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Blood Characteristics Associated with Confinement Stress in Black Chin Tilapia *Sarotherodon melanotheron*

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Abstract: Effect of confinement stress as a result of acclimation on haemotological parameters of *S. melanotheron* was investigated. Two hundred and seventy apparently healthy adult male and female *S. melanotheron* (mean weight, 31.14±0.34 SD) mean length 11.14±0.16 cm SD) were collected from recruitment ponds in the African Regional Aquaculture Centre, Brackish water farm, Buguma and confined in tanks for seven days effects the blood characteristics before and after confinement. Confinement caused reduction ($p<0.05$) on Haemoglobin (Hb); Packed Cell Volume (PCV), Leucocrit (Lct) and Red Blood Cell (RBC) of male and female; Thrombocytes (Thr) and Lymphocytes, (Lym) and of male fish. White Blood Cell (WBC); Mean Corpuscular Haemoglobin Concentration (MCHC) Mean Corpuscular Haemoglobin (MCH); Mean Corpuscular Volume (MCV); Neutrophils (Neut.) and Monocytes (Mon) were raised ($p<0.05$) after confinement. This indicates that confinement exerts some level of stress on the fish and should be taken into consideration when collecting blood for analyses, more so if appropriate values are to be used for determining the heath status of the fish.

Key words: Blood characteristics, confinement stress, chin tilapia s*arotherodon melanotheron*

INTRODUCTION

An inevitable part of intensive aquaculture is the manipulation of the fish which includes handling, confinement, acclimation, anaesthesia, transportation and other operations from the hatchery to the final commercial stage. These indispensable procedures unavoidable stress in intensive aquaculture expose cultured fish species to rapid increase in (Angelids *et al*., 1987). Although the rapid increase in aquaculture production in recent years has come from intensive culture systems characterized by high stocking densities found in sub systems can make fish more susceptible to stress and disease, which cause a decline in yield or cause severe stock loss (Shreck, 1996).

The physiological stress response, although initiated as an adaptive response to destabilizing factors, can have damaging effects if prolonged (Bittencourt *et al*., 2003). It is an established fact that continuous stress affects the normal behaviour and development of fish with reduction in growth (Jobling and Reinsnes, 1986), suppression of reproduction (Gerking, 1980) and an increase in susceptibility to infections through immuno-depression (Shreck and Bradford, 1990). This may lead to mortality (Ajani *et al*., 2006). Hence the need to appraise this aquaculture practice (intensive culture) on fish haematology, because haemotological characteristics are among the most important indices of the status of internal environment of the fish (Edsall, 1999). Also, they act as physiological indicators to changing external environment (Gills and Pant, 1981) the changes are as a result of their relationship with the energetics (metabolic levels), respiration and defense mechanisms of fish cultured. These parameters provide an integrated measure of the health status of an organism which overtime manifest in growth (Yaji and Auta, 2007). However, this study investigated the effects of confinement stress due to acclimation on haemotological parameters of *S. melanotheron* a common and widely cultured brackish water species.

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MATERIALS AND METHODS

Two hundred and seventy male and female *S. melanotheron* (mean weight 30.12±0.32 g SD; total length, 10.22±0.15 cm SD) were samples from the wild (Buguma, Creek Niger Delta). They were weighed, sexed and blood was collected from apparently healthy 18 males and 18 females at the sampling site. The collection was done by kidney punctures using a 21 gauge hypodermic needle and preserved in disodium salt of Ethylene Diamine Tetraacetic Acid (EDTA) bottles for analysis. The remaining fish were stocked in three rectangular 70 fish per tank (0.36 m^3) hatchery tanks and for seven days. They were fed at 3% body weight twice daily. At the end of acclimation period, blood was sampled from 20 males and females and preserved for analysis as was done before acclimation. Physico-chemical parameters: (pH), water temperature, dissolved oxygen, sulphide, nitrite, ammonia and salinity were determined insitu with Chinese-water-quality kits.

Standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (Hb) was done by Cyanomethaemoglobin method, Packed Cell Volume (PCV) by microhaematocrit method. WBC was determined with the improved Neubauer counter; differential count was done on blood film stained with may Grumwald-Giemsa stain. RBC was estimated using the relationship between Hb and PCV . Leucocrit was analysed using the methods of Wedemeyer *et al*., (1983) Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and mean corspuscular haemoglobin (MCH) were calculated according to Hall and Malia. Data obtained from the trial were analysed with ANOVA at 0.05% probability and the means were separated using Duncan means separation.

RESULTS

The value of Hb, PCV, Lct, WBC, RBC, MCHC, MCH, MCV, thrombocytes, neutrophils, lymphocytes and monocytes considered in this study were consistently higher in females than males before and after confinement (Table 1) It was observed that the mean values of Hb, PCV, Lct, RBC, thrombocytes and lymphocytes reduced

Table 1: Haematological characteristics of male *S. melanotheron*, before and after confinement (Mean±SD)

	Before confinement			After confinement		
Variable	Mean*	Min.	Max.	Mean**	Min.	Max.
Hb	6.40±0.39^a	5.80	7.10	5.40±0.76^b	5.30	6.84
PCV	20.96±1.78^a	18.00	23.40	18.87±2.70^b	13.10	21.30
Lct	8.08±2.28^a	4.90	12.60	6.70±2.09^b	4.10	9.60
WBC	28.88±5.71^a	22.00	38.00	21.51±10.96^b	2.60	31.00
RBC	2.43±0.24^a	2.00	2.80	2.17±1.99^b	1.40	7.90
MCHC	31.04±2.84^a	27.23	35.85	33.83±5.15^b	27.70	44.27
MCH	27.71±3.95^a	22.30	34.00	32.67±8.94^b	23.46	50.71
MCV	86.90±10.97^a	70.71	103.00	92.09±37.85^b	25.23	147.14
Thromb	181.33±15.76^a	155.00	202.00	163.66±23.48^b	120.00	191.60
Neut	35.58±6.95^a	20.40	41.30	36.81±5.81^b	30.10	49.60
Lymp	52.37±4.96^a	47.10	60.20	50.47±5.69^b	39.20	58.50
Monocyte	2.01±0.49^a	1.60	3.20	3.02±0.50^b	2.40	3.8

Key: Hb: Haemoglobin (g dL^{-1}), Ht: Haematocrit (1 L^{-1}), Lct: Leucocrit (%) MCV: Mean Corpuscular Valume (Fl) WBC: White Blood Count (10^9 cell L^{-1}); RBC: Red Blood Cells (10^{12} cell L^{-1}). MCH: Mean Corpuscular Haemoglobin 10^{12} cell L^{-1}). MCHC: Mean Corpuscular Haemoglobin Concentration (g dL^{-1}), MCHC: Mean Corpuscular Heamoglobin Concentration (pg) Plt: Platelets (x 10^9 cell L^{-1}); Neut: Neutrophils (%); Lymp: Lymphocytes (%,) Mono: Monocytes (%). Means in the row with asterisks (*, **) with different superscripts are not significantly different ($p<0.05$)s

Table 2: Haematological characteristics of female *S. melanotheron* before and after confinement (Mean±SD)

	Before confinement			After confinement		
Variable	Mean*	Min.	Max.	Mean**	Min.	Max.
Hb	7.29±0.75^a	5.80	8.20	6.83±0.54^b	5.80	7.60
PCV	22.87±3.27^a	18.60	26.90	20.18±3.30^b	16.80	26.40
Lct	8.30±2.28^a	5.40	11.70	6.64±1.99^b	3.80	9.10
WBC	30.11±7.59^a	17.00	38.00	31.11±2.66^b	27.00	35.00
RBC	2.59±0.15^a	2.40	2.80	1.82±0.31^b	1.60	2.20
MCHC	32.72±5.61^a	23.96	39.39	34.67±4.60^b	25.75	40.48
MCH	28.71±3.35^a	24.16	34.16	38.81±7.71^b	30.90	57.51
MCV	88.30±11.30^a	69.62	100.83	112.49±21.82^b	96.67	166.66
Thromb	161.44±18.39^a	138.00	190.00	171.00±15.72^b	140.00	192.00
Neut	34.90±7.76^a	21.40	42.70	40.90±5.10^b	29.80	47.70
Lymp	49.84±7.65^a	36.40	59.60	51.88±4.01^b	44.80	57.40
Monocyte	1.61±0.51^a	1.10	2.40	3.25±0.60^b	2.20	4

Key: Hb: Haemoglobin (g dL^{-1}), Ht: Haematocrit (1 L^{-1}), Lct: Leucocrit (%) MCV: Mean Corpuscular Valume (Fl) WBC: White Blood Count (10 ^{9}cell L^{-1}); RBC: Red Blood Cells (10^{12} cell L^{-1}). MCH: Mean Corpuscular Haemoglobin 10^{12} cell L^{-1}). MCHC: Mean Corpuscular Haemoglobin Concentration (g dL^{-1}), MCHC: Mean Corpuscular Heamoglobin Concentration (pg) Plt: Platelets (x 10^9 cell L^{-1}); Neut: Neutrophils (%); Lymp: Lymphocytes (%,) Mono: Monocytes (%). Means in the row with asterisks (*,**) with different superscripts are not significantly different ($p<0.05$)

Table 3: Mean Values of haematological characteristics of *S.melanotheron* confined in brackish water

	Before confinement			After confinement		
Variable	Mean*	Min.	Max.	Mean**	Min.	Max.
Hb	6.84±0.73[a]	5.80	8.20	6.65±0.67[b]	5.80	8.20
PCV	21.92±2.73[a]	18.00	26.90	19.53±3.00[b]	13.10	26.40
Lct	8.19±2.21[a]	4.90	12.60	6.67±1.98[b]	3.80	9.60
WBC	29.50±6.54[a]	17.00	38.00	26.31±9.18[b]	2.60	35.00
RBC	2.51±0.21[a]	2.00	2.80	2.25±1.46[b]	1.20	7.90
MCHC	31.88±4.40[a]	23.96	39.39	34.25±4.76[b]	25.75	44.27
MCH	28.21±3.59[a]	22.30	34.16	35.75±8.69[b]	23.46	57.51
MCV	87.60±10.83[a]	69.62	103.00	102.29±31.77[b]	25.23	166.66
Thromb	171.38±19.51[a]	138.00	202.00	167.33±19.77[b]	120.00	192.00
Neut	35.24±7.15[a]	20.40	42.70	38.85±5.71[b]	29.80	49.60
Lymp	51.11±6.39[a]	36.40	60.20	51.18±4.83[b]	39.20	58.50
Monocytes	1.81±0.53[a]	1.10	3.20	3.14±0.55[b]	2.20	4

Key: Hb: Haemoglobin (g dL^{-1}), Ht: Haematocrit (1 L^{-1}), Lct: Leucocrit (%) MCV: Mean Corpuscular Valume (Fl) WBC: White Blood Count (10^9 cell L^{-1}); RBC: Red Blood Cells (10^{12} cell L^{-1}). MCH: Mean Corpuscular Haemoglobin 10^{12} cell L^{-1}). MCHC: Mean Corpuscular Haemoglobin Concentration (g dL^{-1}), MCHC: Mean Corpuscular Heamoglobin Concentration (pg) Plt: Platelets (x 10^9 cell L^{-1}); Neut: Neutrophils (%); Lymp: Lymphocytes (%;) Mono: Monocytes (%). Means in the row with asterisks (*, **) with different superscripts are not significantly different ($p<0.05$)

after confinement in male and female, (Table 1 and 2). While WBC, neutrophils, monocytes, leucocytes increased. RBC has the lowest value ($2.59\pm0.15\times10^{12}$ L^{-1}) of all the blood parameters in in male (Table 1) while the highest value was in thrombocytes in female $171.00\pm15.72\times10^9$ cell L^{-1} (Table 2). Generally (Table 3) confinement caused variable responses in the blood parameters. Some as Hb, PCV, RBC, thrombocytes and lymphocytes were reduced after confinement whereas the others were raised in (Table 3).

DISCUSSION

The present investigation showed that confinement of *S. melanotheron* induces a variety of distortions and anomalies in their blood characteristics. The decrease ($p<0.05$) in the number erythrocyte and PCV in this study corroborated haematological changes reported by Sopinska (1985) in carp, Omoregie (1998) and Omoregie and Oyebanji (2002) on tilapia exposed to altered and stressful environmental conditions, which ultimately leads to anaemic conditions in the fishes.

According to Ellis (1981) fish under stressful conditions fish undergo a general adaptation syndrome, reported as stress. During this syndrome, a discharge of corticosteroids and catacholamines occurs after the stimulation of the interrenal and chromaffin cells respectively (Mazeaud *et al*., 1977). Fries (1986) declared that corticosteroids (cortisone) are the most immuno-suppressive factors released during stress. Reduction in the number of lymphocytes may be due to the action of corticosteroids released as a result of the stress (Angelids *et al*., 1987). In fish, destruction of lymphocytes occurs following the injection of a high dose of the corticosteroids (Strom, 1984). This may explain the reduction that stress in the number of lymphocytes in *S. melanotheron* in this study and channel catfish, the observation of Ellsaesser and Clem (1986) similar situation in channel catfish under a stressful high stocking density. Release of corticosteroid also may explain the reduction in the number of thrombocrytes as reported by Roche-Fondeur *et al.* (1986) in rainbow trout, under confinement stress.

The increase in WBC, neutrophils, monocytes, leucocrit may be due to increased leucopolesis as a means of combating stressor in the body system of the fish. Similar findings were recorded by Gabriel *et al.* (2004) in *Clarias garicpinus* under confinement due to acclimated for 7 days. These changes in WBC and differential counts have been reported to play important roles in the assessment of the state of health of *C. gariepinus* (Ezeri, 2001; Gabriel *et al*., 2004). Leucopaenia and leukocytosis have been reported in the fish under exposure to pathogens, heavy metals and chemotherapeutsants (Van Vuren *et al*., 1994; Ezeri 2001; Omoregie and Oyebanji, 2002). Thus, changes in leucocytes differential counts may be an evidence of decreased level of non-specific immunity in fish after exposure to toxic substance (Svoboda *et al*., 2003) confinement, stress as recorded in this and several other studies (Gabriel *et al*., 2004; Roche-Fondeur *et al*., 1986).

This investigation revealed that acclimation has a significant effect on the blood parameters of *S. melanotheron* and hence should be reckoned with when reporting blood characteristics value of the fish.

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