inhibition responsible for hemoglobin synthesis (Pamila*et al.,* 1991). Packed cell volume or hematocrit value showed a declination in *H. fossilis*. According to Ramesh and Saravanan 2008, the decreased level of PCV in Chlorpyrifos treated *C. caprio* is due to rapid oxidation of hemoglobin or release of free oxygen redicals. There are significant decreases in erythrocyte count, haemoglobin content, PCV, MCV, MCH and increase in MCHC, leukocyte and ESR in *Heteropneustesfossilis*after 15 days treatment with devithion, Nath and Banerjee (1995).

Conclusion

The present study in the effects of altered pH has sensitive effects on hematology of *Heteropneustesfossilis* compare to the control group. Therefore, it provides the information that change in pH is found to have hazard effects on the body functions of animals. In addition, the finding provides evidence related to stress can be used as bioindicator of pH pollution in aquatic environment.

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