Effect of Different Anticoagulants on Hematological Parameters of *Oreochromis niloticus*

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Abstract: This study investigated the effect of anticoagulants on the hematological parameters of Nile tilapia (*O. niloticus*). The anticoagulants used were heparin and ethylenediamine tetraacetic acid (EDTA). Packed cell volume (PCV), hemoglobin concentration (HB), and differential blood count were determined. There was no significant difference (p>0.05) in monocytes and eosinophils values in the control and the tests (EDTA and Heparin) respectively. The values of neutrophils were significantly (p<0.05) higher in EDTA (63 ± 2.0) and Heparin (59 ± 0.5) than in the control (39 ± 3.0), whereas the value of leucocytes was significantly (p<0.05) higher in the control (60 ± 0.5) than in EDTA (36 ± 2.0) and Heparin (40±0.5) respectively. The values of PVC and HB were significantly (p<0.05) higher in the control (14 ± 1.0 and 4.7 ± 0.3) respectively than in EDTA (10.5 ± 0.5; 3.5 ± 0.5) and Heparin (8 ± 1.0; 2.7 ± 0.2). Similarly, total blood cell counts were significantly (p<0.05) higher in the control (948,000/mm³) than in EDTA (528,000/mm³) and Heparin (490,000/mm³) respectively. The values for HB and PVC were significantly higher in EDTA than heparin. On the basis of results obtained, it was concluded that hematological indices of *O. niloticus* are influenced by the anticoagulant used. The use of EDTA and Heparin in fish hematological studies should be done with standard references to avoid misleading results.

Keywords: Anticoagulants, hematological parameters, *Oreochromis niloticus*.

I. INTRODUCTION

The Nile tilapia is the most widely cultured species of tilapia in Africa. It is a good fish for warm water aquaculture. They utilized a wide variety of natural food (plankton, aquatic macrophytes, detritus, planktonic and benthic invertebrates and decompose organic matter) as well as artificial feeds. They tolerate poor water quality and grow rapidly in warm temperatures. These attributes, along with relatively low input cost, has made it the most widely cultured fresh water fish in the tropical and subtropical countries [1].

Hematological study of fish has assumed greater significance due to increasing emphasis on fish culture and greater awareness of the pollution of natural fresh water resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes [2].

Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress condition like exposure to pollutant, diseases, metals, hypoxia, etc [3]. Hematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on blood characteristics [4]. Fish leave in very intimate contact with their environment and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components [5].

Hematological profiles are increasingly being used as diagnostic aids in determining the health status of fish [3, 5]. However many factors can influences the result in such a way that it is difficult to establish proper base value for the various parameters to be determined.
II. MATERIALS AND METHOD EXPERIMENTAL FISH

50 adult O. niloticus were obtained from the University of Calabar fish farm and transported in large plastic bags partially filled with air and water to the fish pathology laboratory, Institute of Oceanography, University of Calabar. Fish were acclimatization for 7 days in aerated glass aquaria before the analysis. The fish were fed with normal pelleted fish meal once daily. The water parameters (dissolved oxygen, temperature, pH, nitrite and ammonia) were evaluated and maintained according to [7]. The anticoagulants that were used for this study were ethylenediamine triacetic acid (EDTA) and heparin.

III. BLOOD PARAMETERS DETERMINATION

After one week of acclimatization, the fish were removed from the aquaria with a hand net, carefully to minimize stress. The head of each fish was covered with wet towel to reduce aggression during blood collection. Blood samples were collected by cardiac puncture with the use of a disposable sterile syringe fitted with needle. The blood samples were then put in different anticoagulant bottles (Heparinized bottles (4mg/ml), EDTA (0.5mg/ml)). Bottles were rocked gently to enhance mixture with anticoagulant and to prevent clotting and haemolysis. Blood parameters were measured within an hour of blood collection.

Leukocyte count was performed by aspirating blood samples (diluted in Turk’s solution) with a leukocyte pipette onto counting lamella and counted under a light microscope [8]. The hemoglobin were determined according to the cynomethemoglobin method [9]. Giemsa stained samples were examined under a binocular light microscope at 100 magnifications for the determination of the leukocyte and differential WBC counts.

Data generated were subjected to one way analysis of variance (ANOVA) test at 0.05% probability and differences among mean were indicated using least significant difference (LSD) tests [10].

IV. RESULTS

Pack cell volume was highest in the control blood (14%) followed by EDTA (10%) while Heparin had the lowest value of 8%. Hemoglobin was highest in the control (4.7g/dl), followed by EDTA (3.5g/dl), while Heparin had the lowest value of 2.7 (Table 1.)

<table>
<thead>
<tr>
<th>Anticoagulants</th>
<th>PCV (%)</th>
<th>HB (g/dl)</th>
<th>Total BC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.7±0.3</td>
<td>19,680</td>
<td>984×10³/L</td>
</tr>
<tr>
<td>EDTA</td>
<td>3.5±0.5</td>
<td>10,560</td>
<td>528×10³/L</td>
</tr>
<tr>
<td>Heparin</td>
<td>2.7±0.2</td>
<td>9,800</td>
<td>490×10³/L</td>
</tr>
</tbody>
</table>

EDTA = Ethylenediaminetetraacetic acid
Total BC = Total blood count

Neutrophil was highest in EDTA (63±2.0), followed by Heparin (60±0.5) and was lowest in Heparin (59±0.5). Leucocyte was highest in the control (60±0.5) followed by H (40±0.5), and lowest in EDTA (36±2.0). Eosinophil was highest in EDTA (1) and zero was recorded in the control, and Heparin respectively. Monocyte was highest in the control (1) and Heparin (1) and zero was recorded in EDTA Basophil was zero in all the anticoagulants and the control (Table 2.).

<table>
<thead>
<tr>
<th>Anticoagulants</th>
<th>Eosinophils</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Basophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>39±3.0</td>
<td>60±0.5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>EDTA</td>
<td>63±2.0</td>
<td>36±2.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heparin</td>
<td>59±0.5</td>
<td>40±0.5</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

EDTA = Ethylenediaminetetraacetic acid.

The figures (1, 2 and 3) below shows pictorial representations of blood sample of O. niloticus in the different anticoagulants. The arrow represents white blood cells.
Results of the blood films stained with Giemsa showed normal distribution of cells in the control (Fig. 1) containing no anticoagulants, whereas films of blood with anticoagulants ranged from slight enlargement of cells in heparin (Fig. 2) to cell haemolysis in EDTA (Fig. 3).

V. DISCUSSION

The two anticoagulants selected for this study has been successfully used in routine hematological analysis, but from the results obtained in this study, blood films stained with heparin gave blood pictures that were closer to normal (control) than EDTA. Although slight enlargement of cells was observed in heparin stained blood, a significant cell haemolysis was observed in EDTA stained blood. Although EDTA has been advocated as the best anticoagulant for routine hematological analysis [11], the result of the present study has shown that EDTA is not the best for fish hematology. Heparin has proved to be the preferred anticoagulant for fish blood.

The differential blood count in the study showed a significant deviation between the values obtained in the non-anticoagulated blood (control) and the blood in heparin and EDTA. This deviation may be attributed to environmental factors and stress of handling. Hematological parameters have been reported to have been influenced by handling stress [12, 13]. Another reason that may be responsible for the off-shoot of the values of white blood cells (neutrophil, and leucocytes) in the anticoagulated blood is the recognition of the presence of anticoagulants as foreign bodies within the cells thereby stimulating the production of more cells (WBC) to fight against them as self-defense.

Although hematological parameters such as hemoglobin (HB), packed cell volume (PCV) had deviations that were not significantly different from the control, there is need to exercise carefulness in the use of anticoagulants in the analysis of fish blood because blood parameters are likely to be reduced when anticoagulants are used. The safest way is to pair up all tests with controls from non anticoagulated blood. A low value of blood parameters PCV and HB had been attributed to a reduction in red blood cell volume caused by osmotic changes [14].

VI. SUMMARY, CONCLUSION AND RECOMMENDATION

Hematological parameters of O. niloticus were influenced by the anticoagulants used. Thus some parameters were either high or low in different anticoagulants as compared to the controls with no anticoagulant. The results of this study led to the conclusion that the hematological parameters of O. niloticus and other fish species are influenced by anticoagulants and should be interpreted with standards (without anticoagulants) to avoid misleading results.

REFERENCES


