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# HEMATOLOGICAL AND GLYCEMIC CHANGES IN *Oreochromis niloticus* SUBJECTED TO ACUTE STRESS BY SYPHONING

# ALTERAÇÕES HEMATOLÓGICAS E GLICÊMICAS EM *Oreochromis niloticus* SUBMETIDAS AO ESTRESSE AGUDO POR SIFONAMENTO

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**Resumo:** Este trabalho teve como objetivos determinar a resposta ao estresse agudo causado pela sifonagem e a influência do uso de NaCl não iodado a 1% e da temperatura em tilápias-do-nilo, *Oreochromis niloticus*. Para tanto foram utilizadas 40 tilápias, distribuídos aleatoriamente em 4 grupos (n=10), sendo eles T1 – não sifonado, controle negativo; T2 – sifonado e submetidos a um banho com cloreto de sódio (NaCl) industrial (não iodado) a 1%; T3 – sifonado, controle positivo e T4 – sifonado e com temperatura controlada de 28 ºC. Foram avaliadas as variáveis hematológicas e glicêmicas dos animais. Da análise do esfregaço sanguíneo observou-se predominância de linfócitos seguidos de monócitos e neutrófilos. Entretanto, não houve diferença estatística no leucograma e eritrograma dos grupos tratados em relação ao controle. O índice glicêmico mostrou-se mais eficaz na detecção do estresse agudo, verificando-se hiperglicemia nos animais do grupo controle positivo (T3) e com temperatura mantida em 28ºC (T4). Concluiu-se que o tratamento com NaCl (1% / 60 min), reduz o impacto na glicemia em *O. niloticus* sob situação de estresse causado pelo sifonamento e que a temperatura de 28ºC parece agir como agente estressor em tilápias, esses achados são úteis como ferramenta no controle do estresse durante o manejo em criações comerciais, bem como na manutenção desses animais em ambientes de laboratório.

**Palavras-chaves:** cloreto de sódio, glicose, teleósteos, temperatura, tilápias

**Abstract:** The objective of this work was to determine the response to acute stress caused by siphoning and the influence of 1 % non-iodized NaCl and temperature in Nile tilapia, *Oreochromis niloticus*. For this purpose, 40 Nile tilapias were used, distributed randomly in 4 groups (n = 10), as it follows: T1. Non-siphoned, negative control; T2. Siphoned and subjected to a 1 % industrial (non-iodized) sodium chloride (NaCl) bath; T3. Siphoned, positive control and T4. Siphoned and kept at a temperature of 28 ºC. The hematological and glycemic variables of the animals were evaluated. From the analysis of the blood smear it was observed

predominance of lymphocytes followed by monocytes and neutrophils. However, there was no statistical difference in the leukogram and erythrogram of the treated groups in relation to the control. The glycemic index was more effective in the detection of acute stress, and hyperglycemia was observed in the animals of the positive control group (T3) and with a temperature maintained at 28ºC (T4). It was concluded that treatment with 1 % NaCl reduces the impact on glycemia in *O. niloticus* under stress caused by siphoning and that the temperature of 28ºC seems to act as a stress agent in tilapia. Therefore, these findings are useful as a tool in the control of stress during handling in commercial farms, as well as in the maintenance of these animals in laboratory environments.

**Key words:** sodium chloride, glucose, teleosts, temperature, Nile tilapia

## INTRODUCTION

Aquaculture has increased globally. Brazilian aquaculture is expected to grow over 100 % in 2025 (FAO, 2016). These commercial issues culminate in the intensification of aquaculture. The rapid intensification has inevitably resulted in high densities, poor water quality, transport, sorting, handling, among others (WEDEMEYER, 1996). These practices can lead to physiological stress in fish (HOSHIBA et al., 2009).

Stress can be defined as a condition of loss of homeostasis (SELYE, 1946; SOUSA *et al*., 2015). Several studies have been performed in order to understand the pathophysiological mechanisms of stress and treatments that can reduce this imbalance (MARTINS *et al*., 2000a; BRANDÃO *et al*., 2006; SIGNOR *et al*., 2010).

Glycemia and hematological profile are tools routinely used as stress indicator for teleost fish (MARTINS *et al*., 2000a; SILVA *et al.* 2012).

Thus, the aim of this study was to analyze glucose levels and hematological variables in Nile tilapias, *Oreochromis niloticus,* subjected to acute stress by siphoning and the influence of 1 % non-iodized NaCl and increased temperature in these variables.

## MATERIAL AND METHODS

40 Nile tilapias (80.10 ± 6.3 g) used during the study were placed in four 250 L fiber tanks (10 fish per tank), supplied with constant aeration, and continuous water flow (1 $L.min^{-1}$), fed with commercial fish feed pellets at 3 % of body weight (28 % crude protein and 4000 $kcal\ kg^{-1}$). The water quality conditions were kept within the optimal range for the species (dissolved oxygen: 5.1 $mg.L^{-1}$; temperature: 20.47 ºC; pH: 7.06 and electrical conductivity: 117.96 µS/cm). Fish were randomly assigned into four experimental groups: T1: non-siphoned, negative control; T2: siphoned and subjected to a 1 % industrial (non-iodized) sodium chloride (NaCl) bath of 60 min; T3: siphoned, positive control and T4: siphoned with temperature kept at 28 ºC by using heaters the whole experiment. After stress induction by siphoning (5 min per tank), fish were anesthetized by immersion in benzocaine (1:20000) diluted in alcohol 98° (100 $mg.mL^{-1}$) (WEDEMEYER, 1970) until reaching stage IV of anesthesia (ROSS & ROSS, 2007). Group T2 was anesthetized after bath.

After stress induction, blood sample collection (1,0 mL per fish) was performed by caudal venipuncture and used for glucose determination, hematocrit (GOLDENFARB *et al.* 1971), hemoglobin (PETRILLO *et al.*, 2007), total red blood in Neubauer (MARTINS *et al.*, 2004) and total leukocyte (MARTINS *et al*., 2000b). Hematimetric indexes: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were also assessed (PETRILLO *et al*., 2007). Additionally, blood smears were stained according to Tavares-Dias & Moraes (2007).

## RESULTS AND DISCUSSION

The erythrogram did not show significant differences between treatments ($p>0.05$) (Table 1). The blood smears were identified in increasing order: lymphocytes, monocytes, neutrophils and thrombocytes (Fig. 1). The hematological variables are important diagnostic tools for fish diseases and they are indicators of fish health and stress caused by handling or environment changes (TAVARES-DIAS & MORAES, 2004). However, in the present study, the erythrogram did not show alterations, probably because of the type of stimulus, which could not produce the release of cells from the hematopoietic organs. Similar results were reported in matrinxã *Brycon amazonicus* subjected to stress by chasing, where the authors attributed to the fact that the stimulus was not intense enough to promote alterations in these indicators (HOSHIBA *et al*., 2009).

Table 1. Hematological parameters[1] (mean ± standard deviation) in Nile tilapia *Oreochromis niloticus*.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | T1 | T2 | T3 | T4 |
| Hematocrit (%) | 58.4 ± 3.5 A | 49.7 ± 4.8 A | 60.7 ± 5.8 A | 55.7 ± 6.9 A |
| Hemoglobin (g/dl) | 6.3 ± 0.4 A | 6.8 ± 0.3 A | 6.4 ± 0.8 A | 7.1 ± 0.5 A |
| Erythrocytes ($mm^3$) | 162.25 ± 47.1 A | 103.71 ± 25.8 A | 153.43 ± 25.1 A | 138.66 ± 32.5 A |
| MCV (fl) | 3.5 ± 0.1 A | 4.7 ± 0.1 A | 3.9 ± 0.3 A | 4.1 ± 0.5 A |
| MCH (pg) | 0.38 ± 0.03 A | 0.65 ± 0.04 A | 0.41 ± 0.05 A | 0.51 ± 0.04 A |
| MCHC (%) | 10.7 ± 1.9 A | 13.6 ± 0.9 A | 10.5 ± 0.8 A | 12.7 ± 1.3 A |
| Total leukocytes (x $10^3$/µL) | 16.4 ± 5.5 A | 13.5 ± 1.9 A | 25.8 ± 7.9 A | 15.9 ± 3.7 A |
| Lymphocytes (x $10^3$/µL) | 7.1 ± 2.1 A | 5.1 ± 1.3 A | 8.3 ± 3.8 A | 3.8 ± 0.5 A |
| Monocytes (x $10^3$/µL) | 5.6 ± 1.9 A | 3.2 ± 0.8 A | 8.5 ± 2.2 A | 5.1 ± 2.3 A |
| Neutrophils (x $10^3$/µL) | 2.2 ± 0.6 A | 3.6 ± 0.4 A | 5.1 ± 1.6 A | 4.2 ± 1.3 A |
| Thrombocytes (x $10^3$/µL) | 0.9 ± 0.1 A | 0.8 ± 0.2 A | 1.7 ± 0.9 A | 0.8 ± 0.1 A |

[1] Figure 1. Data are shown as mean ± standard deviation (n=10). Different uppercase letters indicate significantly different values ($P<0.5$) between different treatments. MCV-Mean corpuscular volume, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration. T1- non-siphoned, control; T2 – siphoned and subject to 1% sodium chloride bath (NaCl) for 60 min; T3 – siphoned and T4 - siphoned and temperature kept at 28 ºC by using thermostat the whole experiment (ANOVA and Tukey tests).

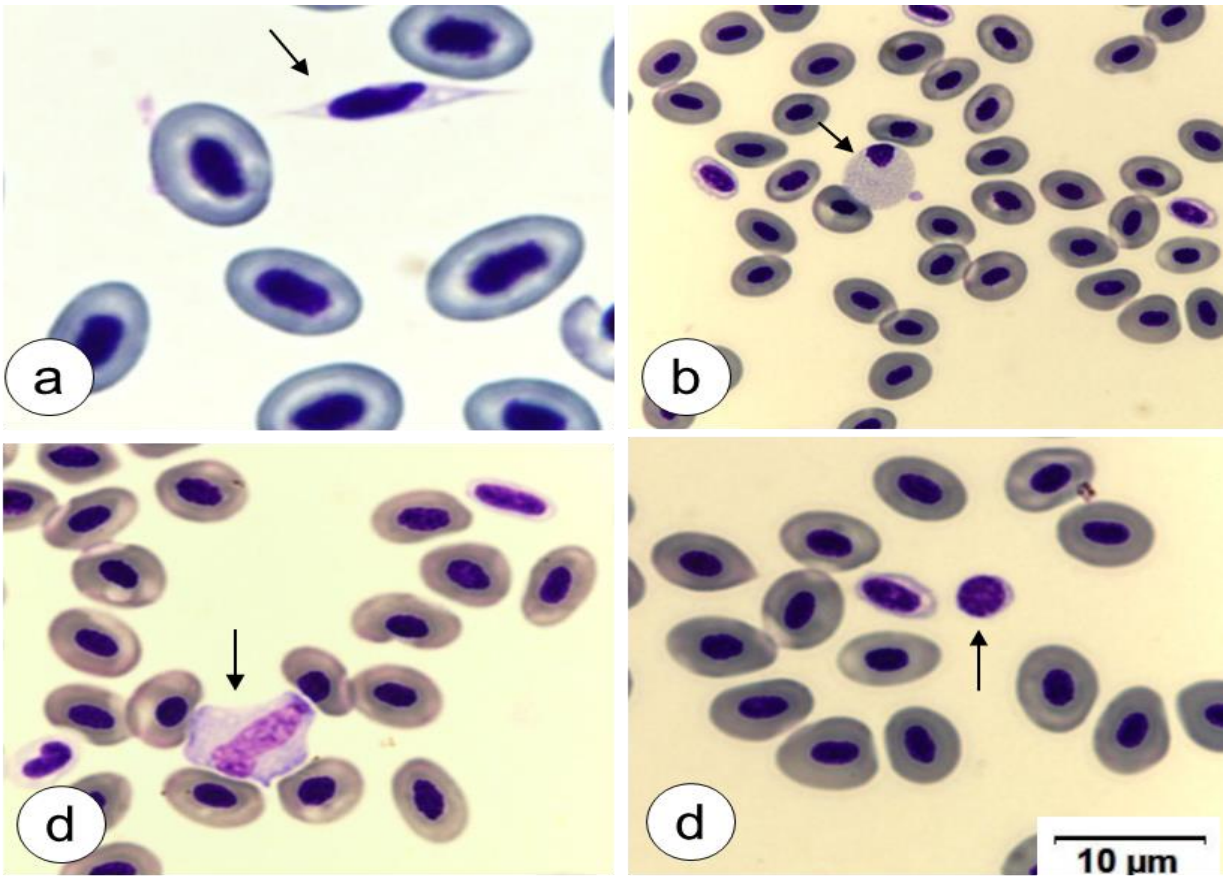


Figure 1: Blood smears stained with rapid panoptic kit in Nile tilapia subjected to acute stress. Thrombocytes (a), Neutrophil (b), Monocyte (c) and Lymphocyte (d).

The results of blood smears showed lymphocyte predominance followed by monocytes and neutrophils, similar to the reported by Martins *et al*. (2000), Tavares-Dias & Moraes (2004), Yunis-Aguinaga *et al*. (2015) and Yunis-Aguinaga *et al*. (2016). The leukogram did not show significant differences between control group and treatments ($p>0.05$), which could be due to the fact that the stimulus was not long enough (TAVARES-DIAS & MORAES, 2004; BOZZO *et al*., 2007).

Glycemia is one of the most common indicators of stress because it is easily detected and the evaluation can be performed by using blood glucose kits (WEDEMEYER *et al*. 1990; MARTINS *et al*., 2000a). Glucose levels were significantly higher in group T4 ($p<0.05$) when compared to control group T1 and group T2. In addition, group T3 did not show significant difference when compared to groups T2 and T4, and control group T1 did not show significant difference when compared to group T2 (Fig. 2).

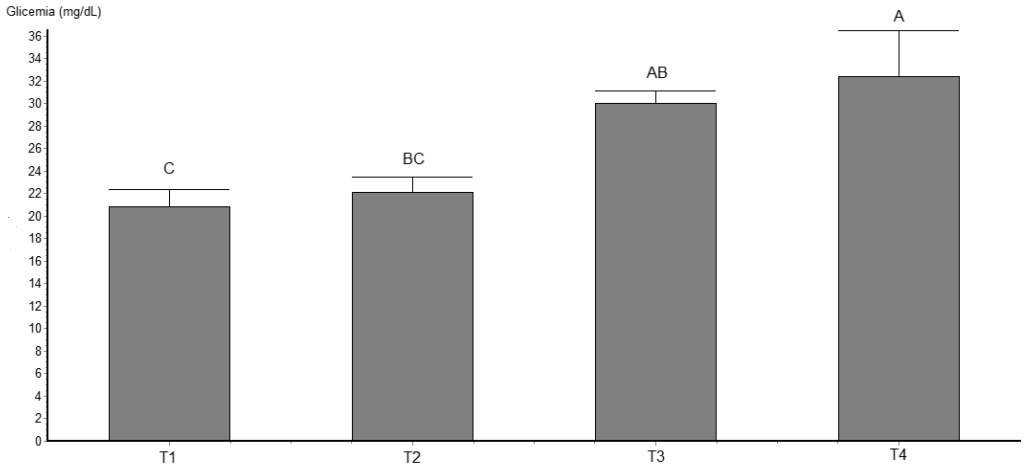


Figure 2. Glycaemia in Nile tilapia (ANOVA and Tukey test). Data are shown as mean ± standard deviation (n=10). Different uppercase letters indicate significantly different values (P<0.5) between different treatments. T1- non-siphoned, control; T2 – siphoned and subject to 1% sodium chloride bath (NaCl) for 60 min; T3 – siphoned and T4 - siphoned and temperature kept at 28 ºC by using thermostat the whole experiment

The results revealed hyperglycemia immediately after the stressing stimulus in groups T3 e T4 when compared to the control group T1. Previous studies obtained same results (RUANE *et al.*, 2001; SLOMAN *et al*., 2001). The increase of glucose levels could be related to alterations of energy metabolism caused by the release of hormones such as cortisol and catecholamines during stressful situations (MCDONALD & MILLIGAN, 1997; MARTINS *et al.*, 2000a). Probably, the higher glucose levels in group T4 could be related to the additional stimulus of the temperature.

The lower glucose levels in group T2 when compared to group T3 showed that the addition of NaCl for a period of 60 min in group T2 reduces the effects of acute stress. The dissociation of NaCl in water replaces the loss of electrolytes during stress. The increase of gill permeability and flux rate can cause a loss of electrolytes (MCDONALD & MILLIGAN, 1997). Temperatures over 27ºC are risk factors associated to disease outbreaks and cause stress (LEAL, 2008; MARCUSSO *et al.*, 2014). This fact explains a higher significant increase of glucose levels in group T4 in relation to the other groups, suggesting that a rise in the temperature added to the siphoning increases the stress.

## CONCLUSIONS

The results observed in this study suggest that siphoning can produce acute stress in animals by increasing glucose levels, which is more severe when added to other stressors such as an increased temperature. In addition, the treatment with 1 % NaCl for a period of 60 min reduces the impact on glucose levels in *O. niloticus* under acute stress. Glycaemia is a variable easy and rapid to detect by fish farmer or researchers and can be used as a tool for stress evaluation in tilapias.

## ETHICAL ISSUES

The present study was approved by the Ethics Committee on Animal Use (CEUA) (protocol number 011688/12).

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