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Haematological Responses of Wild Nile Tilapia *Oreochromis niloticus* after Acclimation to Captivity

Ugwem U. Gabriel[1] , Ojo A. Akinrotimi [2,*] and Funkeye Eseimokumo [3]

[1,3] Department of Fisheries Aquatic Environment, Rivers State University of Science and Technology,[2]African Regional Aquaculture Centers-Nigeria Institute for Oceanography and Marine Research P.M.B. 5122, Port Harcourt, Nigeria

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Abstract

The effects of acclimation to captivity on blood composition of Nile tilapia *Oreochromis niloticus* were investigated. A total of ninety fish, comprising forty five each of juvenile (mean length 10.22cm ±0.26SD; mean weight 54.62g±6.74SD) and adult (mean length 19.4cm±7.21SD; mean weight 346.22g±2041SD) were harvested from a reservoir at African Regional Aquaculture Centre, Aluu, Port Harcourt and acclimated in the experimental tanks for a period of seven days. Before acclimation significant difference ($p < 0.05$) were observed between the blood parameters of adult and juvenile fish with the female consistently having higher values than the males in all the parameters. After acclimation to captivity, there was significant reduction ($p<0.05$) in the values of haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), lymphocytes and thrombocyte, while the values of mean corpuscular volume (MCV), white blood cell (WBC), neutrophils (NEUT) and monocytes (MON) increased significantly ($p < 0.05$). Results from this study therefore suggest that sex and size have some degree of influence on the blood characteristics of *O. niloticus*.

Keywords: Haematology, tilapia, fish, acclimation, aquaculture.

1. Introduction

Fish represents at least 55% of the animals protein consumed in the diet of Nigerians (Anyanwu *et al.*, 2002), with fish demand outstripping the supply this has led pressures on the natural fisheries reserves, which have been subjected to gross over fishing for several years. It is therefore imperative in order to achieve self-sufficiency for the country (Adesulu, 2001).

The choice of species to culture and efficient management practices are very crucial to the overall success of any aquaculture venture, according to Gabriel *et al*. (2007a). Effective handling procedures of species in the culture medium are key practical factors which determine the profitability and sustainability of aquaculture as an enterprise. Effective management practice has 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 the culture environment (Akinrotimi *et al*., 2007a). According to Akinrotimi *et al*. (2007b), one of the production procedures commonly used in aquaculture is acclimation, which is a preconditioning of fish before stocking in ponds and before use for experimental studies. Acclimation is, therefore, the modification of biological structures to minimize deviation from homeostasis, despite change in environmental factors (Gabriel *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*., 2007c).

Haematological variables have been used as indices of fish health status in a number of fish species to detect physiological changes as a result of stress condition such as exposure to pollutants, hypoxia, transportation, anaesthetic and acclimation (Akinrotimi *et al.*, 2009). Haematological indices are therefore ready tools used by fish biologists and researchers in many parts of the world. This is so because fish are closely associated with the aquatic environment and the blood will reveal conditions within the body of the fish long before there is any visible sign of disease (Fernades and Mazon, 2003).

Fish may be stressed when captured and when held in captivity. Effects of acclimation to captivity on haematological parameters of fish have been studied in a number of fish species, *Clarias gariepinus* (Ezeri *et al.*, 2004, Gabriel *et al*., 2004), *Sarotherodon melanotheron* (Akinrotimi, *et al.*, 2006; Gabriel *et al*, 2007d), *Tilapia guineensis* (Akinrotimi *et al*., 2010). There is limited information on the blood parameters of *O. niloticus*, which is a good experimental fish for studying the effect of environmental conditions on blood parameters (Omoregie

[*] Corresponding author: ojoakinrotimi@yahoo.com

and Oyebanji 2002). For this reason, the objective of this study was to assess the effects of acclimation to captivity on haematoligical characteristics of the species.

2. Materials and Methods

A total of 90 *O. niloticus* comprising 45 each adult (mean length 19.48cm±7.21SD; mean weight 246.22g ± 20.41SD) and juvenile (mean length 10.22cm ± 1.26SD; weight 54.62g ± 6.74SD) were harvested from reservoir in African Regional Aquaculture Centre (ARAC) Aluu, Port Harcourt, Rivers State, Nigeria. After harvesting, the fishes were then sorted into adult and juvenile sizes and blood was immediately taken from 10 fish in each sizes, with 21 gauge hypodemic needle and preserved in disodium salt of ethylene diamine tetra-acetic acid (EDTA) bottles for analysis.

The fish were then transferred into indoor hatchery, where they were acclimated in six rectangular tanks (0.6 x 0.6 x $1m^3$). Half of the water in the experimental tanks was exchanged on daily basis. The fish were fed twice a day with pelleted feed (40% crude protein) at 3% body weight. Some water quality parameters were taken also on daily basis. Temperature was measured using mercury in glass thermeter ($^{\circ}$C), pH with pH meter (Model H1 9812, Hannah Products, Portugal). Salinity was determined by using hand held refractometer (Model HRN-2N Atago Product Japan). The dissolved oxygen (DO), nitrate and nitrate levels were determine using the methods described by APHA (1985).

After seven days, another set of blood samples, similar to the first, were collected again, and taken to laboratory for analysis. Standard haematological procedures described by Brown (1980) and Blaxhall and Daisley (1973) were employed in the assessment of haemoglobin, packed cell volume, red blood cell, lymphocytes, neutrophils, monocytes, platelets and erythrocyte sedimentation rate. While the values of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Miale (1982).

The data obtained were collated and analyzed with analysis of variance (ANOVA), where differences existed, mean separation was done with the Turkey HSD test at 0.05% probability (Wahua, 1999).

3. Results

The physico-chemical parameters monitored in the tanks during the trial were within the same range in both the reservoir and experimental tanks with the only difference ($p < 0.05$) recorded in the dissolved oxygen level (Table 1).

The blood characteristics of adult *O. noloticus* before and after acclimation for seven days indicated a significant reduction in the values of Hb, PCV, RBC, MCHC, Lymph and Platelets in both male and female fish after acclimation (Table 3). But the values of MCV, WBC, NEUT, MON and ESR increased in both sexes (Table 3). However, the value of MCH did not change in male fish but it increased significantly ($p < 0.05$) in female fish after acclimation (Table 2).

Acclimation to captivity caused a reduction ($p < 0.05$) in the blood characteristics of male and female juvenile *O. niloticus* (Table 3) of Hb, PCV, RBC, MCH, MCHC, Lymphocytes and Platelets. However, in both sexes the values of MCV, WBC, neutrophils, monocytes and ESR increased ($p < 0.05$). These changes were more pronounced in male fish than the female after acclimation. The pooled data of the blood characteristics of *O. niloticus* irrespective of life stage (Table 4) revealed that the values of al the blood variables were raised (p<0.05) after seven days acclimation.

Table 1. Physico-chemical variable of reservoir and acclimation tanks

Variables	Reservoir	Experimental Tank
Temperature (^{0}C)	29.30±1.26^{a}	28.78±3.14^{0}
Dissolved Oxygen (mg/l)	6.72±0.16^{a}	4.98±0.84^{b}
pH	6.58±0.28^{a}	6.51±0.38^{a}
Salinity(‰)	0.02±0.01^{a}	0.01±0.01^{a}
Nitrite (mg/l)	0.003±0.001^{a}	0.014±0.001^{a}
Nitrate	0.32±0.03^{a}	0.98±0.34^{a}

Means in the same row with different alphabets are significantly different numbers in brackets indicate the S.D, ($P \leq 0.05$).

Table 2. Blood characteristics of adult *O. niloticus* before and after acclimation for seven days (mean ± SD)

Variable	Male		Female	
	Before	After	Before	After
Hb	6.93±0.70[a]	3.99±0.19[b]	7.03±0.24[a]	4.93±1.25[b]
PCV	20.01±0.17[a]	17.68±0.80[b]	21.64±1.32[a]	17.21±0.81[b]
RBC	4.05±0.18[a]	2.27±0.49[b]	4.25±0.22[a]	2.05±47[b]
MCH	17.06±1.00[a]	17.94±0.08[b]	16.60±1.50[b]	23.91±0.45[a]
MCHC	34.62±3.23[a]	22.57±0.08[b]	32.62±3.25[b]	28.93±0.87[b]
MCV	49.42±1.87[b]	79.53±12.12[a]	50.91±0.42[b]	87.00±21.33[a]
WBC	21.73±0.44[b]	23.96±0.43[b]	23.01±0.39[b]	24.00±0.40[a]
Lymph	69.67±0.92[b]	56.95±5.83[b]	60.18±3.86[a]	56.95±5.83[b]
Neutr	36.94±1.06[a]	38.07±0.71[b]	36.42±3.59[a]	38.36±5.80[a]
Mono	3.37±0.14[b]	5.04±0.71[b]	36.42±3.59[a]	38.36±5.80[a]
Platelets	109.26±2.65[a]	100.26±0.80[b]	114.73±3.57[a]	102.75±3.49[b]
ESR	6.57±0.40[b]	9.36±0.27[b]	6.62±0.32[b]	10.42±0.3725[b]

Key: Hb – haemoglobin, (g/dl) PCV – Packed Cell Volume (%); RBC – Red Blood Cells (Cells x $10^{12/1}$); MCH – Mean Corpuscular Haemoglobin (pg); MCHC – Mean Corpuscular Haemoglobin Concentration (g/dl); MCV – Mean Corpuscular Volume (F1); WBC – White Blood Cells (Cells x $10^{0/1}$); LYMP – Lymphocytes (%); MONO – Monocytes (%); Plat – Platelets (cells x $10^{0/1}$); ESR – Erythrocyte Sedimentation Rate (mm/br).

Table 3. Blood characteristics of male and female Juvenile *O. niloticus* before and after acclimation for seven days (mean ± SD)

Variable	Male		Female	
	Before	After	Before	After
Hb	6.37±0.9a	5.04±0.06a	6.44±0.94	5.07±0.94a
PCV	20.01±0.17a	17.86±0.80b	21.64±1.32a	17.21±0.80b
RBC	2.68±0.13a	2.38±0.06b	3.09±0.08a	2.63±0.49a
MCH	23.87±4.57a	21.15±0.27a	20.81±2.49a	19.28±0.06a
MCHC	35.43±5.19a	30.82±0.88a	34.11±5.38a	30.66±6.62a
MCV	67.00±2.13a	68.66±2.91a	61.28±2.55a	67.53±14.41a
WBC	19.25±0.07b	22.04±0.72b	20.72±0.85b	23.43±0.71b
Lymph	63.74±1.16a	59.41±1.20b	64.86±1.27a	60.42±0.69b
Neutr	35.44±0.13B	36.44±0.99a	32.40±1.38b	35.84±0.85a
Mono	2.15±0.13b	4.13±0.21b	2.73±0.11b	3.73±0.16a
Platelets	92.60±7.10a	89.20±7.62a	103.46±1.96a	100.87±2.13a
ESR	6.57±0.40b	9.63±0.27a	6.34±0.19b	9.04±0.34a

Key: Hb – haemoglobin, PCV – Packed Cell Volume; ERS – Erythrocyte sedimentation rate; RBC – Red Blood Cells, MCH – Mean Corpuscular Haemoglobin; MCHC – Mean Corpuscular Haemoglobin Concentration; MCV – Mean Corpuscular Volume; WBC – White Blood Cells; Lymph – Lymphocytes; Neutr – Neutrophils, Mono – Monocytes; ESR – Erythrocyte sedimentation rate (Mean under each of the sexes with different alphabets are not significantly different at 0.05% ($p > 0.05$).

Table 4. Haematological characteristics of adult and juvenile *O. niloticus* irrespective of sex before and after acclimation for seven days

Variable	**Male**		**Female**	
	Before	**After**	**Before**	**After**
Hb	6.69±0.22a	4.76±0.22b	6.41±0.84b	5.05±0.59b
PCV	1965±0.24a	17.13±0.24b	18.47±0.56a	16.63±0.54b
RBC	3.520±0.15a	2.33±0.15b	2.88±0.24a	2.50±0.34b
MCH	19.58±0.92a	75.62±3.58b	22.34±3.69a	20.21±1.04b
MCHC	34.19±1.45a	20.57±0.92b	34.77±5.11a	30.74±4.22b
MCV	57.15±3.58a	75.62±3.58b	64.14±3.77a	68.09±9.31a
WBC	21.18±0.19b	23.36±0.19b	19.99±0.97a	22.73±0.19b
Lymph	62.11±1.00a	60.41±1.00b	64.30±1.25a	59.91±1.03b
Neutr	35.30±0.01b	70.39±0.50a	33.92±1.88a	36.14±0.89b
Mono	2.91±0.7a	4.40±0.75b	2.44±0.33a	3.93±0.22b
Platelets	105. 01 ±1.40a	98.27±1.40b	98.03±7.55a	95.73±8.12b
ESR	6.520±0.15a	9.33±0.13b	5.70±0.72a	8.65±0.75b

Mean in the same row with similar, with different superscripts are not significantly different at 0.05% ($p > 0.05$). Numbers in brackets indicate the S.D,

4. Discussion

Haematological parameters are routinely used for the evaluation of physiological environmental and husbandry stressors in fishes (Fanouraki, *et al*., 2007). In recent years good management practices have been advocated as effective ways of reducing stress in aquaculture (Gabriel, *et al*., 2007c). One of this method is acclimation, which is the sum total of the adjustment which fish makes to changes in the environment.. Hence, the mortality, recorded in this trial could be attributed to handling stress and sudden change in the environment particularly decline in the available dissolved oxygen. There were no significant differences between the physico-chemical parameters of the water (except DO) an indication that acclimation of *0. nitoticus* did not have an adverse effects on the other parameters as reported by Gabriel *et al*. (2004) who studied the effect of acclimation of *Clarias gariepinus* under similar conditions. The differences may be accounted for by the differences in the amount of water used in the previous study and mucus produced from the skin of the latter due to the stress of acclimation.

Several factors have been reported to affect haematological responses in fish. These include sex, age, size, environmental and physiological conditions (Sowunmi, 2003). Before acclimation the female consistently had higher blood values than the males corroborating the observation of Akinrotimi *et al.* (2007a) in *S. melanotheron*. This may be as a result of resting plasma cortisol level which is more in female than male fish (Akinrotimi *et al.*, 2010). After acclimation, the variation in blood parameters was more in male fish than in the female. It appears that the males are more responsive to the stress of acclimation than the females as reported by Gabriel *et al*. (2004) in *C. gariepinus*. Age and size of fish are crucial factors that affect fish response to stressors in aquaculture (Tavares - Dial, 2010). In this study changes in the blood parameters as a result of acclimation were more pronounced in adult compared to juveniles. This is comparable to the observations of Akinrotimi *et al*. (2010) in adult *T. guineensis*. This may be due to the size difference and hormonal interactions which are more in adult than the juvenile (Akinrotimi *et al.*, 2010b).

There was a significant reduction in the values of haemoglobin and red blood cells in both sizes of *0. niloticus* due to acclimation. Similar results were recorded by Gabriel *et al.* (2007a), in *S. melanotheron* subjected to acclimation for seven days. The significant reduction in these parameters is an indication of severe anaemia caused by acclimation in the exposed fish. The anaemic response could be as a result of disruption in erythrocyte production (Wintrobe, 1978; Omoregie, 1995), haemodilution (Sampath *et al*., 1993), and destruction of intestinal cells involved in the production of vitamin B12 used in the production of the haemoglobin portion of the red cells (Gardner and Yevich, 1970). The PCV was reduced ($P < 0.05$) in both sexes of juvenile and adult fish, a situation similar to that experienced by *T. guineensis* (Akinrotimi *et al*., 2010) under similar conditions and red porgy, *Pargus pargus* exposed to consecutive handling stress. (Fanouraki *et al.*, 2007). The low value of PCV in fish exposed to stress was attributed to a reduction in red blood cell volume caused by osmotic changes (Aiwan *et al*., 2009).

Changes in white blood cells and the differential counts neutrophils, lymphocytes and monocytes indicated a stress condition in *0. niloticus*. In *Limanda linianda* (Palsford *et al*, 1994) recorded similar increase in WBC which was believed to be caused by migration of white blood cells from the spleen to the blood circulation. Reduction of circulating lymphocytes caused by acclimation was also observed by in other teleost fishes like *Oncholiriynchus kitsutch* (McLeay, 1973), *Salmo trutta* (Espelids *et al*., 1996) and *Ictalurus punctuatus* (Ellsaesser and Clern, 1987). These were associated to re-trafficking of cells to lymphoid tissues which consequently leads to clearance of these cells from the blood stream (Harris and Bird, 2000). The increase in the neutrophils and monocytes values in acclimated experimental fish was similar to that reported by Omoregie and Oyebanji (2002), in the same fish species. This may be as a result of recruitment of more cells to combat the effect of acclimation in an attempt to maintain external homeostasis.

Erythrocyte sedimentation rate (ESR), is a non-specific haematological parameter that may indicate the presence and intensity of disease state. The values are usually raised with increased tissue destruction as in acute infection and heavy metal poisoning among others (Blaxhall and Daisley, 1973). The mean corpuscular values are concerned with the volume of the average erythrocyte and the amount of. haemoglobin in the average erythrocytes. The values of this indices recorded in this study agrees with the report of Anyanwu *et al*. (2007) in *S. me1anotheron* transferred directly to fresh water.

5. Conclusions

The results of this study reveal that blood parameters of *O. niloticus* varied with age and sex. The haematological parameters of adult fish were generally higher than those of the juvenile; the values blood parameters in females were also higher than those of the males.

Acclimation to captivity caused a reduction in HB, PCV, RBC, MCHC, Lymph and platelets and increase in WBC, MCH, MCV, neutrophils, monocyte and ESR. These effects of acclimation to activity were more pronounced in male fish and in adult fish.

This information should be used in future acclimation experiments and the age as well as the sex of the experimental fish should be factors to be considered in experimental studies using *O. niloticus*.

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