Journal of Agriculture and Environment Vol. 15 No. 1, 2019: 165-177
ISSN: 1595-465X (Print) 2695-236X (Online)

HEMATOLOGICAL PROFILE OF *Clarias gariepinus* REARED IN DIFFERENT CULTURE SYSTEMS

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ABSTRACT

Clarias gariepinus, a typical freshwater clariid species in the coast of Africa is an essential fish species given its immense contribution to the nutritional needs, economic growth and development of any African nation including Nigeria. Knowledge of current health status of *Clarias gariepinus* reared in different culture media is lacking in Nigeria. Therefore, this study was conducted in six randomly selected fish farms in three agricultural zones in Lagos State, South Western Nigeria to determine the hematological variations of juveniles and adults of *C. gariepinus* reared in two culture systems (earthen ponds and concrete tanks). Hematogical results showed that red blood cells, packed cell volume and hemoglobin increased significantly ($p < 0.05$) in fish reared in concrete tanks with highest mean red blood cells (RBC**)** 2.54±0.64 cells x 10^{12}**,** packed cell volume **(**PCV) 36.56±5.56 % and hemoglobin 15.83±7.32 g/L while white blood cells, leucocytes and platelets increased significantly in fish reared in earthen ponds, indicating infection and poor health status. A marked decrease observed in the RBC, PCV, hemoglobin and corpuscular volume (MCV) of fish reared in earthen ponds are diagnostic elements, predisposing factors for microcytic fish anemia, a health phenomenon in cultured catfish.

Keywords: Culture systems; *Clarias gariepinus*; hematological variations

INTRODUCTION

Aquaculture in Nigeria is essentially based on catfish production and the prospects of fish supply in the country depend on its culture and development (Adewumi and Olaleye, 2011). *Clarias gariepinus* (Family Clariidae) is the most commonly cultivated fish species in the country, making Nigeria the largest producer of *Clarias gariepinus* in Africa and third in the world, after Thailand and Indonesia (FAO, 2010).

Aquaculture has been practiced successfully in different culture systems ranging from natural water bodies (pens and cages), earthen ponds, concrete tanks, fiberglass tanks and plastic tanks (Dauda *et al*., 2014). The selection of culture facility depends on the available materials, operation size and the level of expertise of the farm manager (Akinwole *et al*., 2014). The dominant culture facilities in Nigeria remain the earthen ponds and concrete tanks (Akinwole and Akinnuoye, 2012). In terms of facility size, a large proportion of concrete tanks used in culturing fish in Nigeria range between 25 - 40 m^2 and is operated majorly by individuals while earthen ponds range from 0.02-0.2 ha^2 (Anyanwu and Ezenwa, 1998). The production systems have grown globally and the current trend is to move from intensive and semi-intensive fish culture systems practiced mainly in earthen pond with low stocking densities to hyper and ultra-intensive culture systems in highly sophisticated recirculating systems with efficient water management techniques (Dauda *et al*., 2014). The development of aquaculture is not only in output, but also the practices and operations which cut across the chains of activities in production, including culture systems and management practices (water quality management, stocking densities, feed types and feeding regime).

The use of hematological techniques in fisheries is very important in toxicological research which results in monitoring and predicting health conditions of fish (Bittencourt *et al.,* 2003, Akinrotimi *et al.,* 2009; Chukwu and Lawal, 2010). Since fish are so intimately associated with the aqueous environment, blood will reveal rapidly the measurable physiological changes in fish (Ezeri *et al.,* 2004; Chukwu and Lawal, 2010). Hematological parameters are widely used indicators of environmental stress in fish. Indices such as hemoglobin, hematocrit, red and white cell counts, erythrocyte sedimentation rates, and differential blood smears have all been used as indicators of disease and stress (Harikrishman *et al.,* 2003). Many studies have demonstrated changes in blood variables as a result of environmental conditions such as presence of contaminants (Ruas, 2008). Hematological variables such as red blood cell, white blood cell, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and differential counts are widely used to diagnose the effect of external stressors and toxic substances as a result of the close association between the circulatory system and the external environment (Gabriel *et al.*, 2007b).

Blood analysis is crucial in many fields of ichthyologic research, in the area of toxicology and environmental monitoring; as a possible indicator of physiological or pathological changes in fishery management and disease investigation (Adedeji *et al.*, 2011). The changes of hematological indices depend on fish species, age, cycle of sexual maturity of the spawners, and diseases (Gabriel *et al*., 2004). In warm-blooded animals, changes in the blood parameters which occur because of injuries or infections of some tissues or organs can be used to determine and confirm the dysfunction or injuries of the organs or tissues. However, in fish, these parameters are more related to the response of the whole organism, i.e. to the effect on fish survival, reproduction and growth.

MATERIALS AND METHODS

Study Area

This study was carried out in Lagos State, South-western Nigeria (6°35'' N and 3°45''E). The following fish farms were randomly selected for the study: In eastern agricultural zone, TEM fish farm (concrete tank) and TRA fish farm (earthen pond) were selected. TIM fish farm (concrete tank) and BLU fish farm (earthen pond) were selected from western agricultural zone while CHA fish farm (concrete tank) and MOY fish farm (earthen pond) were selected from far eastern agricultural zone. NIOMR concrete tanks and earthen ponds fish farms were used as control (Figure 1). Control farm adopted standard stocking density of 25 fish/m^2 in concrete tanks and 10 fish/m^2 in earthen ponds, 3 times /week for water exchange in concrete tanks, once/month in earthen ponds, and 3 times/day of feeding regime in both concrete tanks and earthen ponds. CHA and BLU complied with the standard while TEM, TIM, TRA and MOY deviated from the standard management practices.

Note: TRA, BLU, MOY, TEM, TIM and CHA are acronyms of the experimental fish farms, for ethical grounds and non-disclosure agreement.

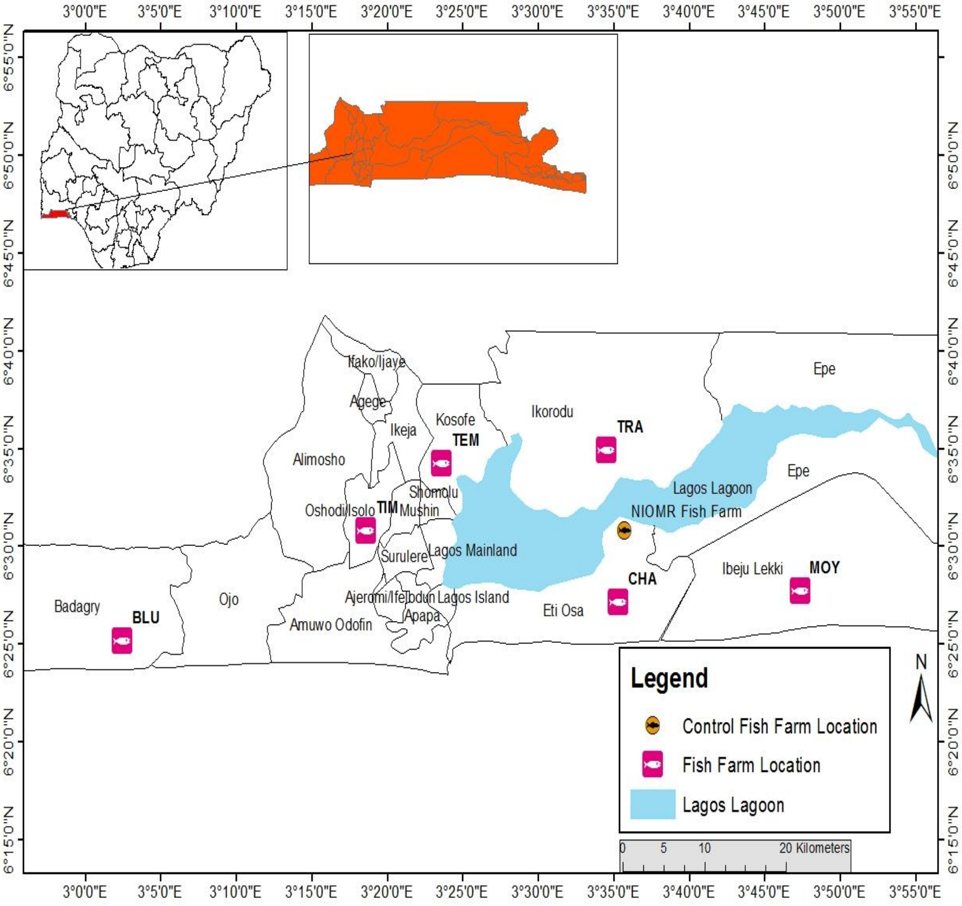

Figure 1: Map of Lagos State showing the experimental and control fish farms

Collection of Blood Samples

Three fish from each replicate tank and pond in six experimental fish farms and two control farms were randomly collected (Kpundeh *et al.*, 2013), giving a total of seventy-two (72) mixed sex of *C. gariepinus* (36 post juveniles weighing 200-250g and 36 adults weighing 400-500g). Blood samples of juveniles were collected at 7^{th} week of stocking while blood samples from the adults were collected at 16^{th} week. All blood samples were drawn from the caudal artery and 2ml of the blood collected was poured into tubes containing anticoagulant potassium salt of ethylene diamine tetra-acetic acid (EDTA) The blood samples were put in ice box and transported to laboratory for analysis within 6 hours of collection.

Analytical Procedure

Red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), packed cell volume (PCV), platelets (Pt), leucocytes (LYM), granulytes (GRANUL) mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volumes (MCV) were determined using automatic hematology analyzer (Swelab Alfa Diluent and Swelab Alfalyser, Serial No: 15452, Lot. No: 1309-240) following standard methods.

Statistical Analysis

The hematological data were expressed in mean and standard deviation and analyzed using one-way analysis of variance (ANOVA) at 5% level of significance. Post-hoc mean comparison was carried out where significant differences exist using Duncan's Multiple Range Test (DMRT). These analyses were carried out based on a computer programme SPSS 22.0 designed and implemented by GeLePattaurel.

RESULTS

Variations in Hematological Values of *C. gariepinus* Juveniles and Adults Reared in Grow-out Earthen Ponds and Concrete Tanks

The hematological parameters in juveniles of *C. gariepinus* reared in grow-out earthen ponds and concrete tanks are shown in Figure 1-10. Red blood cells (RBC), packed cell volume (PCV), hemoglobin (HB) and mean corpuscular hemoglobin concentration (MCHC) observed in earthen ponds recorded higher values in BLU fish farm than other farms when compared to control. The lowest values of the same parameters were obtained in fish reared in MOY farm. Significant increase ($p < 0.05$) comparable to the control values were recorded in white blood cells (WBC), platelets (PLT), procalcitonin (PCT), leucocytes (LYM), granulytes (GRANUL) and mean corpuscular volume (MCV), with the highest values observed in juveniles of *C. gariepinus* reared in MOY farm. The MCH was within the same range across the farms. The adult *C. gariepinus* in earthen ponds revealed a significant difference ($p < 0.05$) of blood parameters in all the farms except GRANUL, MCV, MCH and MCHC. The juveniles of *C. gariepinus* raised in grow-out concrete tanks displayed significant difference ($p < 0.05$) in RBC, WBC, HB, PLT, and LYM comparable to the control values while, PCT, GRANUL and MCHC were within the same range with no significant difference, however, higher values of RBC, HB, and PCV were recorded in CHA farms. For the adults, significant values were recorded in RBC, HB, WBC, LYM, and MCV while PLT, GRANUL, MCH and MCHC were not significantly different ($p < 0.05$) among the farms.

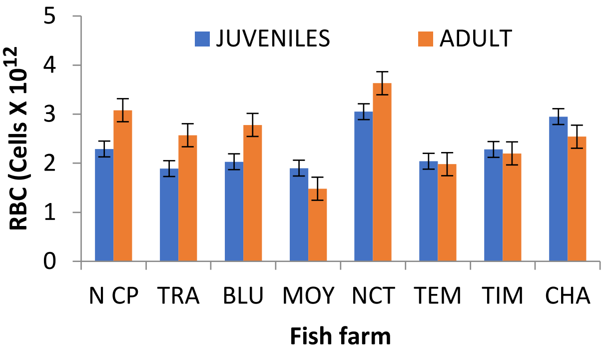

Figure 1: RBC in earthen ponds and concrete tanks

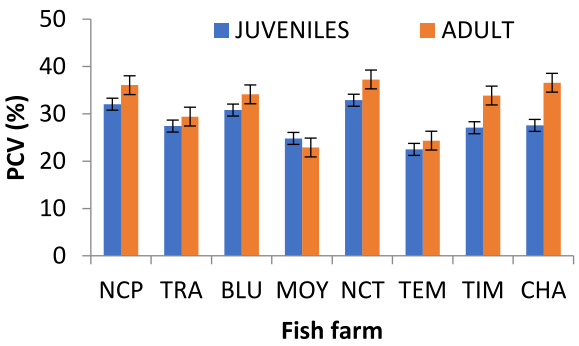

Figure 2: PCV in earthen ponds and concrete tanks concrete tanks

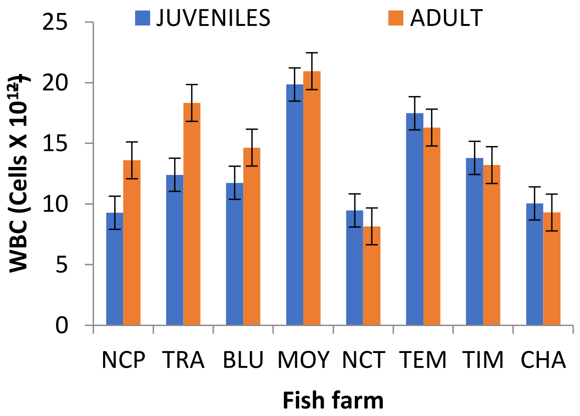

Figure 3: WBC in earthen ponds and concrete tanks

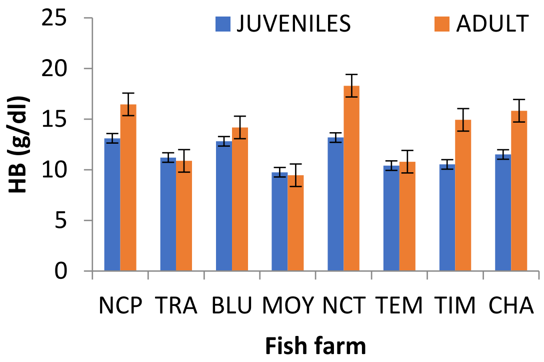

Figure 4: HB in earthen ponds and concrete tanks

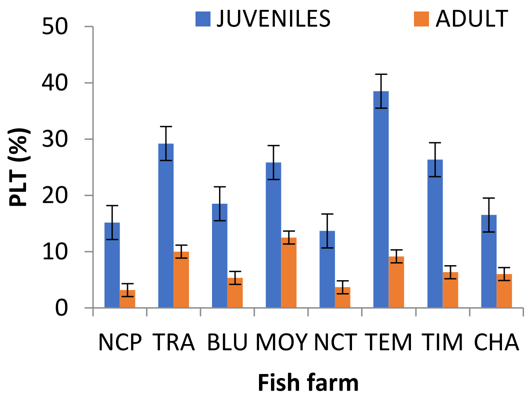

Figure 5: PLT in earthen ponds and concrete tanks

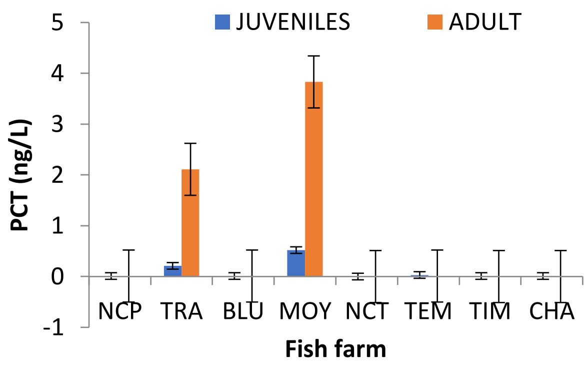

Figure 6: PCT in earthen ponds and concrete tanks

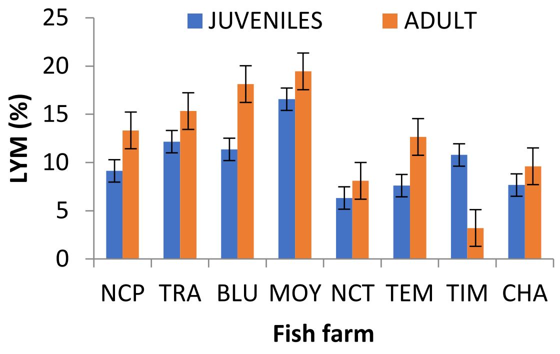

Figure 7: LYM in earthen ponds and concrete tanks

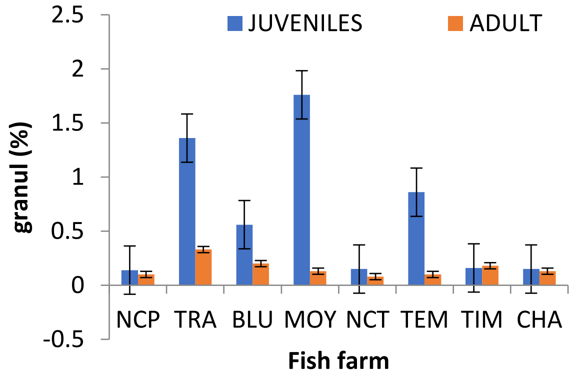

Figure 8: GRANUL in earthen ponds and concrete tanks

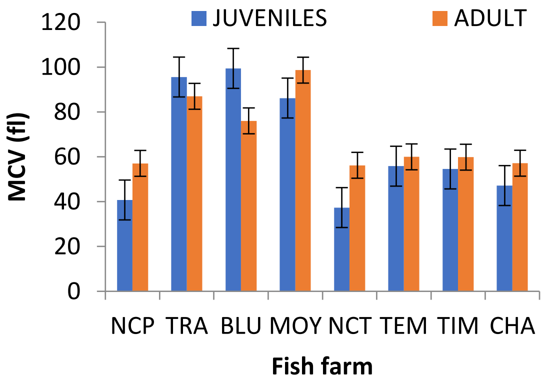

Figure 9: MCV in earthen ponds and concrete tanks

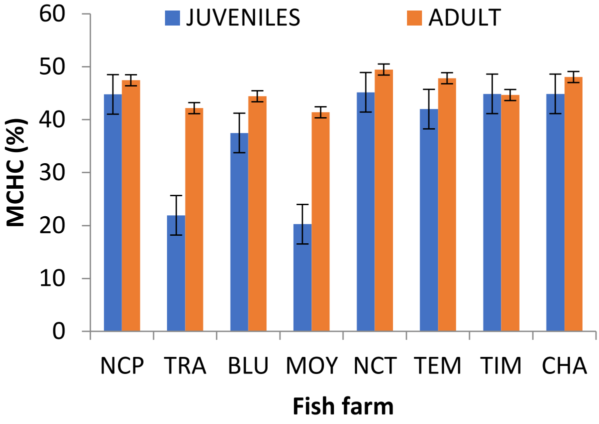

Figure 10: MCHC in earthen ponds and concrete tanks

DISCUSSION

Variations of Hematological Parameters among the Experimental Fish Farms

There is a clear indication that environment where fish lives exert some influence on the hematology. A marked deviation of hematological indices from this study is evident. The values of RBC were higher in the control farms compared to experimental farms. The lower values of RBC observed in experimental farms may be due to poor management practices. These lower values could induce anemia or hemolysis in *C. gariepinus* as a result of decreased synthesis of erythrocytes in the kidney, spleen and bone marrow (Bouck and Ball, 2006). Erythrocytes are the most common type of blood cells and the vertebrate organism's principal means of delivering oxygen (O_2) to the body tissues through the circulatory system (Cameron, 2010) Erythrocyte level was found to be depressed in fishes subjected to stressful conditions. The reduction in erythrocyte may be caused either by the inhibition of erythropoiesis or by the destruction of red blood cells. This is in agreement with the position of Allan *et al*. (2006) who stated that a change in water quality characteristics in specific area affect hematological indices of fish. This result agreed with the submission of Alim (2005) in blood assessment of *Oreochromis niloticus* and *Clarias gariepinus* reared in two culture systems. Iwama *et al.*, (2002) reported that the destruction of hepatic tissue in kidney and spleen result in decreased blood cell production and consequent reduction in erythrocyte count.

Hemoglobin (Hb) in fish is the protein molecule in red blood cells that carries oxygen from the gills to the body's tissues and returns carbon dioxide from the tissues back to the gills. (Adeyemo, 2003). The decreased hemoglobin concentration (Hb) in *C. gariepinus* in other farms when compared to control values obtained in this study is similar to those reported in *Sarotherodon melanotheron* reared in concrete and earthen ponds (Gabriel *et al.*, 2007c). This pattern of response may be attributed to heterolysis which results in hemo-dilution, a means of diluting hemo concentration of the fluid; this reduces the effect of the stressor in the fish system. Besides, it may result from either an increase in the rate of hemoglobin destruction or decrease in its productivity or synthesis (Rauf and Arian, 2013).

Prolong reduction also leads to blood dyscrasias and degeneration of the erythrocytes (Gardiner, 2013).

Packed cell volume (PCV) is the percentage of red blood cells in circulating blood. A decreased PCV generally means red blood cell loss from any variety of reasons like cell destruction, blood loss, and failure of blood production. (Campbell and Ellis, 2007). In the present study also, cultured fish in the experimental farms exhibited anemic condition by recording lower content of PCV than that of the control fish in both culture systems. This may be due to destruction of RBC or inhibition of erythropoiesis or interference of the hemo-metabolism or disturbance in fluid volume balance produced by handling stress which may find support from the previously cited workers. The decrease of hemoglobin leads to hypochromic microcytic anemia, which is attributed to deficiency of iron and its decreased utilization for hemoglobin synthesis.

It is well known that glycolysis is concerned with the reduction of methemoglobin as soon as it is formed, thus maintaining the iron of the hemoglobin in the ferrous form (Fange, 2002). Grue *et al.* (2002) are of the opinion that the disruption of iron-synthesizing machinery due to inhibition of aerobic glycolysis could be the reason for the decrease of blood parameters in the stressed fish. In the present study, a similar type of mechanism may be operating resulting in the reduction of PCV in *C. gariepinus.* It has been shown that the packed cell volume levels may vary with oxygen requirement (Akinrotimi *et al.,* 2012). Therefore, the reduced packed cell volume in *C. gariepinus* is likely to be due to either increased metabolic demand or gill damages resulting in impairment of oxygen transport or both the gill ultra-structure.

Procalcitonin (PCT) is a precursor to the hormone calcitonin (Lovas *et al*., 2014). It is normally produced by the thyroid gland. However, 2 - 4 hours after a severe bacterial infection, immune cells also begin to produce PCT, which leads to increased blood PCT levels. The level of procalcitonin in the blood stream of healthy fish is below the limit of detection (0.01 ng/L) by clinical test (Grace and Turner, 2014). In this study, lower (zero) levels of PCT were observed in the control farms, when compared to other farms. Briel *et al*. (2008) observed that the level of procalcitonin rises in a response to a pro-inflammatory stimulus, especially of bacterial origin. In this case, it is produced mainly by the cells of the gills and the intestine. It does not rise significantly with viral or non-infectious inflammations.

The increase in white blood cells (WBC), lymphocytes, and granulocyte counts recorded in this work when compared to the control farms, may be due to the attempt of the fish to fight against the stressors and this augmented the production of more WBC to improve the health status of the fishes which agreed with the reports of Adeyemo (2003) and Gabriel *et al.,* (2007b). White blood cells are important components in blood. They protect the animal during injury, hemorrhage and attack by foreign compounds. They exhibit phagocytic action. Increase in the leucocytes has an adaptive value to meet the stressful condition and defence mechanism.

Granulocytes are category of white blood cells characterized by the presence of granules in their cytoplasm. They are also called polymorphonuclear leukocytes because of the varying shapes of the nucleus, which is usually lobed into three segments. Granulocytes are phagocytes, that is, they are able to ingest foreign cells such as bacteria, viruses and other parasites. Granulocytes are so called because these cells have granules of enzymes which help to digest the invading microbes. Granulocytes account for about 60 % of our white blood cells. (Sanchez and Porcher, 2009). The farm dependent increase in the percentage subpopulation of lymphocytes and granulocytes may be associated with the nature of immunological challenge to which the fish was exposed at a particular period of time consequent of handling and managerial induced stress. Granulocytes being phagocytic in function, their increase may have been associated with invasion of the system by pathogenic micro-organisms, viruses and debris, which may have been occasioned by tissue and organ damage (Bouck and Ball, 2006).

The increase in lymphocytes counts as observed in this study may be associated with enhanced release of lymphocytes from lymphoid tissues. This could be an adaptive mechanism to boost the immune system of the fish and give it to positive survival value needed in the sub-lethal toxic environment or possibility of leukemia due to prolonged toxic assault (Akinrotimi *et al*., 2015). Gabriel *et al*. (2004) reported culture methods and duration dependent degenerations in the blood of *C. gariepinus* exposed from the wild and culture system. These authors reported that culture systems have ways of affecting blood profiles in fish. Platelets also known as Thrombocytes in fish, are comparable to mammal blood platelets and play an important role in the blood clotting which prevents blood loss from hemorrhaging.

Increase in the values of platelets as observed in this study may be a mechanistic release of the cells to fight or combat the effect of the stressor on the fish (Sanchez and Porcher, 2009). Platelets showed variable responses in *C. gariepinus* reared in different culture systems. However, zero values were observed in adult fish in both culture systems; an indication that platelets in adult fish are more stable during stressful conditions than the juveniles. This is because adult fish have more matured kidney and bone marrow than the juveniles (Akinrotimi *et al*., 2015). Alteration in the rate of production of platelets by the head kidney and bone marrow due to stress effects may be responsible for the variations in the number of platelets.

In the present study, culture of *C. gariepinus* in different aquaculture facilities resulted in significant alterations in hematologic indices. Blood is a pathophysiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to pollutants (Adhikari *et al.*, 2006). The reduced mean corpuscular volume (MCV) and increased mean corpuscular hemoglobin concentration (MCHC) values compared to the control values as observed in this study, have been shown in many species exposed to chronic hypoxia (Davids, 2015).

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