EFFECTS OF DIRECT TRANSFER TO FRESH WATER ON THE HAEMATOLOGICAL PARAMETERS OF *Tilapia guineensis* BLEEKER, 1862

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ABSTRACT

Changes in blood parameters of Tilapia guineensis transferred directly from brackish water (salinity 14.5 ‰) to fresh water (0.12 ‰) was investigated to assess the effect of sudden change in environment on fish blood characteristics. The results obtained indicated significant reduction (p < 0.05) in mean values of haemoglobin (Hb) from 5.50 ± 0.61 to 3.65 ± 0.66 dl^{-1}, packed cell volume (PCV) 20.31 ± 2.14 to 17.18 ± 2.58%, red blood cell (RBC) 2.59 ± 0.72 to 1.58 ± 0.86 x 106, mean corpuscular haemoglobin concentration (MCHC) 27.03 ± 3.66 to 20.99 ± 3.75 pg, mean corpuscular volume (MCV) 78.82 ± 6.12 to 70.92 ± 4.27 fL and platelets (PLT) 175.19 ± 12.32 to 144.46 ± 9.22 10^3uL^{-1}. However, increases were observed in the values of erythrocyte sedimentation rate (ESR) from 3.06 ± 0.16 to 9.71 ± 1.66 mm/hr, white blood cell (WBC) 27.18 ± 2.96 to 29.69 ± 3.75 x 10^9L^{-1}, neutrophils (NEUT) 41.64 ± 3.76 to 50.76 ± 7.27 %, lympphocytes (LYMP) 55.30 ± 3.24 to 59.30 ± 11.26 % and monocytes (MON) 3.06 ± 1.12 to 3.96 ± 1.21 %. These alterations in the blood parameter were more noticeable in adult than juvenile fish. Results from this study therefore suggest that direct transfer of fish to area of lower salinity may have negative impact on the physiology of T. guineensis.

Keywords: Haematology, *Tilapia guineensis*, Salinity, Freshwater, Brackish water

INTRODUCTION

The Guinean tilapia, *Tilapia guineensis* is considered a potential commercial aquaculture species in Niger Delta region of Nigeria, because of a higher consumer acceptance, reasonable market price, and defined culture techniques (Akinrotimi *et al.,* 2009). Adaptation to changes in environmental factors has been identified as one of the major area of research necessary for *T. guineensis* culture development (Akinrotimi *et al.,* 2007a). In the wild, *T. guineensis* are estuarine, fond mostly in the coastal parts of the country. There is the need therefore to assess the possibility of culturing this specie in fresh water. Lemarie *et al.* (2004), reported that the ability of *Tilapia* to live in different environment with fluctuating salinities depends on the species, mean individual weight, methods of transfer, feeding techniques during pre-acclimation, the physiological status of the fish and more generally the effects of environmental factors.

Consistent changes in composition of aquatic environment have been observed to alter the behaviour and physiology of fish (Alkahem *et al.,* 1998). In aquaculture, the performance of cultured fish species is control by not only genetic potential and technological manipulation but also by its immediate environmental conditions (Pickering, 1993). Sudden change in the environmental condition of fish, reportedly cause serious stress, which will eventually disrupt internal homeostatic (Reddy and Leatherland, 1998).

Teleosts exposed to hypoosmotic environments experience osmotic water gain coupled with diffusive ion loss. To balance the influx of water in the body of the fish and ultimately survive in water of low salinity, fish must actively absorb ions from the surrounding water (Sardella *et al.,* 2004), in the intestine water is absorbed osmotically, following active absorption of salts across the gill epithelium (Wilson *et al.,* 1996). Fish face osmotic challenges in both freshwater and sea and to a greater degree when moving between these environments (Evans *et al.,* 2005). The requirements for salt and water homeostasis in freshwater or seawater can be quite large due to the large respiratory surface area of the gill (Marshall, 2002). When demands for oxygen uptake increase, it decreases blood perfusion distance in the gill with accompanying alterations in haematological parameters of the fish, thus affecting its metabolism (Sakamoto and McCormick, 2006). The disrupted blood components involved in maintaining or expanding the capacity of water, ion transport, and homeostasis are now limited in performing their functions, thus leading to immunodepression, low productivity disease outbreaks and ultimately mortality (Akinrotimi *et al.,* 2007b). Hence, proper knowledge of haematological response in fish to changes in environment will help in improving production of fish and control of stress in aquaculture.

This work therefore examine the effect of direct transfer from brackish to fresh water on the haematological indices of *T. guineensis* a popular fish for culture among local farmers in the Niger Delta regions of Nigeria.

MATERIALS AND METHODS

One hundred and twenty *T. guineensis* comprising of 60 juvenile size (length 12.64 ± 2.11 cm and weight 42.61 ± 6.21 g) and 60 adult size (length 20.24 ± 3.24 cm and weight 384.26 ± 28.64 g) were collected from brackish water in Buguma creek at low tide. They were immediately transferred to the outdoor hatchery where the initial blood samples were taken from the fish and taken to laboratory for analysis. They were later kept 20 each in three rectangular tanks (0.36 m^3) filled to half capacity with fresh water for a period of seven days for each experimental fish sizes. The fish were fed twice daily with 35 % crude protein pelleted feed at 2 percent body weight.

Physico-chemical parameters namely temperature pH, ammonia nitrogen, nitrate, dissolved oxygen, sulfide and salinity in the creek (brackish water) and in the experimental tanks (fresh water) were monitored. Temperature was taken with mercury in glass thermometer, pH with pH meter while ammonia, nitrate, sulfide and dissolved oxygen were determined with Horiba U-7 water checker. And salinity was measure with hand held refractometer (Model HRN-2N Adago Products, Japan).

Blood samples were collected from a total of 40 fish, that is 20 each before and after transfer, comprising of 10 fish each for both juvenile and adult sizes. Blood samples were obtained with heparinized plastic syringe fitted with 21 gauge hypodermic needle and preserved in disodium salt of ethylene diamine tetraacetic acid (EDTA) bottles for analysis. The blood samples collected at the hatchery were labeled initial and those collected after seven days of transfer were labeled final samples.

The packed cell volume (PCV) was determined using microhaematocrit method (Snieszko, 1960), haemoglobin (Hb) by cyanomethaemoglibin method (Brown 1980), red blood cell (RBC) and white blood cell counts (WBC) were enumerated using improved Neubaeur haemocytometer (Miale, 1982). The erythrocyte sedimentation rate (ESR) was done using Wintrobe method (Wedemeyer *et al.,* 1983), platelet (PLT) by Rees and Ecker method (Seiverd, 1983), while the red blood cell indices; mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume were calculated

Table 1: Physico-chemical parameters of water before and after transfer of *T. guineensis* from brackish to freshwater environment

Parameters	Before Transfer (Brackish water)	After Transfer (Freshwater)
pH	6.60 ± 0.34^a	6.89 ± 0.41^a
Temperature (^{0}C)	28.14 ± 1.64^a	28.32 ± 1.78^a
Ammonia (mgL^{-1})	0.45 ± 0.01^a	0.71 ± 0.01^a
Nitrate (mgL^{-1})	0.0046 ± 0.02^a	0.0067 ± 0.01^a
Dissolve oxygen (mgL^{-1})	4.36 ± 0.06^a	4.01 ± 0.02^a
Sulfide (mgL^{-1})	0.04 ± 0.01^a	0.02 ± 0.01^a
Salinity (‰)	14.50 ± 2.61^a	0.04 ± 0.01^b

Means with the same superscript within the row under before and after trial are not significantly different (p>0.05)

Table 2: Seven day mortality of *T. guineensis* transfer from brackish to freshwater environment

Days	Fish Sizes	
	Juvenile	Adult
1	13.00 ± 1.21^a	36.00 ± 6.11^b
2	10.00 ± 4.11^a	31.00 ± 7.22^b
3	9.00 ± 2.14^a	29.00 ± 4.22^b
4	7.00 ± 1.21^a	25.00 ± 3.12^b
5	6.00 ± 2.16^a	21.00 ± 3.66^b
6	3.00 ± 1.10^a	16.00 ± 4.14^b
7	2.00 ± 1.11^a	10.00 ± 3.76^b

Means with the same superscript within the row under before and after trial are not significantly different (p>0.05)

from the equation of Anderson and Klontz (1965). The differential counts were done on blood film stained with Grumwald-Giemsa stain as described in Hrubec *et al.* (2000).

The mortality was determined by counting the number of the dead fish in each experimental tank for each size from the fist day to the seventh day.

All data obtained were subjected to Analysis of variance (ANOVA) at 0.05% probability and differences among means were separated with the significant difference using SAS software (SAS, 1999).

RESULTS

The salinity of water in the creek (before transfer) and the experimental tanks (after transfer) were significantly different ($p < 0.05$) (Table 1). Other physioco-chemical parameters were statistically similar ($p > 0.05$) (Table 1). After transfer the highest mortality (86.00 ± 6.11) was recorded in day 1 for adult fish while the lowest (2.00 ± 1.11) was observed in day 7 for juvenile fish (Table 2).

The results on the haematological parameters of juvenile and adult *T. guineensis* transfer to freshwater environment indicated a consistent reduction in Hb, PCV, RBC, MCHC, MCH and PLT values, while the values of ESR, WBC, MCV, Neutrophils, lymphocytes and monocytes increased significantly ($p < 0.05$) and was more pronounced in adult than in juvenile fish (Table 3 and 4).

The pooled data for the changes in haematological parameters irrespective of fish size indicated that the lowest value was observed in RBC while PLT had the highest value (Table 5).

DISCUSSION

The knowledge of the haematological characteristics is an important tool as well as environmental factors that can infer with the fish population health. Fish respond in a different way depending on the stressor stimuli, as observed by Martins *et al.* (2004) and Gomes *et al.* (2003). According to Akinrotimi *et al.* (2007c), the haematological response of fish to stress is dictated by the nature of the stress itself, that is specific stress elicit specific responses. According to Luskova (1996) and Gbore *et al.* (2006) the significance of the haematological status of a fish as a biological

Table 3: Haematological profile of juvenile *T. guineensis* before and after transfer from brackish to freshwater environment

Blood Parameters	Before Transfer Brackish water	After Transfer Freshwater
Hb (gld1)	5.01±0.49[a]	3.99±6.11[b]
PCV (%V)	19.38±1.21[a]	18.24±3.11[b]
ESR (mm/hr)	2.98±0.12[a]	8·74±1.12[b]
RBC (x10^6/μL)	2.31±0.66[a]	1.51±0.71[b]
WBC (x10^9/L)	26.41±2.24[a]	29.26±1.21[b]
MCHC (%)	25.85±2.71[a]	21.88±2.61[b]
MCH (g/dl)	21.68±6.14[a]	20.15±1.86[b]
MCV/(fl)	83.89±6.14[a]	92.12±4.24[b]
PLT/(10^8/μL)	171.74±9.13[a]	150.21±12.61[b]
Neutrophils (%)	40.64±3.64[a]	44.88±3.21[b]
Lymphocytes (%)	56.24±2.79[a]	60.38±13.12[b]
Monocytes (%)	3.12±1.74[a]	3.99±0.82[a]

***Key:** Hb – Haemoglobin; PCV – Packed Cell Volume; ESR – Erythrocyte Sedimentation Rate; RBC – Red Blood Cell; WBC – White Blood Cell; MCHC – Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Haemoglobin PLT – Platelets*

Table 4: Haematological profile of adult *T. guineensis* before and after transfer from brackish to freshwater environment

Blood Parameters	Before Transfer Brackish water	After Transfer Freshwater
Hb (gld1)	5.99±0.24[a]	3.24±0.82[b]
PCV (%V)	21.24±1.46[a]	16.11±2.32[b]
ESR (mm/hr)	3.14±0.64[a]	10.68±2.14[b]
RBC (x10^6/μL)	2.88±0.21[a]	1.64±0.14[b]
WBC (x10^9/L)	27.94±3.41[a]	30.12±3.22[b]
MCHC (%)	28.20±2.61[a]	20.11±4.61[b]
MCH (pg)	20.79±2.61[a]	19.76±3.12[b]
MCV/(fl)	73.75±6.21[a]	49.72±4.14[b]
PLT(10^8/μL)	178.64±4.64[a]	138.71±6.21[b]
Neutrophils (%)	42.64±3.89[a]	56.64±11.24[b]
Lymphocytes (%)	54.36±7.11[a]	58.22±9.22[b]
Monocytes (%)	3.00±0.89[a]	3.92±0.14[b]

***Key:** Hb – Haemoglobin; PCV – Packed Cell Volume; ESR – Erythrocyte Sedimentation Rate; RBC – Red Blood Cell; WBC – White Blood Cell; MCHC – Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Haemoglibin PLT – Platelets.*

indication of stressful condition was established and various haematological parameters were shown as very sensitive to intoxication with trace metals, micro contaminants, diseases, and changes in environmental factors.

The present study demonstrates different haematological responses of *T. guineensis* subjected to direct transfer from brackish to fresh water. The sudden change in the environment induced a significant (P < 0.03) reduction in the following parameters of blood; haemoglobin, packed cell volume, red blood cell, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and platelets. The count of old disintegrated erythrocyte was significantly higher in adult than the juvenile fish. Patterns of changes in these parameters found by other authors depended on the salinity level, duration of exposure, mode of transfer and fish species. The reduction in blood parameters as observed in this study is in line with the results of Gabriel *et al.* (2007a) in black chin tilapia *Sarotherodon melanotheron* where similar reductions in blood parameters were reported. This may be due to the drastic effect of stressor (change in environment) which leads to haemodilution, as a result of impaired erythropoiesis (production of erythrocytes) consequent of disrupted osmoregulation (Gabriel *et al.*, 2007b). Zhiteneva *et al.* (1989) reported inhibition of erythrocyte production, splitting of the cytoplasm of erythrocytes in tilapia exposed to sudden change in environment.

Direct transfer of *T. guineensis* into new environment also elicit an increase in erythrocyte sedimentation rate, white blood cell, mean corpuscular volume, neutrophils, lymphocytes and monocytes. The erythrocyte sedimentation rate which measure the speed at which red cell settle out of their plasma, increased significantly, is an indicative

Table 5: Haematological profile of *T. guineensis* (juveniles and adults) before and after transfer from brackish to freshwater environment

Blood Parameters	Before Transfer Brackish water	After Transfer Fresh water
Hb (gld1)	5.50±0.61[a]	3.65±0.66[b]
PCV (%V)	20.31±2.14[a]	17.18±2.58[b]
ESR (mm/hr)	3.06±0.16[a]	9.71±1.66[b]
RBC (x10^6/µL)	2.59±0.72[a]	1.58±0.86[b]
WBC (x10^9/L)	27.18±2.96[b]	29.69±4.21[b]
MCHC (pg)	27.03±3.66[b]	20.99±3.75[b]
MCH (g/dl)	21.24±4.11[a]	19.96±4.22[a]
MCV(fl)	78.82±6.12[b]	70.92±4.22[a]
PLT(10^8/µL)	175.19±12.32[a]	144.46±9.22[b]
Neutrophils (%)	41.64±3.76[b]	50.76±7.22[a]
Lymphocytes (%)	55.30±3.24[b]	59.30±11.26[b]
Monocytes (%)	3.06±1.12[b]	3.96±1.21[a]

Key: *Hb – Haemoglobin; PCV – Packed Cell Volume; ESR – Erythrocyte Sedimentation Rate; RBC – Red Blood Cell; WBC – White Blood Cell; MCHC – Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Haemoglibin PLT – Platelets. Mean within the row with different superscript are significant (P < 0.05)*

of anaemia, due to fragility of the erythrocytes as a result of stress induced disruptions in the formation and release of erythrocytes from haematopoeitic tissue (Akinrotimi *et al.,* 2009). The present study demonstrated significant increase in the number of white blood cell (WBC) this agreed with the findings of Davids *et al.* (2002) who reported increase in WBC in *Sarotherodon melanotheron* after exposure to new environment. Also, number of WBC increased in goldfish *Carassius auratus* after exposing the fish to different type of stressors (Vosyliene and Kazlauskiere, 1999). An increase in the count of WBC may be caused by a release of cells accumulated in the spleen, when fish is exposed to stressors (Houston *et al.,* 1996).

The differential counts namely monocytes, neutrophils and lymphocytes increased considerably as seen in this study, this support the findings of Girling *et al.,* (2003) in green back flounder *Rhombosulea tapirina* exposed to fresh water. The increased observed may be due to release of more of the white cells to combat the stressor (Ajani *et al.,* 2007).

Conclusion: The present study confirmed that haematological parameters are very sensitive indicators of fish organism response to changes in the environmental factors. The study revealed that a sudden change in the fish environment can distress fish physiological status. Therefore it is necessary and crucial to culture fish in a suitable environment for its adaptive and functional physiological mechanism. When there is the need to culture *T. guineensis* in fresh water it should undergo gradual acclimation rather than direct transfer which may leads to reduction in production potential of fish and in some cases lead to mortality.

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