Science World Journal Vol 5 (No 4) 2010
www.scienceworldjournal.org
ISSN 1597-6343

Full Length Research Article

EFFECTS OF ACCLIMATION ON HAEMATOLOGICAL PARAMETERS OF *Tilapia guineensis* (Bleeker, 1862)

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ABSTRACT
The effect of acclimation on blood composition of juvenile and adult sizes of Tilapia guineensis was investigated. A total of eighty (80) fish, comprising forty each of juvenile and adult sizes were collected from the wild (Buguma Creek) and acclimated in the experimental tanks for a period of seven days. The pooled data of the results indicated alterations in the blood parameters with significant reduction ($p < 0.05$) in the values of Haemoglobin (Hb); Packed Cell Volume (PCV); Red Blood Cell (RBC); Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelets while there were significant ($P < 0.05$) increase were observed in the mean values of Erythrocyte Sedimentation Rate (ESR); White Blood Cell (WBC); Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Lymphocytes; Neutrophils and Monocytes. The results of this study thus highlighted the stress to which fish are subjected to during the process acclimation.

Key words: Acclimation; Haematology, Stress, Aquaculture, *Tilapia guineensis*

INTRODUCTION
Effective management practices have been recognized as a key to profitable and sustainable fish farming in Nigeria. This has led to the application of various manipulation strategies to maximize fish production in a cultured environment for the benefit of mankind (Akinrotimi *et al.,* 2007a). One of the production techniques commonly used in brackish water species is acclimation, which is a pre condition for stocking of ponds, both for culture and experimental purposes (Akinrotimi, 2006). Acclimation therefore, is the modification of biological structures to minimize deviation from homeostasis despite change in environment and physico-chemical parameters (Gabriel *et al.,* 2001; Akinrotimi *et al.,* 2007b). It is a general practice to subject fish species to be used in laboratory experiment to a minimum acclimation period of seven days (Gabriel *et al.,* 2004). It is believed that during this period the fish may show symptoms of hidden disease that may assist in the separation of apparently healthy fish for any trial or culture (Gabriel *et al.,* 2007a).

The use of haematological parameters in assessment of fish physiology was proposed by Hesser (1960), since then haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful condition such as handling, pollutants, metals, hypoxia, anaestehtics and acclimation (Blaxhall, 1972; Duthie & Tort, 1985; Ogbulie & Okpowasili, 1999; Alwan *et al.,* 2009). Fish are known to be in close relationship with the aqueous environment, hence, the blood will reveal conditions within the body of the fish long before there is any visible manifestation of disease (Musa & Omoregie, 1999; Okechukwu *et al.,* 2007), haematological indices are therefore widely used by fish biologists and researchers the world over.

Fernades & Mazon (2003) reported that fish blood are closely related to its response to changes in the environment where it lives, natural or artificial. The responses of fish to particular stressor vary according to their characteristics, however there are features of stress reaction common to the majority of most forms of environmental stressors which are known to alter their blood characteristics thereby leads to disruptions in metabolic activities (Ajani *et al.,* 2007), reduced growth rate and impairment of reproductive process (Mgbeka *et al.,* 2005) suppression of immune system (Auta, 2001) and in extreme cases results in mortality (Akinrotimi *et al.,* 2009).

Effects of acclimation on haematological parameter of fish have been studied in some *species* such as *Clarias gariepnus* (Ezerie *et al.,* 2004; Gabriel *et al.,* 2004), *Sarotherodon melanotheron* (Gabriel *et al.,* 2007b; Anyanwu *et al.,* 2007 and Akinrotimi *et al.,* 2007b). However, reports on haematological response of *T. guineensis* to the process of acclimation are scanty, thus necessitating the need for this work. It focuses on response of this species to conditioning of acclimation in an artificial environment.

MATERIALS AND METHODS
Eighty *T. guineensis* consisting of forty juvenile fish (mean length 14.64±3.22SD; mean weight 92.38g±5.79SD) and forty adult fish (mean length 24.77cm±4.66SD; mean weight 402.12g±6.23SD) were harvested from the recruitments ponds of African Regional Aquaculture Centre, brackish water research fish farm, Buguma, Rivers State, Nigeria. Their blood was taken immediately and stored in bottles containing Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant. The fish were immediately transferred to eight 30 m^3 concrete tanks (four for each size), with stocking rate of ten fish per tank for a period of seven days.

The fish were fed twice daily and the water in the experimental tanks was renewed every two days. At the end of the experimental period, another set of blood was taken from the fish from the caudal vessels using heparinized syringes with 21gauge needle and stored in EDTA bottles

Standard haematological procedures described by Blaxhall & Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (Hb) concentration was estimated as cyanmethemoglobin (Brown, 1980), Packed Cell Volume (PCV) was determined using microhaematocrit method of Snieszko (1960). The Red Blood Cell (RBC) were counted using haemocytometer (Improved Neubauer Weber Scientific Ltd), according to Wintrobe (1978). Also the total White Blood Cell Counts (WBC) was enumerated with an improved Neubauer Haemocytometer using Shaw's diluting fluid (Miale, 1982). The Erythrocyte Sedimentation Rate (ESR) was done by Wintrobe method (Wedemeger *et al.,* 1983). Platelet (PLT) count was performed according to Rees & Ecker method (Seivered, 1983). The Red Blood Cell indices that include Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume were calculated using the formula mentioned by Dacie & Lewis (2001).

Some physico-chemical parameters of the ponds and experimental tank were measured before and at the end of the experiment. The concentrations of nitrite, ammonia, dissolved oxygen and sulphide were evaluated according to APHA (1985).

Data generated were subjected to one way analysis of variance (ANOVA) test at 0.05% probability and differences among mean were indicated using least significant difference (LSD) tests (Zar, 1984).

RESULTS

The parameters for the water quality measured before and after acclimation (Table 1) were within the same range with no significant difference ($P > 0.05$). The haematological components of juvenile and adult sizes of *T. guineensis* before and after acclimation are presented in Table 2 and 3. The result indicated a consistent reduction in the values of Hb, PCV, RBC, MCHC, and platelets, while the values of ESR, WBC, MCH, MCV, Lymphocytes, neutrophils and monocytes increases significantly ($p < 0.05$), which was more observable in adult fish than the juvenile (Table 2 and 3). When the data were pooled for the juvenile and adults, there were significant differences ($p < 0.05$) in all the blood parameters (Table 4).

TABLE 1. WATER QUALITY PARAMETER DURING THE EXPERIMENTAL PERIOD

Parameter	Before Acclimation	After Acclimation
	Mean ± SD	Mean ± SD
Temperature (^{0}c)	28.21 ± 1.66^{a}	28.46 ± 3.14^{a}
Ammonia (mg L^{-1})	0.37 ± 0.02^{a}	0.39 ± 0.01^{a}
Nitrite (mg L^{-1})	0.0036 ± 0.01^{a}	0.0040 ± 0.01^{a}
pH	6.58 ± 1.14^{a}	6.88 ± 1.21^{q}
Dissolved Oxygen (Mg L^{-1})	4.66 ± 1.26^{a}	4.64 ± 1.32^{a}
Sulfide (Mg L^{-1})	0.05 ± 0.01^{a}	0.07 ± 0.01^{a}
Salinity (‰)	17.00 ± 6.21^{a}	17.00 ± 4.21^{a}

Mean with different superscript in the same row are significantly different ($p < 0.05$)

TABLE 2. HAEMATOLOGICAL RESPONSE OF *T. GUINEENSIS JUVENILE TO ACCLIMATION*

Parameter	Before Acclimation	After Acclimation
	Mean ± SD	Mean ± SD
Hb (g/dl)	4.98 ± 1.26^{a}	4.00 ± 1.11^{b}
PCV (%)	18.64 ± 4.18^{a}	16.22 ± 42.2^{b}
ESR (mm/hr)	5.24 ± 1.24^{a}	6.78 ± 2.46^{b}
RBC ($x10^6/\mu^L$)	2.62 ± 1.10^{a}	1.98 ± 1.02^{b}
WBC ($x10^6/\mu^1$)	19.34 ± 4.71^{a}	21.62 ± 5.61^{b}
MCHC (%)	26.73 ± 5.68^{a}	22.32 ± 4.69^{a}
MCH (pg)	19.00 ± 3.78^{a}	20.20 ± 3.92^{b}
MCV (μ^3)	71.14 ± 6.45^{a}	80.29 ± 11.34^{b}
Platelets ($10^6/\mu^L$)	86.22 ± 10.22^{a}	80.21 ± 12.11^{b}
Lymphocyte (%)	62.42 ± 7.80^{a}	44.07 ± 3.98^{b}
Neutrophils (%)	35.36 ± 4.26^{a}	52.21 ± 6.74^{b}
Monocytes(%)	2.22 ± 1.21^{a}	3.63 ± 1.12^{b}

Mean with different superscript in the same row are significantly different ($p < 0.05$).

TABLE 3. HAEMATOLOGICAL RESPONSE OF *T. GUINEENSIS ADULT TO ACCLIMATION*

Parameter	Before Acclimation	After Acclimation
	Mean ± SD	Mean ± SD
Hb (g/dl)	5.6 ± 1.12^{a}	3.90 ± 1.22^{b}
PCV (%)	20.24 ± 4.68^{a}	16.22 ± 2.11^{b}
ESR (mm/hr)	4.28 ± 1.28^{a}	9.78 ± 3.42^{b}
RBC ($x10^6/\mu^L$)	2.99 ± 1.02^{a}	1.78 ± 0.21^{b}
WBC ($x10^6/\mu^1$)	21.34 ± 4.24^{a}	22.46 ± 6.81^{b}
MCHC (%)	28.06 ± 5.61^{a}	25.62 ± 4.11^{b}
MCH (pg)	18.99 ± 3.76^{a}	21.91 ± 3.72^{b}
MCV (μ^3)	67.69 ± 8.92^{a}	76.86 ± 12.46^{b}
Platelets ($10^6/\mu^L$)	90.24 ± 11.21^{a}	70.22 ± 11.21^{b}
Lymphocyte (%)	64.37 ± 4.21^{a}	46.10 ± 7.81^{b}
Neutrophils (%)	33.64 ± 7.21^{a}	50.11 ± 6.21^{b}
Monocytes(%)	2.91 ± 1.02^{a}	3.79 ± 1.12^{b}

Mean with different superscript in the same row are significantly different ($p < 0.05$)

TABLE 4. MEAN VALUES OF HAEMATOLOGICAL RESPONSE OF *T. GUINEENSIS TO ACCLIMATION*

Parameter	Before Acclimation	After Acclimation
	Mean ± SD	Mean ± SD
Hb (g/dl)	5.33 ± 1.17^{a}	3.76 ± 1.24^{b}
PCV (%)	19.44 ± 3.12^{a}	15.72 ± 3.64^{b}
ESR (mm/hr)	4.76 ± 1.79^{a}	8.28 ± 2.11^{b}
RBC ($x10^6/\mu^L$)	2.81 ± 0.96^{a}	2.00 ± 1.01^{b}
WBC ($x10^6/\mu^1$)	20.34 ± 3.46^{a}	22.04 ± 5.79^{b}
MCHC (%)	27.40 ± 4.28^{a}	23.97 ± 7.21^{b}
MCH (pg)	18.99 ± 4.12^{a}	21.64 ± 3.98^{b}
MCV (μ^3)	69.41 ± 3.22^{a}	78.58 ± 6.22^{b}
Platelets ($10^6/\mu^L$)	88.23 ± 7.22^{a}	75.22 ± 7.14^{b}
Lymphocyte (%)	63.53 ± 8.91^{a}	47.09 ± 4.22^{b}
Neutrophils (%)	33.76 ± 3.66^{a}	49.16 ± 3.78^{b}
Monocytes(%)	2.60 ± 1.08^{a}	3.71 ± 1.10^{b}

Mean with different superscript in the same row are significantly different ($p < 0.05$)

DISCUSSION

The physiological stress resulting from acclimation is clearly reflected by blood patterns of the experimental fish. There was significant reduction in the values of Haemoglobin and Red blood cells in both sizes exposed to acclimation. The reduction observed may be as a result of stress which affects the metabolism and normal functioning of the fish physiology. Red Blood Cells, is composed mainly of haemoglobin surrounded by a flexible protein membrane and an outer lipid bilayer. And the energy required for the maintenance of red cell shape, flexibility and osmotic pressure is provided by adenosine triphosphate (ATP), generated by anaerobic glycolysis (Cheesbrough, 2005). The depletion of ATP as a result of imposed stress due to acclimation, results in inability of the red cells to transport excess sodium out of the cell membrane and subsequent haemolysis of the red cells (Emelike *et al.*, 2008). Thus the red cell life span becomes so short that cells are destroyed much faster than they can be formed (Guyton & Hall, 2005).

The Packed Cell Volume (PCV), reduced in both sizes of *T. guineensis* consequent of acclimation. This is in line with the findings of Ezeri *et al.,* (2004) who observed similar findings in African Catfish *Clarias gariepinus* acclimated for seven days. The reduction in PCV values was providusly recorded by Poleo & Hytterod (2003) for Atlantic Salmon *salmon salar* exposed to heavy metals. The low values of PCV in fish exposed to stressors has been explained by a reduction in RBC volume caused by osmotic changes due to ion losses from the blood plasma and on the other hand by reduced number of RBC as a result of adrenergic – splinic expansion in hypoxic conditions (Alwan *et al.,* 2009).

The erythrocyte sedimentation rate (ESR) is the speed at which red cells settled out of their plasma (Seiverd, 1983). Hence ESR is a non specific haematological parameter that may indicate the presence and intensity of a disease state (Gabriel *et al*., 2004). The values are usually raised with increased tissue destruction as in acute infections and heavy metal poisoning among others (Blaxhall & Daisley, 1973). The increased value of ESR in this study corroborated the reports of Onusiriuka & Ufodike (2000), in *Clarias gariepinus* exposed to some plant extracts under laboratory conditions. The increase ESR value may be due to fragility of ertythrocytes as a result of stress induced disruptions in the formation and release of erythrocytes from haematopocitic tissue (Akinrotimi *et al.,* 2007c).

The mean corpuscular values are concerned with the volume of the average erythrocyte and the amount of haemoglobin in the average erythrocyte and the three types are Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC), which measures the Volume, Weight and the Concentration of Haemoglobin respectively (Wedemeyer *et al.,* 1983). The values of this indices recorded in this study agrees with the findings of Anyanwu *et al.,* (2007) who observed same in black jaw tilapia transfer directly from brackish water to fresh water. The decreased value of MCHC and the increase values of MCH and MCV indicated the extent of the shriking cell size of erythrocytes stress induced by acclimation.

Changes in White Blood Cell (WBC) and the differential counts, neutrophils, lymphocytes and monocytes indicated a stress condition in *T. guineensis.* In this study, the increased percentage of neutrophils and monocytes and decrease percentage of lymphocyte in the circulating blood of *T. guineensis* agrees with the report of Johansson-Sjobeck *et al.,* (1978) in *Anguilla anguilla* exposed to handling stress, but contrary to the findings of Salonius & Iwama (1993), who reported increased in lymphocytes percentage in Atlantic salmon subjected to handling stress. This disparity may be due to duration of the stress. The salmons were only exposed for a period of four hours, and the *T. guineensis* for seven days.

Finally, the present study suggests that the perturbations in these blood indices attributed to a defence reaction against stress imposed by acclimation process due to the disturbances that occurred in both metabolic and haem activities of fish exposed to acclimation.

In conclusion, this study has provided valuable information for fish biologist in the assessment of fish health and in monitoring stress response we assume that variation in values of blood indices may be a defensive mechanism against stress induced by acclimation. Also the shrink also revealed that size of the fish exert some degree of influence on the haematological parameters of *T. guineensis* and hence the need to reckon with these in the assessment and reporting of the haematological characteristics of this species.

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