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ORIGINAL ARTICLE

Effects of Photoperiod on the Haematological Parameters of *Clarias Gariepinus* Fingerlings Reared in Water Recirculatory System

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Haematological analyses has been routinely used in determining the physiological state of animals and known to be affected by different environmental factors, the present study was therefore designed to assess the effect of 24 hours of light (00D: 24L), 24 hours of darkness (24D: 00L) and 12 hour light / 12 hours darkness (12D: 12L) photoperiod on the haematological parameters of the African Catfish. At the end of the six weeks experiment, it was observed that some haematological parameters such as Mean Corpuscular Haemoglobin Concentration (MCHC), the Mean White Blood Cells (WBC), Mean Red Blood Cells (RBC), Haemoglobin content (HGB), Platelet count (PLT) showed significant difference ($P<0.05$), while Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) did not differ significantly ($P>0.05$). However MCHC and MCH were noticed to increase as the light period increased while the other parameters reduced as the light period increased. The findings indicate that exposure of the African Catfish to continuous light for six weeks duration elicits response in the haematological profile of the fish.

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Haematological analyses have been used as a guide in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human beings. Also it is routinely used to assess the level of stresses due to environmental and nutritional factors. Haematological studies involve the assessment of various blood parameters such as total red blood cell counts (RBC), haemoglobin content (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Erythrocyte sedimentation rate (ESR), Total white blood cell counts (WBC), differential leukocyte counts and coagulation time (Mmereole, 2009). This parameter have been reported to vary significantly with the application of different environmental stressors like temperature, salinity, pH, water currents, dissolved oxygen etc. However studies on the effect of photoperiod as a stressor on haematological

variable is scarce and where available highly varied among different species, size and illumination intensity of light phase of the photoperiod used, Previous studies on the effect of photoperiod to the fingerlings of *Clarias gariepinus* had focused on growth, feed conversion, survival and aggressive behaviour but only a few assessed haematological response of this important tropical species.

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, anesthetics and acclimatization (Blaxhall, 1972; Duthie and Tort, 1985; Ogbulie and Okpowasili, 1999; Alwan *et al.,* 2009). Decreased lymphocyte numbers were observed under stressed conditions – hypoxia, cortisol induced or during handling and transport by Ellsaesser and Clem (1986, 1987). Valenzuela *et al.,* (2008) reported that lymphocytes numbers diminished in trouts exposed to constant illumination, also Akinrotimi *et al.,*(2010) reported reduction in the values of Haemoglobin (Hb); Packed Cell Volume (PCV); Red Blood Cell (RBC); Mean Corpuscular Haemoglobin Concentration (MCHC) and Platelets of *Tilapia guineensis* subjected to seven days of acclimation. Photoperiod regimes are alterations in the natural light: dark cycles and any alteration or manipulation of environmental parameters such as temperature or light results in abrupt changes in the environment which may cause stress thus compromising the welfare and general well-being of the fish (Barton and Iwama 1991; Wendelaar Bonga 1997). Therefore this study was designed to assess the effect of the stress induced by photoperiod on the haematology of the African Catfish.

MATERIALS AND METHODS

Fingerlings of *Clarias gariepinus* used were obtained from homogenous source through induced breeding at the Department of Fisheries and Aquaculture research farm and acclimatized for two weeks. They were fed twice daily during the period of acclimatization with 0.8mm Coppens starter feed. The fishes were maintained in the water re-circulatory system (dissolved O_2-7.5-11.5 mg/l; pH 7.1-8.5; water temperature 25-30ºC) with an average flow rate of 4 L min^{-1}.

25 *C. gariepinus* fingerlings of mean weight of 9.92g±0.12 were selected at random weighed and placed in the six rearing tanks connected to the water re-circulatory system. The six tanks were assigned to three photoperiods namely twenty-four hours of light (00D: 24L), twelve hours of light twelve hours of darkness (12D: 12L) and twenty-four hours of darkness (24D: 00L). The light phase was achieved with the aid of an energy bulb (60W) emitting 150 lux intensity of light measured at the surface of water.

The fishes during the course of the experiment were fed 5% of their body weight with Coppens 2mm feed (8.2% Moisture, 9.5% Ash, 45% Crude protein, 12% Ether extract, 1.5% Crude fiber) for 42 days. The experimental fishes were weighed weekly for 6weeks that the experiment lasted.

Blood collection and Analysis

At the end of the experiment, blood was collected from anaesthetized fish by cutting the caudal peduncle. Blood of two to three fish were pooled to obtain enough samples for hematological analysis. The collected blood was placed in coded 1.5mL heparinized plastic tubes, stored on ice

according to the procedures established by Campbell and Murru (1990), standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameters. Haemoglobin (HGB) 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). Platelet (PLT) count was performed according to Rees and Ecker method (Seivered, 1983). The Red Blood Cell indices that include Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated using the formula mentioned by Dacie and Lewis (2001).

Statistical Analysis

The results gotten were subjected to Analysis of variance using a computer software Gen stat discovery edition.

RESULTS

Table 1 shows the blood indices of the fishes in the 3 treatments. Haematological parameters like Mean Corpuscular Haemoglobin Concentration (MCHC), the Mean White Blood Cells (WBC), Mean Red Blood Cells (RBC) where significant different ($P<0.05$) among the treatments, while Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) did not vary significant. TR 3 had the highest value for WBC, HGB, RBC, PCV, and PLT (215.2±10.0; 10.75±0.55; 2.35±0.15; 34.15±1.55 and 17.55±0.55 respectively) compare to TR 1 (160.0±2.35; 8.05±0.15; 1.68±0.07; 23.80±3.3 and 13.5±0.5 respectively) and TR 2 having the least values (44.6±24.7; 3.3±1.0; 0.52±0.18; 6.75±2.35 and 11.5±1.50). However MCHC and MCH increased as the light period increased with TR 2 having the highest value of 49.70±2.5 and 79.75±17.45 respectively compared with TR 1 (34.35±4.15 and 48.05±1.25 respectively) and TR 3 (31.05±0.15 and 47.6±1.3 respectively).

Table 1: Haematological profile of fishes at different photoperiod

	PHOTOPERIOD			
	12D: 12L	***00D: 24L***	***24D: 00L***	***P***
WBC (10^9/L)	160.0±2.35^{ab}	44.6±24.7^{c}	215.2±10.0^{a}	**0.01**
HGB (g/dL)	8.05±0.15^{ab}	3.3±1.00^{c}	10.75±0.55^{a}	**0.009**
RBC (10^{12}/L)	1.68±0.07^{b}	0.52±0.18^{c}	2.35±0.15^{a}	**0.006**
PCV (%)	23.80±3.3^{ab}	6.75±2.35^{c}	34.15±1.55^{a}	**0.01**
MCH (pg)	48.05±1.25	79.75±17.45	47.6±1.3	**NS**
MCV(fl)	141.8±13.4	131.9±0.65	152.8±4.2	**NS**
MCHC (g/dL)	34.35±4.15^{bc}	49.70±2.5^{a}	31.05±0.15^{c}	**0.035**
PLT (10^9/L)	13.5±0.50^{ab}	11.5±1.50^{c}	17.55±0.55^{a}	**0.046**

Means in the same roll with different superscripts differ significantly ($P<0.05$)
NS- Not significant; S.E.-standard error

DISCUSSION

Studies relating to the influence of photoperiod on haematological parameters (including blood cell indices) are rather few in fishes and responses observed are quite variable (Srivastava and Sanjeev 2010). The present study shows reduced WBC, HGB, RBC, HCT, and MCV, for the 24L: 00D photoperiod compared to the other photoperiods, however increased MCH and MCHC was observed compared with the other photoperiod, Ali Bani (2009) reported that, juvenile of great sturgeon (*Huso huso*) fish reared under a 12L:12D photoperiod had higher haemoglobin values and erythrocyte (RBC) numbers than in the other photoperiods, while no differences were found between groups with regard to haematocrit values or leucocyte numbers, however similar to the result of the present study; lowest levels of haemoglobin and erythrocytes were observed in the 24L: 00D photoperiod although Mean corpuscular volume, MCH and MCHC did not differ significantly ($P>0.05$) among the various photoperiods which is in contrast to the result obtained in the present study. The reduction in RBC observed in the present study might be as a result of depletion of ATP as a result of imposed stress (Emelike *et al.,* 2008), this according Guyton and Hall (2005) results in inability of the red blood cells to transport excess sodium out of the cell membrane and subsequent haemolysis of the red cells. Thus the red cell life span becomes so short that cells are destroyed much faster than they can be formed.

The Packed Cell Volume (PCV) reduction in 24L 00D: photoperiod is in line with Ezeri *et al.,* (2004) and Akinrotimi *et al,*. (2010) findings in African Catfish *Clarias gariepinus* and *T. guineensis* acclimated for seven days respectively, also reduction in PCV values was previously recorded by Poleo and 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 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). Akinrotimi *et al.,* (2010) reported a decreased in the value of MCHC and increased MCH in African Catfish *Clarias gariepinus* acclimated for seven days respectively they however expressed that these variations are indicator of the extent of the shrinking cell size of erythrocytes stress induced by acclimation. Slightly opposite result were gotten with respect to the present study with an increase in MCH and MCHC and decrease in MCV; however it should be noted that the experiments were performed under the different laboratory conditions using different stressors.

Martem'yanov (1995) demonstrated that the erythrocyte number in fish blood decreases in response to stress, which was in accordance to the results of the present study. Therefore, the hypothesis that the number of circulating erythrocytes is developed under a continuous 24L: 00D light regime (Valenzuela, *et al.,* 2006) would not hold for the African Catfish. However, it should

be noted that the present study was performed on a different species and in a different age group compared with that reported by Valenzuela *et al*., (2006). This study therefore demonstrate the fact that different photoperiod lead to significant changes in haematological profile of a fish.

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