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# Hematological Changes in Brazilian's *Oreochromis niloticus* (Nile tilapia) Strain Infected with *Saprolegnia parasitica*

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**A B S T R A C T**

Experiments were conducted on specimens of the Brazilian's *Oreochromis niloticus* strain infested with *Saprolegnia parasitica*. This was done in order to determine the effects of this infestation on hematological parameters. To do this, specimens of *O. niloticus* with a weighing 37,2 ± 12,7 g and having a size of 12.9 ± 0.9 cm, were exposed to concentrations of *S. parasitica* ranging from $10^5$ to $10^7$ spores/mL, and hematological parameters were measured after 48 h, taking into account controls, infested fish, and sex differences. The results show a decrease in the measured parameters in infected specimens compared to uninfected specimens. However, an increase in mean red blood cell counts and mean corpuscular volume was observed in infected females, as well as increasing in white blood cell counts in males and females. This could indicate an immune response or blood abnormalities, such as leukemia. The decrease in other parameters suggests the presence of anemia and deterioration of the hematopoietic organs, indicating an inflammatory state related to damage caused by infection and stress in the fish. Variations in hematological parameters can be attributed to individual differences, the origin of the fish, and genetic factors. These results highlight the importance of monitoring these parameters to assess the physiological status of farmed fish.

## Introduction

Tilapia is the most commonly farmed fish in over one hundred countries worldwide (El-Sayed, 2002). It is therefore one of the most important fish and plays a vital role in all types of livestock farming. It has a global production of over two million tons after carp and salmon (Fitzimmons, 2006). In Côte d'Ivoire, Nile tilapia is the most consumed fish, both in terms of aquaculture production and overall consumption (Koné, 2015). While the country has a high demand for fish, it currently imports a large portion of its needs, highlighting the potential for increased domestic tilapia farming to meet demand. However, environmental and physiological factors can affect health status, in particular, many parameters in the blood. These environmental factors cause stress in animals. Hematological assessment in animals is of primary interest in defining the diagnosis of many diseases (Diaby *et al.,* 2016).

Hematological studies also have ecological and physiological interests (Anderson, 2003). These studies help to understand the link between blood characteristics and the species' ability to adapt to the environment.

The Oomycete Saprolegnia parasitica belongs to the Saprolegniaceae family, which contains the most important pathogens of freshwater fish (Khoo, 2000). They cause the destruction of spawn in aquaculture (Caruana *et al.,* 2012) and are responsible for serious damage to various stages of fish development (Ghasemi Pirbalouti *et al.,* 2009), with the early stages being the most vulnerable.

This stage of development is characterized by the destroyment of eggs and larva within a few days or even hours. From an economic perspective, fungal infections represent one of the main sources of loss in aquaculture (Han-Ching, 2006). The same source reports that health problems can indeed lead to serious economic damage, jeopardizing the viability of fish production.

The Nile tilapia, *Oreochromis niloticus*, Brazil strain, was chosen as the model for this study. This strain is the result of a genetic improvement program, hence its resilience in Côte d'Ivoire aquatic environments. The objective of this study is to experiment the effects of *Saprolegnia parasitica* infection on some hematological parameters in Nile tilapia *O. niloticus* of the Brazil strain. This was done taking into account the sex of the different specimens. The analysis of this investigation focused on hematological parameters due to their relationship with respiration (red blood cell count, hematocrit and hemoglobin levels), defense mechanism (white blood cell count) and energy (blood glucose levels).

## Materials and Methods

### Study Setting and Livestock Monitoring

The experiments were conducted at Nangui Abrogoua University in Abidjan, Côte d'Ivoire, within the Fisheries and Aquaculture Research Center. The design consisted of six glass aquaria, four of which were 0.48 $m^2$ for the various tests and two of 0.14 $m^2$ for the control groups. The fish used were Nile tilapia (*O. niloticus*), weighing 37.5 ± 12.5 g, measuring 12.9 ± 0.9 cm, from the Blondey fish farm in the Agnéby Tiassa region. The aquaria were continuously aerated, and 25% of the water were renewed daily. Fish were acclimatized for 15 days before experimental infestations (Effiong and Alatise, 2009; Jin *et al.,* 2010). They were fed five times a day with a commercial food "RAANAN" representing 5% of their biomass, and with a stocking density of 15 fishes per square meter.

### Inoculation Preparation and Infection

Experimental inoculation trials were conducted on Nile tilapia *O. niloticus*. The oomycete *Saprolegnia parasitica* was used for infestations. It was prepared on SDA (Sabouraud Dextrose Agar) supplemented with chloramphenicol in sterile distilled water.

A constant contact inoculation technique was used, consisting of placing a culture fragment under the fish's skin as recommended by Afzali *et al.,* (2015). The fish were anesthetized with a 0.01% $MS_{222}$ (Methane Sulfonate Tricaine) solution before injection, which lasted 20 to 30 s. The fish were then returned to the experimental aquarium. Recovery occurred after 4 to 5 min.

A culture fragment of the microbial suspension between 0.1 and 0.3 ml of the strain with a concentration of $1.10^5$ to $1.10^7$ spores/mL (Rheka *et al.,* 2014) was then slid along the scalpel and placed at the bottom of the pocket made. They were then returned to the experimental tanks and closely monitored for 48 h to detect clinical signs and mortality. The control groups did not receive injections, and all moribund fish were isolated and another mycological identification was made.

### Blood Collection and Analysis

Blood samples were collected from infected and uninfected fish 48 h post-infection using the method described by Sakandé *et al.,* (2003) to minimize interference with anemia assessments. The fish were re-anesthetized with $MS_{222}$, and blood was collected from the caudal vein using a 25 mL syringe, using EDTA as an anticoagulant. Various hematological parameters were analyzed, including red and white blood cell counts, hemoglobin levels, and hematocrit, using a URIT-3000Plus hematology analyzer. Glucose levels and a biochemical parameter were determined using a Clima MC 15 automated system.

## Statistical Analysis

The Kruskal-Wallis test was applied due to non-compliance with ANOVA assumptions, while the Mann-Whitney U test was used for pairwise group comparisons. Statistical analyses were performed using STATISTICA 7.1 (Stat Soft France, 2005).

## Results and Discussion

The different mean values of hematological parameters measured on uninfected and infected *O. niloticus* specimens of the Brazil strain during the experiments are reported in Table I.

The means of parameters such as red blood cells, hemoglobin, and hematocrit are respectively 1.94 ± 0.52 $10^3$/mm$^3$ (uninfected) and 1.17 ± 0.14 $10^3$/mm$^3$ (infected) for males while the values for females are 1.62 ± 0.2 $10^3$/mm$^3$ for uninfected and 2.26 ± 0.15 $10^3$/mm$^3$ for infected; of 4.85 ± 0.65 g/dL (uninfected fish) and 3.77 ± 0.45 g/dL (infected fish) for males and 5.6 ± 1 g/dL (uninfected fish) and 4.4 ± 0.8 g/dL (infected fish) for females; of 15.77 ± 1.35% for uninfected males and 16.8 ± 3.4% for uninfected females and the values noted for infected fish are 12.37 ± 1.12% (males) and 14.3 ± 3.4% (females).

Note that at the level of these parameters, the values all decreased except those of the red blood cells in which there was an increase when moving from uninfected females to infected females.

Regarding the mean corpuscular volume (MCV), the mean corpuscular hemoglobin content (MCHC), the mean corpuscular hemoglobin concentration (MCHC), there is also a decrease in all the parameters when passing from the uninfected state to the infected state of the specimens of *O. niloticus*. However, we note an increase in the Mean Corpuscular Volume (MCV) in infected females compared to uninfected females.

The mean values obtained for these parameters are respectively 98 ± 3.34 fL and 89.8 ± 0.4 fL, respectively, for uninfected males and females while the infected males and females have a mean corpuscular volume of 83.23 ± 0.91 fL and 99.5 ± 4.5 fL; of 30.5 ± 0.53 pg for uninfected males, 25.8 ± 0.44 pg for infected males, 26.5 ± 0.2 pg for uninfected females and 21.67 ± 1.75 pg for infected females; of 30.23 ± 0.55%, 27.4 ± 1.54% respectively in uninfected and infected males and 30.2 ± 0.5% and 28.53 ± 0.15% for uninfected and infected females. Regarding blood platelets, the mean values determined decreased from uninfected to infected individuals for both sexes. They were 136 ± 65.82 $10^3$/mm$^3$, 171.33 ± 118.36 $10^3$/mm$^3$, respectively, for uninfected males and females, and 102.4 ± 98.47 $10^3$/mm$^3$ and 118.66 ± 50.36 $10^3$/mm$^3$ for infected males and females.

The mean blood glucose levels of male and female *O. niloticus* specimens taken as controls, therefore uninfected, were 6.85 ± 1.25 g/L and 9.17 ± 1.7 g/L. While this number is 2.35 ± 0.45 g/L and 4.7 ± 0.4 g/L, it has decreased in infected males and females.

For white blood cells, which represent the body's defense, they include the leukocyte formula composed of neutrophils, eosinophils, basophils, monocytes, and lymphocytes. Thus, the average values noted are 2.5 ± 0.05 $10^3$/mm$^3$ for uninfected males, 3.15 ± 0.05 $10^3$/mm$^3$ for infected males, 2.1 ± 0.2 $10^3$/mm$^3$ for uninfected females, and 2.5 ± 0.3 $10^3$/mm$^3$ for infected females. White blood cell counts increased in both male and female *O. niloticus* fish when infected.

Statistical tests showed a significant difference between white blood cell counts in infected and uninfected males. The same was true for MCV and TCMH (Mann Withney U test; $p < 0.05$).

The oomycete *Saprolegnia parasitica*, isolated from fish farm waters, was used to infest *Oreochromis niloticus* of the Brazil strain. These infestations were performed to demonstrate the effects of this aquatic fungus on the hematological parameters of the fish's blood. The study of these blood parameters allows for a better assessment of the health of fish subjected to infestation, disease, and changes in environmental conditions (Blaxhaa and Daisley, 1973; Achuthan-Nair and Balakrishnan-Nair, 1983).

During these experiments, it emerged that infestation of *O. niloticus* with *S. parasitica* caused several changes in all blood parameters measured in both males and females of the Brazil strain.

Thus, most of the measured parameters decreased when moving from the uninfected to the infected state. The decrease in these parameters is thought to be linked to the presence of *S. parasitica* in the bodies of infested specimens. This infestation induces anemia in infected individuals, which is a major clinical sign (Steinhagen *et al.,* 1990; Zuo and Woo, 2000). Reduced red blood cell, hemoglobin, and hematocrit concentrations caused anemia in infected specimens, which may be due to damage to hematopoietic tissues.

These results were also demonstrated by Tavares Dias *et al.,* (2002); Bittencourt *et al.,* (2003) and Zaki *et al.,* (2003; 2008) on *S. parasitica* infestations of *O. niloticus*. The same was true in various other fish species such as *Clarias gariepinus* (Atamanalp *et al.,* 2002a, b; Atamanalp and Yanik, 2003; Shah and Altindag, 2004; Shah, 2010). These authors also observed a decrease in the levels of these parameters after infestation in the specimens studied. Indeed, the reduction in the level of hematological parameters could be due to a decrease in their production and an increased loss through the destruction of red blood cells.

Another reason for the reduction in red blood cells could also be the deterioration of hematopoietic tissue or organs such as the kidney, spleen and bone marrow which play a crucial role in the formation of blood cells, and also in transporting oxygen via hemoglobin. On the other hand, the increase in red blood cells in infected females could be a sign of a blood abnormality or disease. This could be due to dehydration of the infected body.

Since MCV, TCMH and CCMH values are dependent on the hematocrit level, as well as the number of red blood cells and the hemoglobin content, they were influenced by them. This confirms the existence of anemia due to the decrease in the red blood cell content (hypochromic) and the fact that the red blood cell diameter is larger than normal (macrocyte). This decrease in the hemoglobin trend could result from swelling of the red blood cells as well as poor mobilization of hemoglobin from the spleen. In addition, the hematocrit level seems to be positively correlated with the red blood cell counts, hence a decrease in hematocrit. Similar results have been reported for several freshwater fish such as *Oreochromis niloticus* and *Clarias gariepinus*, respectively, exposed to infection with *Ichthyophthirius multifiliis* and *Saprolegnia* sp. (Atamanalp and Yanik, 2003; Rekha *et al.,* 2014; Chodavadiya *et al.,* 2015). In terms of the leukocyte count, the results of the studies carried out showed an increase in white blood cell counts from uninfected to infected specimens. This increase in these blood cells reflects a general inflammatory state due to the damage caused by the *S. parasitica* infestation. This could be explained by the stimulation of the immune system leading to these increases.

Furthermore, this increase could also characterize leukemia, the appearance of abnormal cells, as well as other factors such as stress and environmental variations on the animals' physiology. These results corroborate those of Qureshi *et al.,* (2001); Buckham-Sporer *et al.,* (2008); and Adenkola *et al.,* (2009). These authors also reported changes in these parameters in similar models.

Regarding glucose, a decrease was recorded in infected specimens. This would be primarily due to the administration of anesthesia ($MS_{222}$) and the blood collection technique. This reduction would be explained by a state of stress in the fish caused by the introduction of the fungal strain into the body. This stress would be based on the decrease in the level of this parameter because it is one of the secondary parameters of the stress response that is most sensitive to the methods of handling the fish. This observation was also made by Khalfallah (1993) who showed that phenoxyethanol anesthesia was the basis for the reduction in glucose in *Dicentrarchus labrax* L. (1758).

Regarding sex, almost all values are higher males than in females. These results corroborate those of Ibrahim *et al.,* (2003) and Jawad *et al.,* (2004) who made the same observations in their studies. These higher hematological values in males would be mainly due to the fact that they are biochemically and nutritionally richer than in females (Joshi, 1973). Indeed, in females, a large part of the metabolites and nutrients are continuously depleted in the development of the ovary. During the period before spawning, males feed with almost the same intensity, while feeding decreases in females due to the gradual decrease in space in the abdominal cavity following the rapid development of the gonads. Naturally, although metabolic demands may be higher in females during the spawning period, all factors make the female poorer in various hematological parameters at the same time and, temporary microcytic anemia may be present (Bindu Bhaskaran, 2011).

**Table.1** Mean values of HEMATOLOGICAL parameters observed in O. niloticus of the Brazil strain uninfected and infected with *S. parasitica*.

| Parameters | Status of Oreochromis niloticus strain Brazil | | | |
|---|---|---|---|---|
| | Uninfected males (n = 15) | Infected males (n = 15) | Uninfected females (n = 15) | Infected females (n=15) |
| Hematology | | | | |
| Globular Numeration | | | | |
| WBC ($10^3$/mm$^3$) | $2.5 \pm 0.05^a$ | $3.15 \pm 0.05^b$ | $2.1 \pm 0.2^{ab}$ | $2.5 \pm 0.3^{ab}$ |
| RBC ($10^3$/mm$^3$) | $1.94 \pm 0.52^a$ | $1.17 \pm 0.14^a$ | $1.62 \pm 0.2^a$ | $2.26 \pm 0.15^a$ |
| Hb (g/dL) | $4.85 \pm 0.65^a$ | $3.77 \pm 0.45^a$ | $5.6 \pm 1^a$ | $4.4 \pm 0.8^a$ |
| HCT (%) | $15.77 \pm 1.35^a$ | $12.37 \pm 1.12^a$ | $16.8 \pm 3.4^a$ | $14.3 \pm 3.4^a$ |
| MGV (fL) | $98 \pm 3.34^a$ | $83.23 \pm 0.91^{ab}$ | $89.8 \pm 0.4^a$ | $99.5 \pm 4.5^{ac}$ |
| MCHR (pg) | $30.5 \pm 0.53^a$ | $25.8 \pm 0.44^{ab}$ | $26.5 \pm 0.2^a$ | $21.67 \pm 1.75^{ac}$ |
| MCHC (%) | $30.23 \pm 0.55^a$ | $27.4 \pm 1.54^a$ | $30.2 \pm 0.5^a$ | $28.53 \pm 0.15^a$ |
| Pla ($10^3$/mm$^3$) | $136 \pm 65.82^a$ | $102.4 \pm 98.47^a$ | $171.33 \pm 118.36^a$ | $118.66 \pm 50.36^a$ |
| Leucocyta Formula | | | | |
| Neutrophils (%) | $37 \pm 7^a$ | $38 \pm 5^a$ | $44 \pm 4^a$ | $45.33 \pm 0.58^a$ |
| Eosinophils (%) | $2 \pm 0^a$ | $1 \pm 0^a$ | $2 \pm 0^a$ | $2 \pm 0^a$ |
| Basophils (%) | $0^a$ | $0^a$ | $0^a$ | $0^a$ |
| Monocytes (%) | $6 \pm 2^a$ | $8 \pm 0^a$ | $8 \pm 0^a$ | $9.33 \pm 1.53^a$ |
| Lymphocytes (%) | $55 \pm 5^a$ | $53 \pm 5^a$ | $46 \pm 4^a$ | $44.33 \pm 1.15^a$ |
| Biochimical Parameters | | | | |
| Glucose (g/L) | $4.45 \pm 3.13^a$ | $3.92 \pm 5.09^a$ | $6.95 \pm 5.14^a$ | $3.03 \pm 3.52^a$ |

**WBC**: White Blood Cells; **RBC**: Red Blood Cells; **Hb**: Hemoglobin Concentration; **HCT**: Hematocrit; MGV: Mean Globular Volume; **MCHR**: Mean Corpuscular Hemoglobin Rate; **MCHC**: Mean Corpuscular Hemoglobin Concentration; **Pla:** Platelets. For each value, the different alphabetical letters (a, b and c) on them indicate a statistically significant difference ($p < 0.05$).

This study shows that infestation of *O. niloticus* with *S. parasitica* affects hematological and biochemical parameters, leading to blood disorders and anemia in exposed fish.

The variability between these parameters appears to be related to individual differences, as well as fish origin and genetic factors, highlighting the validity of using the physiology to assess the welfare of farmed fish.

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## Author Contributions

N'guessan Francis: Conceived the original idea and designed the model the computational framework and wrote the manuscript.; Keita Gaoussou: Formal analysis, writing review and editing.; Kouassi Affoue Edwige: Validation, methodology, writing—reviewing

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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