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Characterization of Some Blood Parameters of African Catfish (*Clarias gariepinus*)

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Abstract: The aim of this study was to establish the haematological profile of Juvenile African catfish (*Clarias gariepinus*). Hematological indices (RBCs, WBCs, PCV, Hb, MCV, MCH, MCHC and leucocyte differential count) were measured in blood samples from 120 juvenile African catfish. The results in mean values were; RBCs (x 10^6 µl) 2.88±0.7011, WBCs (10^3µl) 41.1±11.048, PCV (%) 36.00±9.036, Hemoglobin (g/dl) 6.044±25.572, MCV(g/dl) 113±15.635, MCH (g/dl) 23.381±6.238, MCHC(g/dl) 26.881±30.332, PLT(10^3µl) 41.9875±17.864, Lymphocyte (10^3µl) 34.121±17.512, Monocyte (10^3µl) 1.675±0.642, Neutocyte (10^3µl) 1.086±0.385. Knowledge of these haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes.

Key words: *Claris gariepinus* · Haematology · RBCs · WBCs

INTRODUCTION

Haematological characteristics are important tools that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Hematological indices are very important parameters for the evaluation of fish physiological status. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases [1-4]. Normal ranges of various blood parameters in fish have been established by different investigators [5, 6]. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting [7]. Blood biochemistry parameters can be also used to detect the health of fish [8]. Exogenous factors, such as management [9], diseases [10] and stress [11], always induce major changes in blood composition. For example, significant fluctuations were detected in the concentrations of cortisol, glucose, cholesterol and other basic components in response to handling and hypoxic stress [12]. The levels of cortisol and glucose are considered specific indicators of sympathetic activation during stress conditions [13]. Basic ecological factors, such as feeding regime and stocking density, also have a direct influence on certain biochemical parameters [14]. Blood parameters truly reflects physical and chemical changes occurring in organism. Therefore, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. Early diagnosis is also possible when evaluating hematological data, particularly blood parameters [1, 3, 15].

Clarias spp. is a widely distributed fish in Asia and Africa. In these areas, the fish is extremely popular on account of its tasty flesh, its unparalleled hardness, its rapid growth and high market price. It inhabits tropical swamps, lakes and rivers, some of which are subject to seasonal drying. Furthermore, *Clarias* fishery constitutes one of the major fisheries in many river basins in Africa. Awachie and Ezenwaji [16] estimated that *Clarias* fishery contributes about 17% of over 6,000 tons of annual fish production from all fisheries sectors [17].

In Nigeria, *Clarias* spp. is an indigenous fish occurring in freshwater throughout the country. It is suspected that apart from tilapia, *Clarias* is the most abundant cultivated fish species in Nigeria. The common species found are *Clarias gariepinus*, *Clarias anguillaris* and *Clarias buthupogon*.

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Clarias sp., are generally strong fishes. They possess accessory air breathing organs that enable them to tolerate adverse aquatic conditions where other cultivated fish species cannot survive [18].

MATERIALS AND METHODS

Fish Sampling: One hundred and twenty normal post juvenile *Clarias gariepinus* of both sexes with a mean weight of 135.44±1.99 g and mean length of 28.32±0.844 cm were purchased from Osayi farms in Benin City, Edo state. They were kept in 60 L aquaria at 27.5±0.4 °C, pH 7.3, with 12:12 h photoperiod and an average density of 6.4 g/l. They were left unfed in the first 2 days to adapt to a change in environment before feeding them with the fish diet. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. Fish were normally fed once a day with pelleted commercial food (Durante Aquaculture fish concentration-2mm). They were allowed to acclimate to captivity conditions for two month prior to taking the blood samples. Careful netting and handling was implemented to minimize stress. The peripheral blood was collected by puncture of the genital opening with a heparin-coated 21 gauge×0.5 inch needle, attached to a 2 ml syringe. After sampling, fish were placed in separate tanks of freshwater for recovery. Right after each extraction, blood samples were processed for microscopy as follows: For light microscopy, a blood smears form every fish was fixed in absolute methanol for 3 min at room temperature or formalin vapor at 37 °C for 1 h and stained with 10% Giemsa in PBS, hematoxylin-eosin or PAS [19, 20]. The slides were subsequently examined and photographed under a Nikon Micropho

The blood samples were processed for RBCs, WBCs and PCV as follows: RBCs [21] and WBCs + thrombocytes [22] were determined using a Neubauer hemocytometer. Differential white cell and thrombocyte counts were done on blood films stained with Giemsa. For every 1200 erythrocytes counted at random, the number of thrombocytes and the different types of leucocytes was determined on each blood smear and a mean relative percent calculated. The absolute Value was then obtained, multiplying this percent value by the WBCs + thrombocytes from the hemocytometer. Thrombocyte numbers were subtracted from the WBCs + thrombocytes count to obtain a total WBCs. Replicate counts were made for each blood sample. Hematocrit value was determined by the standard microhematocrit method and expressed in percentage. Duplicate blood samples were loaded into standard heparinized capillary tubes, spun in a microhematocrit centrifuge at 12,000 rpm for 5 min and measured on a microcapillary reader. Later Hb in erythrocytes was determined using the cyanmethemoglobin method. Prior to reading the absorbance, hemoglobin test samples were centrifuged to remove dispersed nuclear material.

The following indices: mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to Seiverd [23].

Blood Analysis: The haemoglobin concentration of the blood samples was determined in duplicate by the cyanmethemoglobin method [24]. The microhaematocrit method of Snieszko [25] was used to determine the haematocrit. The red blood cells were enumerated in an improved Neubaeur haemocytometer, using diluting fluid [26]. The total white blood cell counts were similarly enumerated in an improved Neubaeur haemocytometer using Shaw's diluting fluid. The hematological indices: mean erythrocytic haemoglobin concentration (MEHC), mean erythrocytic hemoglobin (MEH) and mean erythrocytic volume (MEV) were calculated from the equations given by Anderson and Klontz [27].

$$\text{MEHC (\%)} = \frac{\text{Hemoglobin (g\%)}}{\text{Haematocrit (\%)}} \times 100$$

$$\text{MEH (g)} = \frac{\text{Hemoglobin (g\%)}}{\text{Erythrocyte count (per/L)}} \times 10$$

$$\text{MEV } (\mu^3) = \frac{\text{Haematocrit (\%)}}{\text{Erythrocyte count (per/L)}} \times 10$$

RESULTS

Erythrocytes, thrombocytes and four types of leucocytes: lymphocytes, monocytes, heterophils and eosinophils were distinguished and characterized by light microscopy.

DISCUSSION

There is growing interest in the study of hematological parameters and structural features of fish blood cells regarded as important for aquaculture purposes. A combination of quantitative and morphological methods is needed if the classification of fish blood cells is to advance from its present state [28].

Table 1: Hematological profile of *Clarias gariepinus*

Parameter	Mean± Standard error	Sample Size (n)
RBC (x 10^6 µl)	2.88±0.7011	40
WBC(10^3µl)	41.1±11.048	40
PCV (%)	36.00±9.036	40
Hemoglobin (g/dl)	6.044±25.572	40
MCV(g/dl)	113±15.635	40
MCH(g/dl)	23.381±6.238	40
MCHC(g/dl)	26.881±30.332	40
PLT(10^3µl)	41.9875±17.864	40
Lymphocyte (10^3µl)	34.121±17.512	40
Monocyte (10^3µl)	1.675±0.642	40
Neutocyte (10^3µl)	1.086±0.385	40

Results of haematological analysis shown in Table 1, includes the mean and sample size for each of the different parameters evaluated. A low count of monocyte and neutrocyte were observed. The percent of heterophils was found to be high in relation to the percent of lymphocytes.

Erythrocytes are the dominant cell type in the blood of the vast majority of fish species. It is widely accepted that fishes, like most other vertebrates, have a common leucocyte pattern consisting of granulocytes, monocytes, lymphocytes and thrombocytes.

In the present study, *C. gariepinus* blood cells were characterized microscopically and haematological indices were analyzed. The mature erythrocytes of *C. dimerus* show an average size and ultrastructural features similar to those described for mature erythrocytes of other fish species and, like in all the species examined so far.

Hematological parameters such as haematocrit, hemoglobin, number of erythrocytes and white blood cells are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals [29, 30].

Several scientists have investigated hematological changes of fish exposed to pesticides [31-36]. Knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Normal ranges of various blood parameters in fish have been established previously by different investigators [5, 9]. The analysis of blood indices has proven to be a valuable approach for analysing the health status of farmed animals as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before they are present in a clinical setting [7].

CONCLUSION

Establishment of the blood characteristic of *Clarias gariepinus* makes it easy for further studies to carried out to determine the difference caused by exposure to toxicant

REFERENCES

1. Luskova, V., 1997. Annual cycles and normal values of hematological parameters in fishes. Acta. Sc. Nat. Bibo., 31: 70-78.
2. Golovina, N.A. and I.D. Trombicky, 1989. Haematology of Pond Fish. Kishinev, Shtiinca, pp: 158.
3. Golovina, N.A., 1996. Morpho-functional characteristics of the blood of fish as objects of aquaculture. PhD Thesis, Moscow, pp: 53.
4. Zhiteneva, L.D., T.G. Poltavceva and O.A. Rudnickaja, 1989. Atlas of normal and pathological cells in the blood of fish. Rostou-on Don, Rio de Janeiro, Brazil, pp: 112.
5. Rambhaskar, B. and K. Srinivasa Rao, 1986. Comparative haematology of ten species of marine fish from Visakhapatnam Coast. J. Fish Biol., 30: 59-66.
6. Xiaoyun, Z., L. Mingyun, A. Khalid and W. Weinmin, 2009. Comparative of haematology and serum biochemistry of cultured and wild Dojo loach *Misgurnus anguillicaudatus*. Fish Physiol. Biochem., 35: 435-441.
7. Bahmani, M., R. Kazemi and P. Donskaya, 2001. A comparative study of some hematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). Fish Physiol. Biochem., 24: 135-140.
8. De Pedro, N., A.E. Guijarro, M.A. Lopez-Patino, R. Marinez-Alvarez and M. Delgado, 2005. Daily and seasonal variation in hematological and blood biochemical parameters in touch, Tinca Yince. Aquaculture, 36: 85-96.
9. Svobodova, Z., H. Kroupova, H. Modra, M. Flajshans, T. Randak, L.V. Savina and D. Gela, 2008. Haematological profile of common carp spawners of various breeds, J. Appl. Ichthyol., 24: 55-59.
10. Chen, Y.E., S. Jin and G.L. Wang, 2005. Study on blood physiological and biochemical indices of *Vibrio alginolyticus* disease of *Lateolabrax japonicas*. J. Ocean Tai. Str., 24: 104-108.

11. Cnaani, A., B. Tinman, Y. Advidar, M. Ron and G. Hulate, 2004. Comparative Study of Biochemical parameters in response to stress in *O. aureus*, *O. Mossambicus* and two strains of *O. Silaticus*. Aquaculture, 35: 1434-1440.
12. Skjervold, P.O., S.O. Fjæra, P.B. Østby and O. Einen, 2001. Live-chilling and crowding stress before slaughter of Atlantic Salmon (*Salmo salar*). Aquaculture, 192: 267-282.
13. Lerman-Sagie, T., N. Watemberg, C. Vinkler, J. Fishhof, E. Leshinsky-Silver and D. Lev, 2004. Familial hyperekplexia and refractory status epilepticus: a new autosomal recessive syndrome. J. Child Neurol., 19: 522-525.
14. Coz-Rakovac, R., I. Strunjak-Perovic, M. Hacmanjek, N. Topic Popovic, Z. Lipej and B. Sostaric, 2005. Blood chemistry and histological properties of wild and cultured sea bass (*Dicentrarchus labrax*) in the north Adriatic Sea. Veterinary Research Communications, 29: 677-687.
15. Folmar, L.C., 1993. Effects of chemical contaminants on blood chemistry of teleostean fish: a bibliography and synopsis of selected effects. Environ. Toxicol. Chem., 12: 337-375.
16. Awachie, J.B.E. and H.M.G. Ezenwaji, 1998. The Importance of *Clarias* species in the Fishery Development of Anambra River Basin. CIFA Technical Paper, 8: 212-224.
17. Kori-Siakpere, O., J.E.G. Ake and E. Idoge, 2005. Hematological characteristics of the African snakehead, *Parachanna obscura*. African Journal of Biotechnology, 4: 527-530.
18. Olatunde, A.A., 1983. Length weight relationship and diets of *Clarias lazera* (Cuiver and Vallenciennes) in Zaria Nigeria. Proceedings of the 3rd Annual Conference of Fisheries Society of Nigeria (FISCON), Maiduguri. 22nd and 25th February, 1983.
19. Martoja, R. and M. Martoja-Pierson, 1970. TBcnicas de histología animal. toray-Masson S.A. darcelona. pp: 176-208.
20. Rambhaskar, B. and K. Srinivasa Rao, 1986. Comparative haematology of ten species of marine fish from Visakhapatnam Coast. J. Fish Biol., 30: 59-66.
21. Kaplow, L.S., 1955. A histochemical procedures for localizing and valuating leukocyte alkaline phosphatase activity in smears of blood and marrow. Blood, 10: 1023-1029.
22. Natt, M.P. and C.A. Herrick, 1952. A new blood diluent for counting erythrocytes and leucocytes of the chicken. Poultry Science, 31: 735-738.
23. Seiverd, C.E., 1964. Hematology for medical technologist's. Lea and febiger, Philadelphia, pp: 946.
24. Larsen, H.N. and S.F. Snieszko, 1961. Comparison of various methods of determination of hemoglobin in trout blood. Prog. Fish Cult., 23: 8-17.
25. Snieszko, S.F., 1960. Microhaematocrit as a tool in fishery research and management. U. S. Wildl. Serv. Sci. Rep. Fish., 341: 15.
26. Hendricks, L.J., 1952. Erythrocyte counts and hemoglobin determinations for two species of Suckers, Genus Catostomus, from Colorado, ”Copeia, pp: 265-66.
27. Anderson, D. and G.W. Klontz, 1965. Basic hematology for the fish culturist. Annual Northwestern Fish Culture Conference, 16: 38-41.
28. Vazquez, G.R. and G.A. Guerrero, 2007. Characterization of blood cells and hematological parameters in *Cichlasoma dimerus* (Teleostei, Perciformes). Tissue and Cell, 39: 151-160.
29. Sancho, E., J.J. Cerón and M.D. Ferrando, 2000. Cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla anguilla*. Ecotoxicology Environment Safe, 46: 81-86.
30. Barcellos, L.J.G., L.C. Kreutz, L.B. Rodrigues, I. Fioreze, R.M. Quevedo, L. Cericato, J. Conrad, A.B. Soso, M. Fagundes, L.A. Lacerda and S. Terra, 2003. Hematological and biochemical characteristics of male jundiá (Rhamdia Quelen, Quoy and GaimaRDT, Pimelodidae): changes after acute stress. Aquacul. Res., 34: 1465-1469.
31. Santhakumar, M., M.B. and K. Ramudu, 1999. Effect of sub-lethal concentration of monocrotophos on erythropoietic activity and certain haematological parameters of fish *Anabas testudineus* (Bloch). Bull. Environ. Contam. Toxicol., 63: 379-384.
32. Das, B.K. and S.C. Mukherjee, 2000. Sublethal effect of quinalphos on selected blood parameters of *Labeo rohita* (Ham.) fingerlings. Asian Fishery Sci., 13: 225-233.
33. Aengwanich, W. and A. Tanomtong, 2004. Hematological and serum biochemical values of white ibis (*Threskiornis melanocephalus*). Songklanakarin J. Sci. Technol., 26: 823-828.

34. Adedeji, O.B., O.A. Adedeji, O.K. Adeyemo and S.A. Agbede, 2008. Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*). Afr. J. Biotechnol., 7: 651-654.
35. Ramesh, M. and M. Saravanan, 2008. Hematological and biochemical responses in a freshwater fish, Cyprinus carpio exposed to chlorpyrifos. IJIB., 3: 80-83.
36. Ojezele, M.O. and O.M. Abatan, 0009. Toxicological effects of chlorpyrifos and methidathion in young chickens. African Journal of Biochemistry Research, 3: 48-51.