# EFFECT STRESS PH ON SOME BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS IN NILE TILAPIA OREOCHROMUS NILOTICUS

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## ABSTRACT

Nile Tilapia, (*Oreochromus niloticus*) is the most common cultured freshwater species worldwide, however, changes in water pH from the normal range affect fish survival and production. Therefore this study was carried out to assess the effect of pH change on some haematological and biochemical parameters of *O. niloticus*. Ninety six of healthy *O. niloticus* were obtained from Manzala Lake, Egypt, they were allowed to adapt for one week, and fish were divided into six groups which then exposed to a range of pH levels. Analysis of *O. niloticus* blood revealed no significant differences on pH concentration, HCT percentage, MCH, MCHC, RBCs, WBCs, as well as MDA, CP, GR, sodium, glucose, cortisol level and IgM levels between the treated groups and the control group., The MCV, SOD CAT total and protein level were significantly lower in pH 5.8 and pH 6.4groups ($P<0.05$) compared to the control group. However thrombocytes count was significantly higher in all treated groups compared to the control group, whereas, potassium concentration was significantly higher on pH 7 and pH 7.8 compared to the control group.

***Keywords:*** Tilapia, pH, Biochemical, Haematologic Parameters.

## INTRODUCTION

*O. niloticus* is a member of Cichlidae family, it is an economically valuable cultured species in several areas of the world (El-Saidy and Gaber, 2005; El-Husseiny et al., 2007). However stress affects Nile tilapia growth and reproduction (Schreck, 2010). In fish stress may be induced by different abiotic factors such as changes in water temperature, pH, salinity, oxygen concentration and water pollutants (Lebelo et al., 2001). The pH is one of the main factors that affect water quality, in aquatic animals; as water pH plays an important role in maintenance of the homeostasis and physiological state in their bodies (Das et al., 2006). In fact the normal physiological processes operate normally in fish under a relatively wide range of pH from 6.5-9 (Mohamed et al., 2020). However, sudden changes in pH levels cause ammonia excretion, disturbances in acid-base and ion regulation (Das et al., 2006) and kill aquatic life, even when the changes occur within a pH range (Geetha, 2014). El-Sherif and El-Feky (2009) showed that the growth of *O. niloticus* fish exposed to acidic water has been reduced.

An increase in environmental pH affects osmoregulation in fish and has been associated with an increase in mucus secretion (Val et al., 1998). The mucus of fish exposed to acid water has been shown to have increased in experiments on Sarotherodon melanotheron (Chindah et al., 2008) and zebra fish (Daniorerio) (Zahangir, 2014). In addition, low pH waters causes a decrease in the ability of fish to extract oxygen (Sailaja and Bhaskar, 2001), increased pH also affects oxygen transfer to tissues (Freda and McDonald, 1988). Mcdonald and Wood (1981) found that a reduction in water pH is correlated with blood acidosis, due to high CO2 arterial pressure. Also acidity and alkalinity are responsible on many changes in the haematological and biochemical parameters of freshwater fish. El-Sherif and El-Feky, (2009) showed a decrease in HCT and Hp in *O. niloticus* associated with the increase in pH levels. Due to the economic importance of *O. niloticus* in aquaculture, the present study, focused on the response and effect of different levels of pH on some haematological and biochemical components, homeostasis and antioxidant defense responses in *O. niloticus*.

# MATERIALS AND METHODS

Ninety six of healthy *O. niloticus* were obtained from Manzala Lake, Egypt. They were fed on fish diet and allowed to adapt for one week in dechlorinated water and fed on a basal fish diet. After acclimation, the fishes were divided into six groups with 8 fishes in each. Each group was treated at different pH (pH (5.8) group, pH (6.4) group, pH (7) group, pH (7.4) group, pH (7.8) group and control group) and each treatment was run in duplicates.

The desired alkalinity or acidity was prepared by mixing 28.39g sodium mono hydrogen phosphate (Na2HPO4, MW=141.96) and 27.59g sodium dihydrogen phosphate (NaH2PO4, MW=137.99) they were dissolved in 1000ml of dechlorinated tap water.

## Blood sampling

About 3 ml of blood were collected from the caudal vein within less than 3 minutes to minimize handling stress. The collected blood was divided into 2 tubes, one tube was for haematological assessment and the other tube was for plasma preparation which then assayed for biochemical parameters.

## Determination of hematological parameters

For the experiments on the effect of pH on hematological parameters of O. niloticus blood cell automated counter was used to determine red blood cells (RBCs), haemoglobin (Hb)content haematocrite (HCT%). thrombocytes count, and white blood cells count (WBCs). In addition, varies blood indices, like the mean corpuscular volume (MCV), mean corpuscular Heamoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated Determination of biochemical parameters:

To see what the effect of pH on biochemical components in O. niloticus plasma samples were prepared and used to evaluate malondialdehyde (MDA), level of protein carbonyl (PC), superoxide dismutase (SOD) activity, catalase (CAT) activity, Glutathione reductase (GR) activity, sodium level (Na+), potassium level (K+), glucose level (GLU), total protein level (TP),cortisol level and immunoglobulin M level (IgM). Using an UV spectrophotometer the absorbance values of samples and standards were measured.

**Statistical data analysis:**

XLSTAT program was used to preform all statistical analyses, the mean and standard error of the mean (SEM) for each treated group compared to control ones were obtained. One way analysis of variance (ANOVA) followed by Tukey test was used to investigate whether there is a significant difference between the treated groups whereas, Dennett two sided test was used for comparisons with the control group. A significance level of $p<0.05$ was used in these statistical tests.

## RESULTS

**Haematological parameters**

One of the aims of this experiment was to study the effect of pH change on haematological component. Table 1 summarizes the effect of pH on some haematological component of O. niloticus exposed to different pH values for a period of 11 days. From the table no significant difference was found in HCT Hb concentration, MCH and MCHC percentage between the treated groups and the control group. However HCT% was significantly higher in pH 7.4 group compared to pH 7 group ($P<0.05$). On the other hand, the

MCV was significantly lower in pH 5.8 and pH 6.4groups ($P<0.05$) compared to the control group and that with pH 7, pH 7.4 and pH 7.8. Thrombocytes count was significantly higher in all treated groups compared to the control group, whereas, RBCs and WBCs did not show any significant differences. The highest level of RBCs was at pH 7.4 while the lowest level of WBCs was at pH 7.

**TABLE 1: HAEMATOLOGICAL PARAMETERS OF NILE TILAPIA O. NILOTICUS SUBJECTED TO DIFFERENT PH DEGREES FOR 11 DAYS.**

| Parameter | pH | | | | | |
|---|---|---|---|---|---|---|
| | **Control** | **pH 5.8** | **pH 6.4** | **pH 7** | **pH 7.4** | **pH 7.8** |
| Erythrocytes ($10^6/\mu l$) | *1.89± 0.13* | *2.08± 0.16* | *2.07±0.15* | *1.46± 0.11* | *2.40± 0.19* | *1.69± 0.23* |
| HCT (%) | $22.2\pm0.78^{ab}$ | $21.6\pm 1.3^{ab}$ | $22.2\pm1.4^{ab}$ | $18.20\pm0.49^{a}$ | $24.8\pm1.59^{b}$ | $19.78\pm1.7^{ab}$ |
| Hb(g/dl) | 6.31±0.38 | 6.53±0.42 | 6.41±0.46 | 5.32±0.17 | 6.8±0.53 | 5.56±0.59 |
| MCV(fl) | $117.5\pm7.45^{a}$ | $104.75\pm5.1^{b}$ | $107.96\pm4.76^{b}$ | $126.98\pm8.8^{a}$ | $104.10\pm3.86^{a}$ | $124.5\pm10.79^{a}$ |
| MCH(Pg) | 33.59±0.75 | 32.07±1.15 | 31.31±1.39 | 37.34±3.40 | 28.42±1.08 | 34.23±2.36 |
| MCHC(g/dl)) | 27.71±1.36 | 30.21±0.58 | 29±0.54 | 29.26±1.01 | 27.40±1.13 | 27.98±1.12 |
| Leucocytes ($10^3/\mu l$) | *4.68±0.23* | *3.74±0.17* | *4.25±0.29* | *3.72±0.41* | *4.12± 0.39* | *3.84± 0.21* |
| Thrombocytes($10^3/\mu l$) | $120\pm 1.5^{a}$ | $197.3\pm7.7^{b}$ | $198\pm 8.4^{b}$ | $201.6\pm14^{b}$ | $235\pm 15^{c}$ | $198.3^{b}\pm 9.9$ |

**Data are means ±S.E. Values with different superscript letters within each row are significantly different (ANOVA, P<0.05).**

In addition, the results presented on table 2 shows the effect of pH change on some biochemical parameters in O. niloticus exposed to different pH degrees for a period of 11 days. It can be seen that significant difference exist between the control group and pH 5.8 group in SOD, also SOD activity was significantly lower in pH 5.8 compared to the pH 6.4and up, except for pH 7.8. At this pH, the significance level is not high enough, but still visible. For all pH treated groups, no significant differences were seen in MDA, CP, GR, sodium, glucose and IgM compared to the control group. Compared to the control group, the CAT was significantly lower in pH 5.8 group and pH 6.4 compared to the control group. While, no significant difference was found between pH 7, pH 7.4, pH 7.8, groups and control group. On the other hand, potassium concentration was significantly higher in pH 7 and pH 7.8 groups compared to the control group. Whereas, total protein level was significantly lower in pH 5.8, pH7 and pH 7.8than the control group. The plasma cortisol level was significantly higher in pH 6.4 and pH 7.8 treated groups compared to pH 5.8, pH 7 and pH 7.4 groups but no difference was found between these groups and the control group.

**TABLE 2: BIOCHEMICAL PARAMETERS OF NILE TILAPIA O. NILOTICUS SUBJECTED TO DIFFERENT PH DEGREES FOR 11 DAYS**

| Parameter | pH | | | | | |
|---|---|---|---|---|---|---|
| | Control | pH 5.8 | pH 6.4 | pH 7 | pH 7.4 | pH 7.8 |
| MDA(nmol) | 29.06± 5.67 | 37.62±4.62 | 35.93±4.74 | 26.62±5.1 | 31.96± 3.55 | 41.72± 4.14 |
| CP(nmol/mg) | 301.73±20.27 | 301.11±37.78 | 305.63±18.07 | 192.61±7.84 | 419.20±10.4 | 229.32±48.24 |
| SOD(U/ml) | $362.74\pm3.1^{a}$ | $336.9\pm6.1^{b}$ | $354.9\pm5.1^{a}$ | $355.8\pm 8.6^{a}$ | $364.6\pm 3.3^{a}$ | $347.8\pm9.9^{ab}$ |
| CAT(U/L) | $619\pm56.4^{a\,c}$ | $367.5\pm54.4^{*b}$ | $421.4\pm35.5^{*b}$ | $437.5\pm111^{ac}$ | $709.4\pm 56.5^{a}$ | $577\pm75.7^{a\,c}$ |
| GR(U/L) | 38.18± 4.43 | 42.79± 9.92 | 39.3±6.83 | 40.24± 8.21 | 25.19± 6.70 | 42.7±8.19 |
| Sodium (mEq/L) | *150.4±1.34* | *157.5± 9.8* | *146.7±2.2* | *149.2± 2.7* | *151.9± 1.6* | *144.± 5.6* |
| Potassium (mEq/L) | $7.44\pm1.01^{a}$ | $10.14\pm1.31^{a}$ | $8.87\pm 1.17^{a}$ | $14.5\pm0.8^{b}$ | $11.16\pm 2.40^{a}$ | $15.42\pm 2.98^{b}$ |
| Glucose (mg/d) | $72.9\pm2.9^{ab}$ | $80.3\pm 6.7^{ab}$ | $66.6\pm 3.3^{ab}$ | $59.9\pm 7.6^{b}$ | $70.9\pm 7.3^{ab}$ | $94.3\pm 8.93^{a}$ |
| Total protein (g/dl) | $6.85\pm0.14^{a}$ | $5.79\pm 0.23^{b}$ | $6.09\pm 0.41^{a}$ | $5.5\pm 0.5^{b}$ | $6.15\pm 0.67^{a}$ | $5.82\pm 0.56^{b}$ |
| Cortisol (ng/ml) | $194.2\pm1.21^{a}$ | $94.32\pm6.32^{a}$ | $159.9\pm 5.9^{ab}$ | $63.3\pm 13.3^{a}$ | $73.3\pm 15.7^{a}$ | $238.6\pm 24.3^{ab}$ |
| IgM (mg/dl) | *193.5±4.71* | *180± 17.7* | *172.7±10.6* | *208.3±32.5* | *183.8±22.1* | *194.3± 20.2* |

**Data are means ±S.E. Values with different superscript letters within each row are significantly different (ANOVA, P<0.05).**

## DISCUSSION

Nile tilapia O. niloticus is one of the most common cultured freshwater species worldwide (El-Saidy and Gaber, 2005). PH plays a fundamental role in biodiversity of fish in the aquatic environment, it is one of the most important water quality characteristics effecting fish growth and physiology (Kulkarni and Barad, 2015). Therefore, for a better understanding of the effect of changes in pH in O. niloticus this study was carried out.

The results showed a significant effect of different pH values on some studied haematological and biochemical parameters. The present study, recorded non-significant differences in HCT% and Hb content of treated groups compared to the control group, similar results were obtained by Vosyliene and Kazlauskiene (2004); El-Sherif and El-Feky (2009) in O. niloticus fingerlings and Yilmaz et al., (2015) in tilapia, O. mossambicus.

However, HCT% was significantly high at pH 7.4 compared to that at pH 7 and pH7.8 groups. Similar results were observed in O. niloticus fingerlings stocked by different levels of pH for 60 days (El-Sherif and El-Feky, 2009) and Colossoma macropomum treated with three water pH levels (4.0, 6.0 and 8.0) for 40 days (Aride et al., 2007).The increase of HCT % in the current study may be due to typical spleen response to adrenaline as it has been shown in Oreochromis mossambicus (Ginneken et al., 1997). Exceed HCT % may be results from excess RBCs lowed after p/t College. Also, current study showed a decrease in hemoglobin content of pH 7.8 treated group compared to the pH 5.8 group. This result is similar to the findings reported by Wilkie et al. (1996) in Oncorhynchus clarki henshawi exposed to pH 8.0 which could be explained by the effect of alkaline conditions on homeostasis (Aride et al., 2007), as Hb content is a reflection of oxygen level in blood during acidic stress, the body undergoes anoxic conditions which disturb the process of energy production within the organism (Iftikhar and Hashmi, 2021).The Decrease in the Hb content may be explained by occult blood loss (Goldstein et al., 2011).

RBCs play an essential role in the circulation of oxygen that is required for normal body functions (Iftikhar and Hashmi, 2021), current study observed a decrease in RBCs counts in pH 7 and pH 7.8 treated groups compared to the control group, similar reduction was observed in Zebrafish (Daniorerio) at pH 9.0 and pH 10.0 (Zahangir, 2014) and in common carp (Cyprinus carpio) at pH 8.5 and pH 9.0 (Ghanbari et al., 2012). This might be due to haemolysis and shrinkage of blood cells by the stress effects of pH (Zahangir, 2014).

The MCV provides an indication of red blood cells status that reflects a normal or abnormal cell division during erythropoiesis (Misra et al., 2020). Current study showed that MCV was significantly lower in pH 5.8 and pH 6.4 groups compared to the control group. These results are in agreement with that obtained by Amarasinghe and Attygalle (2013) in O. mossambicus exposed to low water pH 4.0, these results could be due to the effect of acidic pH on erythrocytes count (RBCs) and HCT values. While, no significant differences were found in MCH and MCHC between the treated groups and control group. According to Amarasinghe and Attygalle (2013) these results could be due to exceed RBCs production or decline in HCT % in pH treated groups.

WBCs are the main immune cells which provide a defense mechanism to the body and change in the number of WBCs indicate an infection in the organism that can be caused by stress (Iftikhar and Hashmi, 2021). The present study did not find a significant difference in WBCs of treated groups compared to the control group. However, the number of WBCs was the highest in pH 6.4 treated group compared to the other treated groups, these finding is in line with Zebrafish (Zahangir, 2014) and Sarotherodon melanotheron (Chindah et al., 2008) placed in pH 5.0 and 6.0.

In fish thrombocytes have critical role in immune response because they have the ability to produce a wide range of bioactive proteins (Semple et al., 2011) as well as their capacity of phagocytosis (Stosik et al., 2001). The results showed that, thrombocytes count was significantly higher in all treated groups compared to the control group; these results are in agreement with that of Chindah et al., (2008) who found that thrombocytes count of Sarotherodon melanotheron are increased as the pH increase. Elevation in thrombocytes count may indicate a non-specific immune response due to stress induced by the change in pH.

SOD and CAT have an essential role in protecting cells against H2O2 production (Karadag et al., 2014). In this study, both the SOD and CAT activity was significantly lower in pH 5.8 compared to the control group. While SOD activity was significantly higher in pH7.4 than pH5.8. Similar results were observed in Oreochromis niloticus and Oreochromis aureus at alkaline pH 8.8, these results are suggesting protection against liver damage by alkaline pH (Han et al., 2016). CAT activity was significantly higher in pH 7, pH 7.4 and pH 7.8 groups than pH 5.8 and pH 6.4groups.These could be due to that CAT can hardly play its role under alkaline stress (Han et al; 2016). Change in water pH is responsible for alterations in ion profiles of fish (Matsuo and Val, 2002).

Non-significant decrease was found in plasma sodium in pH 6.4 compared to the control group; similar results were observed by Amarasinghe and Attygalle (2013) on Oreochromis mossambicus that exposed to low water pH. This may be due to that acidic stress caused inhibition in active Na+ uptake coupled with increased rates of passive Na+ loss, leading to a decrease in plasma Na+ level, (Evans et al., 2005). However, plasma potassium was high in pH 7, pH 7.4 and pH 7.8 compared to the control group. This result is similar to the findings reported by Aride et al. (2007), this could be due to inhibition of potassium uptake where pH controls the solubility of metal is associated with competition between H+ and K+ for the transport area of the branchial epithelium (Tayel, 2003).

It was demonstrated that glucose level is a good indicator of stress status which show changes in metabolism of carbohydrate (Evans et al., 2003) and

(Kamal and Omar, 2011). Current study has reported that the plasma glucose was significantly higher in pH 7.8 group compared to pH 7 group. Similar results were observed by Ghanbari et al. (2012) who studied the effect of pH change in fingerlings of common carp (Cyprinus carpio) and found that plasma glucose has increased by 58% at pH 9. Another study conducted by Zahangir (2014) has observed elevated blood glucose levels at pH 10 on Zebrafish (Danio rerio).The increase in plasma glucose level may be associated with high rate of glycogenolysis required to overcome the increase in energy demands imposed by the increase in pH. (Yilmaz et al., 2015), and (Dos Santos Miron et al., 2008).

Plasma total protein is also thought to be related to a stronger innate immune response in fish (Yılmaz and Ergun, 2012). In this study, the plasma total protein was significantly lower in pH 5.8, pH7 and pH7.8 compared to the control group, this decrease in plasma proteins may be explained on the basis of high requirement of energy production after acidic. Moreover, this could be attributed to the damage of liver cells by pH increase which lead to decline in protein synthesis (Shiku et al., 2003).

Cortisol is well known to suppress fish immune system in relation to different stresses (Harris and Bird, 2000). The results showed increased plasma cortisol in pH 7.8 treated group compared to the other groups. Similar results were observed in Tambaqui Colossoma macropomum (Cuvier) when treated with three pH levels (4.0, 6.0 and 8.0) for a period of 40 days (Aride et al., 2007). These results suggest that cortisol is necessary for the adaptation to alkaline pH and change in energy metabolism (El-Khaldi, 2010).

IgM is a main class of antibody molecules that released as a result of varies stresses (Louis-Cormier et al., 1984). The current results showed that the highest level of IgM was seen at pH7. These results may be due to the effect of cortisol on IgM production (Nagae et al., 2001).

## CONCLUSION

From the present results it can be concluded that the changes in water pH cause alterations in fish hematological and biochemical parameters to overcome the stress effect.

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