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# Effect of Low pH on Electrolytes Evaluation of *Oreochromis mossambicus*

**Kumar D.*, M. Narayanan** and **A. Ibrahim**

Aquatic Biodiversity Centre, St. Xavier's College, (Autonomous) Palayamkottai – 627 002, Tamil Nadu, India
* devadosskumar@yahoo.co.in
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**ABSTRACT** The fish *Oreochromis mossambicus* was selected for this present investigation. *O. mossambicus* were exposed to different degrees of acid exposures such as, pH 4.9, 5.8 and control and they were fed with beef meat. The objective of this present study was to quantify the electrolytes (Na, K, CI and Ca) present in the serum of the blood of acid stressed *O. mossambicus*. Results obtained in the present investigation clearly indicated that the electrolytes drastically decreased from the control and led to circulatory collapse. The results exhibited the reduction of Na from the control and led to circulatory collapse. The results exhibited the reduction of Na from the control 17.93mmol/1 in pH 4.9. Similarly and pH 5.8 the reduction of Na + was 9.6 mmol/1 from the control. Similarly the reduction of chloride and potassium were 103. /1 mmol/1, 112.53 mmol/1, 130.1 mmol/l, respectively, Calcium also reduced from the control 1.9mg/mmol/1 respectively, and 567 in the fishes exposed to pH 4.9 and 5.8 respectively.

(pH, electrolytes, beef meat)

## INTRODUCTION

Acidic precipitation is likely to cause changes in pH of surface waters in regions, where waters are low in alkalinity [1]. It is a well established fact that acidic precipitation is a result of environmental pollution that is mainly due to the burning of fossil fuels and industries which result in the release of mainly $CO_2$, $SO_2$ and $NO_2$. Acid rain kills mainly aquatic life forms such as plankton and fish and affects the productivity of aquatic ecosystems. Many bacteria and blue green algae are killed due to the acidification disturbing the whole ecological balance.

Acidification of water body by acid precipitation has severe effects on endemic fish population. Observed effects include acute mortality [2] skeletal deformities [3] reproductive failure [4] reduced growth [5] and accumulation of potentially toxic trace elements [6]. However the mechanisms by which acidification affects fish are not completely understood. It is suggested that adverse effects may result from increased hydrogen ion or increased metal concentration [7]. Hydrogen ion or the metal may affect fish through effects on respiration, osmotic balance and gametogenesis [8].

Acid stress results in an immediate stimulation of the secretion of cortisol and significant increase in secretary activity of the corticosterol is observable at least on the first day following acid exposure [9]. It is possible that the increase in plasma $Na^+$ may be upset by osmoregulating adjustment mediated by adrenocortico steroid hormone. The increased metabolic cost of maintaining plasma electrolyte during condition of acid stress may be a significant factor contributing to the reduction in growth [9]. The loss of $Na^+$ leads to the mortality especially on the site of toxic action on gills.

The maintenance of Ca homeostasis in fish is crucial throughout life but it is especially important in young fish; ca is also critical for growth. The acid exposure results in increased bronchial permeability to water and ions [10, 11]. The literature regarding effect of low pH on Indian fishes in Indian environment is scanty. This is the first attempt on *Oreochromis mossambicus* an exotic fish that has invaded almost all the freshwater bodies in India. Though

fish farmers regard it as an intruder, it is supposed to be the cheapest animal protein for the poor people. Since it is a prolific breeder and can withstand extremes of environmental factors, this species was chosen for the present investigation.

## MATERIALS AND METHODS

Healthy individuals of *O. mossambicus* were collected from the ponds and carefully brought to the laboratory and acclimated for a period of 15 days. During the period of acclimation the fish were fed *ad libitum* with chopped beef liver.

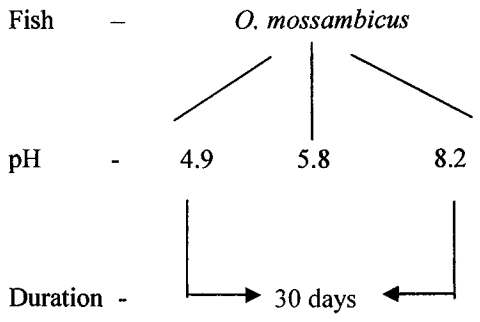
*Oreochromis mossambicus* were segregated into three groups namely A (17.33 □ 1.16g), B (17.38 □ 0.932g) and C (17.3 □ 0.0953g). Fishes of the group A were introduced into experimental medium of pH 4.9, fishes of the group B were introduced into the experimental Media of pH 5.8 and the fishes of the group C were treated as control. The experimental duration was 30 days. Acid media were prepared by the addition of sulphuric acid and the experimental media were changed every day. Experiments were done in triplicates. During the experimental period the fishes were fed with chopped beef liver. The fishes were kept under the respective pH medium throughout the experimental period. After the completion of the experimental period, the electrolytes estimations were carried out in the blood of experimental fishes.

***Experimental design***

Different groups of *O. mossambicus* were subjected to three different pH media in the acidic ranges namely pH 4.9, 5.8 and control pH 8.2.

***Preparation of low pH medium***

Acidic media for experimental were prepared by dissolving sulphuric acid in tap water. The pH of the media was checked using a pH meter. (Digital pH meter, DPH 500).




## METHODOLOGY

Serum samples of all experimental fish were analyzed for *in-vitro* quantitative determination of Sodium, Potassium Chloride, calcium and protein. Using ECA*-3 kit (Dr. Reddy's Laboratories Diagnostic Division)

## RESULTS

***Sodium ($Na^+$)***

The electrolyte $Na^+$ content present in the serum of *O. mossambius* exposed to the acidic environments (pH 4.9 and 5.8) revealed a drastic Hyponatremia. For instance the decrease of $Na^+$ in fish exposed to 4.9 pH medium was 17.93mmol/l from the control (Table 1, Figure 1) and at pH 5.8 the reduction of $Na^+$ was 9.6 mmol/1 from the control.

***Potassium ($K^+$)***

Low pH attributed the decreased potassium level in the blood of fishes. For instance, fishes tested in pH 4.9 and 5.8 and control serum $K^+$ content was 16.7 mmol/1 and 22.3 mmol/l and 25.03 mmol/l respectively (Table 1, 2 and Figure 1).

***Chloride ($Cl^-$)***

The chloride content in the serum of fishes varied with different degrees of acidic pH. The observed results showed that fishes exposed to lower pH 4.9, 5.8 and control pH 8.2 exhibited the depletion of chloride content were 103.1mmol/l, 112.533 mmol/l, and 130.1 mmol/l respectively.

***Calcium ($Ca^+$)***

The observed results displayed the decrease of calcium in fishes exposed to low pH media pH 4.9 and 5.8. Among the experiments, the fishes tested in experimental media pH 4.9 showed the higher depletion of calcium than the fishes exposed to pH 5.8. The decline of calcium in the fishes exposed to pH 4.9 was 1.9 mg% from the control and the fishes tested in the experimental media pH 5.8 was 0.567mg% (Table 1, Figure 1).

***Serum Protein***

The fishes tested in the pH 4.9 showed a remarkable increase of serum protein than the fishes exposed to pH 5.8. The serum protein level was 0.94mg in pH 4.9 and at pH 5.8 .The serum proteins was 0.8mg. The control fishes showed the maximum serum protein content to the tune of 1.6 ± 0.061 mg (Table 2, Figure 2).

**Table 1.** Effects of low pH on electrolytes evaluation of *Oreochromis mossambicus*

| pH | Na (mmol/L) | K (mmol/L) | Ca mg % | Cl (mmol/L) |
|---|---|---|---|---|
| 4.9 | 149.266±0.124 | 16.7±0.0816 | 8.133 ±0.047 | 103.1±0.081 |
| 5.8 | 157.566±0.047 | 22.3±0.081 | 9.466±0.047 | 112.533±0.169 |
| 8.2 | 167.2 ±0.081 | 25.033±0.047 | 10.033±0.047 | 130.1±0.0816 |

**Table 2.** Effects of low pH on the serum Protein of *O. massambicus*

| pH | Serum Protein (g %) |
|---|---|
| 4.9 | 0.94 ±0.016 |
| 5.8 | 0.8 ±0.032 |
| 8.2 | 1.6 ± 0.061 |

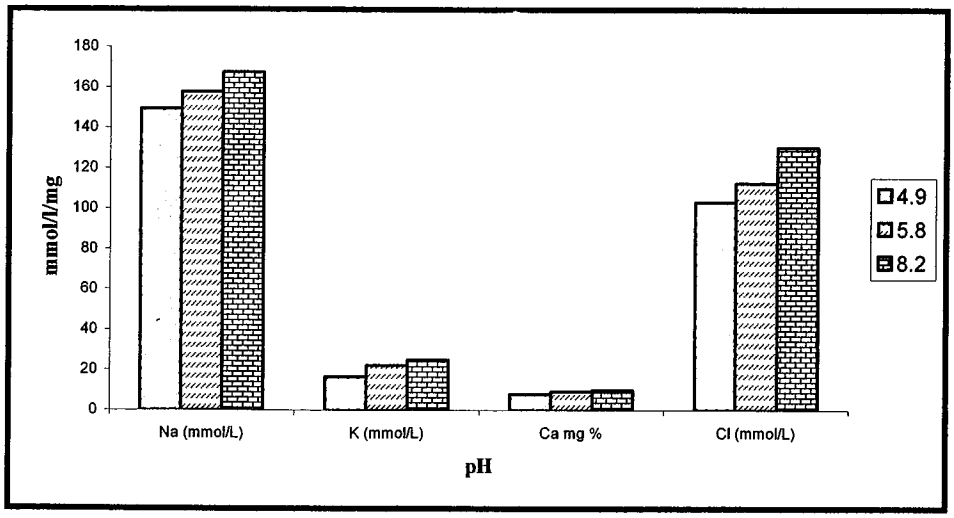



**Figure 1.** Electrolyte evaluation of Sodium, Potassium, Calcium and Chloride of *Oreochromis mossambicus* exposed to different acidic pH media.

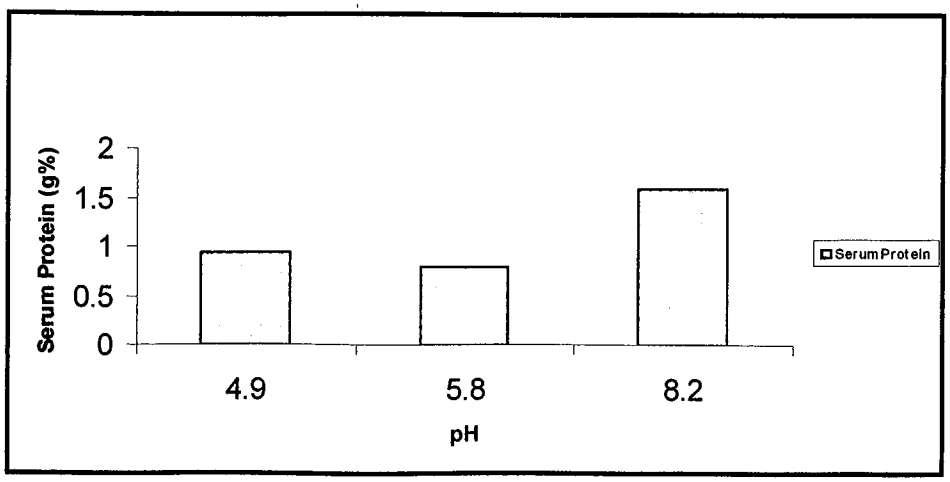



**Figure 2.** Effect of low pH on the serum Protein of *O. mossambicus*

## DISCUSSION

The impact of acidity has highly influenced on the electrolytes status on the blood serum of the fish, which were stressed under severe acidity at pH 4.9.

The results revealed a significant ($p<0.05$) depletion of $Na^+$ in fishes exposed to pH 4.9. The loss of plasma sodium in brown trout was reported by [12]. These electrolytes are lost from muscle tissues and plasma. The depletion of $Na^+$ might also be due to the effect of acidity on aldosterone depress the $Na^+$ concentration. Aldosterone has an antagonistic action with $Na^+$ electrolyte [13].

Similarly like that of $Na^+$, $Cl^-$ was also depleted in the serum of the fishes, when exposed to low environmental acidic media (Leivested and Muniz [12]) reported the loss of $Cl^-$ in brown trout exposed to acidic stress (Fraser and Harvey [14]) reported in rainbow trout exposed to pH 4.9 exhibited that 14% of $Cl^-$.was observed.

The present results are highly corroborated with [13, 14] when the extra cellular fluid become exceedingly acidic the renal tubule reabsorb large quantities of bicarbonate ions and $Cl^-$ ions, reabsorption become greatly diminished. So the $Cl^-$ depletion in the fishes may be due to the failure of acid base balance. The excessive reabsorption of bicarbonate ions shifts the pH of the buffer system in the extra cellular fluid toward a normal pH.

The depletion of $Ca^+$ might be due to the acid stress response in fish and these results corroborate with the results of Leivestad *et al.*, [12] Mc Donald *et al.*, [10]. Mc Donald [10] had reported the depletion of $Ca^{++}$ in Atlantic salmon exposed to chronic acidic stress.

Many biochemical nutrients and hormones are involved in $Ca^+$ regulation. The fat-soluble vitamin D is very essential and it increases the rate of calcium absorption from the gastrointestinal tract. The acidity affected the Vitamin D and it leads to the depletion of $Ca^+$ level [13]. The hyper secretion of parathyroid hormone feed back mechanism operates whereby decreased $Ca^+$ ions concentration increases parathyroid hormone secretion. This may be the cause for the depletion of $Ca^+$. Further more calcitonin plays the important role in regulation of $Ca^+$ level. Decreased secretion of calcitonin in the fish in acidic environments resulted in the decreased plasma ion concentration [13].

When the fish are exposed to a low pH, chloride cells in the gill tissue take up bicarbonate ($HCO_3$)$^-$ ion from the outside to neutralize the hydrogen ($H^+$) ion flowing in the body. At this time, the losses of sodium ($Na^+$) and chloride ($Cl^-$) ions from the body fluids occur, and plasma osmotic pressure decreases (Iwata *et al.*, 1990). This process is considered to be one of the major reasons, why fresh water fish die under acidic conditions. In Tilapia *Oreochromis niloticus*, *O. mossambicus*, and medaka *Oryzias latipes*, $Na^+$, $K^+$ ATP-ase activity in chloride cells increases in association with $Na^+$ loss when exposed to low pH. This suggests that Na, K – ATP- ase may act to affect $Na^+$ uptake under an acidic hypo tonic environment (Yada and Ito 1997, 98).

In particular, great numbers of Atlantic salmon *Salmo salar* and brown trout *S. trutta* were destroyed by the acidification induced by the rapid inflow of acid pollutants into rivers during spring snow-melts (snow-melt acid shock) in Scandinavian countries. This phenomenon is called "Fish Kill" [12]. Fish have the ability to regulate their acid-base balance in order to maintain normal pH of their body studies under acidic ambiance. Plasma $Na^+$ levels could be used as an indicator to estimate the acute effects of acidification on fish. When *rainbow trout Oncorhynchus mykiss* were exposed to various acidic conditions, the fish showed lower plasma $Na^+$ levels and the $Na^+$ levels and pH were found to be significantly correlated (Yada *et al.*, [18]).

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