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Alterations in the hematological parameters of Juvenile Nile Tilapia (*Oreochromis niloticus*) submitted to different salinities

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Abstract. *Oreochromis niloticus* is considered an excellent alternative for the cultivation after the great drop in the production of marine shrimp (*Litopenaeus vannamei*) in Northeast Brazil. However, environmental factors exert a great influence on the health of aquatic animals and can be decisive for the success or failure of an aquaculture enterprise. Thus, the aim of this study was to evaluate in juvenile *Oreochromis niloticus*, submitted to different salinities (15, 25 and 35), the following hematological parameters: percentage of hematocrit, hemoglobin, glucose, total leukocyte and erythrocyte counts. Forty fish were used, kept in 8 tanks, and were exposed to the different salinities. Hematological parameters were subjected to analysis of variance (ANOVA) and Tukey's multiple comparison test (P <0.05). Changes were observed in the values of the blood parameters measured as the salinity increased. Statistical differences were observed in saline treatment 35 for blood glucose, hemoglobin, and total count of erythrocytes and leukocytes. There was no statistical difference in hematocrit percentage. Salinity has an influence on hematological parameters of Nile tilapia, indicating physiological changes.

Keywords: fish; saline environment; blood

Resumo. Alterações nos parâmetros hematológicos de juvenis de Tilápia do Nilo (*Oreochromis niloticus*) submetidos a diferentes salinidades. *Oreochromis niloticus* é considerada uma excelente alternativa para o cultivo após a grande queda na produção de camarão marinho (*Litopenaeus vannamei*) no Nordeste do Brasil. Contudo, fatores ambientais exercem grande influência sobre a saúde dos animais aquáticos e podem ser decisivos para o sucesso ou fracasso de um empreendimento de aquicultura. Assim, o objetivo deste estudo foi avaliar em juvenis de *Oreochromis niloticus*, submetidos a diferentes salinidades (15, 25 e 35), os seguintes parâmetros hematológicos: percentual de hematócrito, hemoglobina, glicose, contagem total de leucócitos e eritrócitos. Quarenta peixes foram utilizados, mantidos em 8 tanques, e foram expostos às diferentes salinidades. Os parâmetros hematológicos foram submetidos a análise de variância (ANOVA) e teste de comparação múltipla de Tukey (P <0,05). Observaram-se alterações nos valores dos parâmetros sanguíneos à medida que a salinidade aumentou. Observaram-se diferenças estatísticas no tratamento da salinidade 35 para a glicemia, hemoglobina e contagem total de eritrócitos e leucócitos. Não houve diferença estatística no percentual de hematócrito. A salinidade tem influência nos parâmetros hematológicos da tilápia do Nilo, indicando alterações fisiológicas.

Palavras-chave: peixe; ambiente salino; sangue

Introduction

In recent years, tilapia farming has intensified in Brazil reaching a production of 253,824.10 tons in 2011 (Brasil, 2013), with the Northeast region being one of the largest producers.

Tilapia belongs to the family of cichlids, and there are more than 70 species grouped into three genera: *Oreochromis, Sarotherodon,* and *Tilapia*. The species of the genus *Oreochromis*, which belongs to Nile tilapia, are omnivorous and microphagous, however, the initial stage of fingerlings are phytoplantophages (Kubitza, 2000; Zaniboni- Filho, 2004; Côa *et al*., 2017) which is a favorable feature for farming.

Originally from Africa, the Nile tilapia (*Oreochromis niloticus*) has had a great impact on aquaculture (Moreira, 2007; Campos-Garcia *et al*., 2016). This species has been constituted as an excellent alternative for cultivation after the big drop in the production of marine shrimp (*Litopenaeus vannamei*) in the Northeast of Brazil (Barbieri *et al*., 2014). It has been titled as the "new white fish" because it presents the typical requirements of fish preferred by the consumer market such as: white meat of firm texture, easy filleting, lack of spine "Y" (mioseptum) and no unpleasant odor (Vanuccini, 1999).

It is known that environmental factors that have a major influence on the health of aquatic animals may be decisive for the success or failure of an aquaculture venture. Several studies have evaluated the physiological status of Nile tilapia in different salinities, demonstrating high tolerance, including; antioxidant status (Gan *et al*., 2016), percentage fertilization and hatching (Hui *et al.*, 2014), osmoregulatory functions (Güner *et al*., 2006), etc. However, much work still needs to be done to evaluate different aspects of the immune system.

Therefore, we need to monitor them adequately and know what influences the variations in the immune response of cultured animals. One way to facilitate this is to study the blood components in fish, which is currently considered an important tool for the diagnosis of animal health (Martins *et al.*, 2004; Ranzani-Paiva & Silva-Souza, 2004).

Another environmental consequence that occurs in fish is the variation in haematological characteristics responsible for immunosuppression in the organism (Yada & Nakanishi, 2002; Barbieri *et al.*, 2016). The study of blood components and their functions is important for the understanding of normal and pathological conditions. The evaluation of these components helps in determining the influence of pathophysiological conditions that may affect homeostasis, which contribute to the diagnosis of adverse conditions (Tavares- Dias & Moraes, 2003).

The cells in the peripheral blood of teleost fish are erythrocytes (red blood cells or erythrocytes), thrombocytes, and leukocytes (white blood cells).

The fish erythrocytes are oval-shaped, with a central nucleus following the cell shape, with condensed chromatin in their nucleoli. These hemoglobin-containing cells, transport O_2 and CO_2 in the blood. Deficiencies in erythrocytes reflect a lack of O_2 in the tissues (Ranzani-Paiva & Silva-Souza, 2004; Barbieri & Bondioli, 2013). Although there are large amounts of erythrocytes, their sizes vary between species. One study shows an inverse relationship between the size of red blood cells and the swimming ability of marine teleost, as well as the size of the species (Tavares-Dias, 2003).

The size and number of erythrocytes reflect their position on the evolutionary scale. The largest, less prevalent erythrocytes are observed in more primitive vertebrates (Wintrobe, 1934; Tavares-Dias, 2003). In Tilapia, they have a rounded shape; similar to grains of beans.

Leukocytes are colorless corpuscles involved in cellular defense and immunity in the organism, and their physiological significance is partially conditioned by their ability to perform selective migration and diapedesis (Ranzani-Paiva & Silva-Souza, 2004).

In fish, it is more difficult to identify cells of the leukocyte series, compared to those of erythrocyte and thrombocytic series. This is mainly due to the difficulty in distinguishing thrombocytes from lymphocytes and monocytes from neutrophils, especially when comparing young cells of different lineages (Ranzani-Paiva & Silva-Souza, 2004).

Lymphocytes are spherical cells of varying sizes, of which they are predominantly rounded, having a rounded core that follows the shape of the cell and strongly compressed chromatin without any nucleoli. The cytoplasm-nucleus ratio is high, as seen by a very sparse cytoplasm, and is strongly basophilic with no visible granulations (Ranzani-Paiva & Silva-Souza, 2004; Tavares-Dias *et al.*, 2002). These cells are very similar among different species of fish.

When the fish is exposed to an environmental and/ or social stressor, they initiate changes that trigger the hypothalamus by way of the senses, which occurs by activating two neuroendocrine axes: the hypothalamic- sympathetic nervous system - cells chromaffin and the hypothalamus–sympathetic nervous system–chromaffin tissue, which results in the release of catecholamines that stimulate the release of cortisol and cortisone steroids, of which the main one is cortisol. The action of these hormones (a secondary effect) stimulates the hydrolysis of glycogen reserves in the liver, thus increasing glucose levels in the blood, decreasing muscle proteins, increasing heart rate, and marking the start of a secondary response (Smith, 1982; Perry & Laurent, 1993; Wendelaar-Bonga, 1997).

A tertiary response is marked by a decrease in fish resistance to diseases (Mazeaud *et al.*, 1977), with changes in growth rates and changes in reproductive behavior (Barton & Iwama, 1991).

It is hypothesized that the increase in salinity causes stress, consequentially altering haematological parameters. Thus, the objective of this study was to evaluate the health conditions of tilapia in saline environments, as well as their adaptability to it, through the verification of haematological parameters (percentage of hematocrit, hemoglobin, glucose, total leukocyte and erythrocyte counts).

Material and Methods

Forty fish in groups of five were randomly placed in 50 Liter tanks with an average weight (± SD) of 38.5 g (± 4.3), and were fed with commercial feed (32% protein) for 1 day during their adaptation (24 hours). After this period the fishes were exposed for 15 days on the following salinities: 0, 15, 20 and 35. The salinities were changed gradually. Water flow and aeration were maintained in the tanks throughout the experimental period, and were held at 20°C.

After 15 days of exposition the haematological parameters were evaluated on five fish per treatment. The fish were anesthetized (1.5 g benzocaine: 15L water), and blood samples were drawn from the caudal vein using a tuberculin syringe, rinsed on 3% EDTA. The treatments were distributed according to the following salinities: control (0), 15, 25 and 35.

The hematological parameters were estimated following routine clinical methods (Wintrobe, 1934), the blood samples were used for total count of red blood cells using a Neubauer chamber. The percentage of hematocrit following the methodology of Goldenfarb *et al.* (1971), determined by micro-centrifugation and the hemoglobin concentration was determined by spectrophotometry following Drabkin's method (1949). Leukocyte differentiation was performed in blood extension stained with May-Grünwald-Giemsa-Wright stain according to Jain (1986). Differential counting was performed on a light microscope under immersion (100 x). Two hundred cells were counted to establish percentages for each target cellular, targeted component. The samples Blood glucose was immediately stored in Eppendorf tube and centrifuged 3000 rpm to separate the plasma, which was maintained in freezer (-20°C) until determination of glucose by enzymatic method - glucose oxidase (Laborlab® kit), with absorbance evaluated in a spectrophotometer at 505nm.

The data were analyzed regarding normality distribution using Shapiro-Wilk's test and Levene's test was used for homogeneity of variance (homocedasticity). Seeing as the results were normal and homocedastic, differences between means of treatments were evaluated using a variance analysis (ANOVA) followed by Tukey's multiple comparisons test with a confidence interval of $p<0,05$.

Single and combined exposure to the same concentration of trace elements was compared by the t test ($p<0,05$).

Results

An increasing trend was observed in blood glucose at the higher salinity; that is, there was a significant difference in salinity 35 (Figure 1).

Concomitantly, a decreasing tendency was observed regarding the percentage of hematocrit (Figure 2), however, there was no statistically significant difference.

A decreasing trend was observed in the percentage of hemoglobin from the increased salinity; there was a significant difference in salinity 35 (Figure 3).

Following the tendency to decrease the percentage of hemoglobin, erythrocytes, carrying hemoglobin, also decreased with increasing salinity; there was a significant difference in salinity 35 (Figure 4).

An increase in white blood cell count (Figure 5) was measured with a statistically significant difference in salinity 35.

Table I summarizes our data from the haematological values measured in Tilapia Nile in various situations of stress, being: semi-intensive

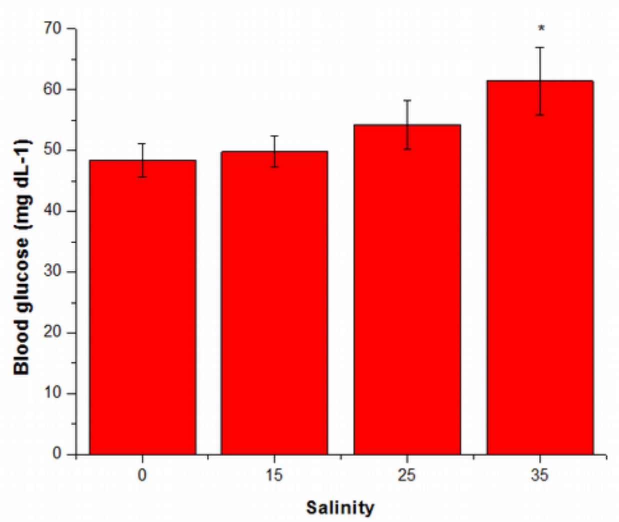

Figure 1. Mean values of glucose in the blood of juvenile tilapia submitted to different salinities. The bars represent the standard deviation. *Indicates statistical difference related to the control group.

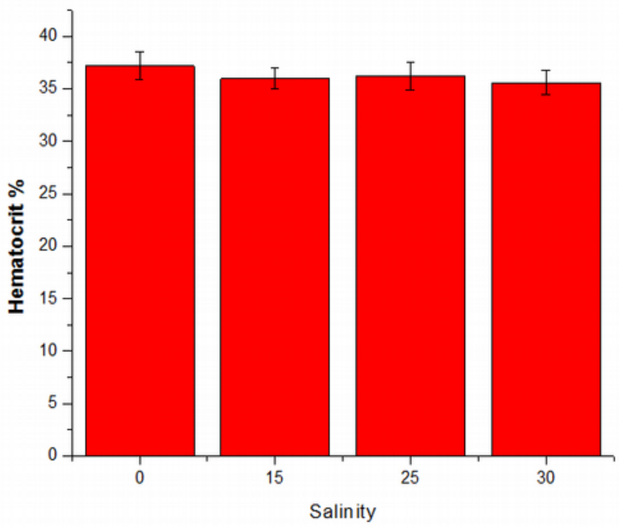

Figure 2. Mean values of hematocrit percentage in the blood of juvenile tilapia submitted to different salinities. The bars represent the standard deviation. There was no statistical difference between treatments.

cultivation in excavated ponds, super-intensive in net cages and fishing ponds.

Discussion

According to Barton (2002), the stress response is considered an adaptive mechanism that allows the fish to face or perceive the stressor, while maintaining its normal state. Stress can be considered a threat to homeostasis which is established by a complex set of adaptive responses. If the intensity of the stressor is overly severe or prolonged, the physiological response mechanisms may be compromised, thus becoming harmful to the

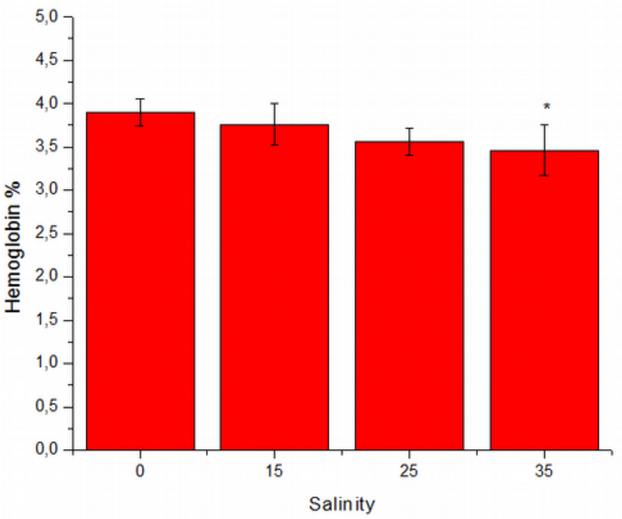

Figure 3. Mean values of hemoglobin percentage in the blood of juvenile tilapia submitted to different salinities. The bars represent the standard deviation. *Indicates statistical difference related to the control group.

Figure 4. Mean values of the total count of erythrocytes in the blood of juvenile tilapia submitted to different salinities. The bars represent the standard deviation. *Indicates statistical difference related to the control group.

animal's health and welfare.Fish raised, especially in intensive production conditions, are under prolonged stress and the physiological response in these circumstances affect energy-dependent processes like reproduction, growth, and disease resistance. The main mortality factors in fish farming are fish diseases, and natural immunity plays an important role in disease resistance (Wedemeyer, 1996; Roed *et al.*, 2002; Cnaani *et al.*, 2004; Hoeger *et al.*, 2005).

It is possible to establish a relationship between fish health and the environment by

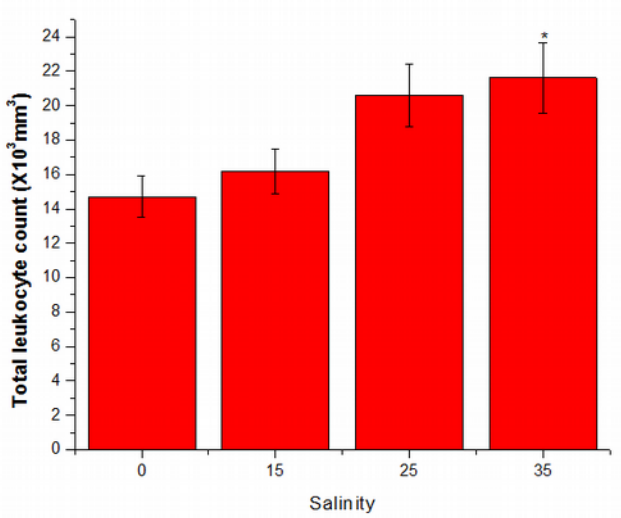

Figure 5. Mean values of total count of leukocyte in the blood of juvenile tilapia submitted to different salinities. The bars represent the standard deviation. *Indicates statistical difference related to the control group.

observing the impact on homeostasis (Tavares-Dias & Moraes, 2004). One way of measuring this is by observing the fluctuations of hematological values in the fish caused by factors like salinity.

The biochemical composition of plasma and blood serum is considered an important tool in the diagnosis of fish health. Through this analysis, it is possible to detect alterations in the functioning of organs and animal adaptation in the face of physiological challenges and metabolic imbalances (González & Scheffer, 2002).

Among the analyses of the biochemical composition, blood glucose level stands out. Hyperglycemia is a good indicator of physiological stress (Wedemeyer *et al.*, 1990) and changes are easily detected by means of easy-to-use glucose meters (Simões & Gomes, 2009).

The results of this study show that juvenile Nile tilapia exposed to more intense salinities (ex. salinity 35) result in a secondary stress response associated with adjustments in energy metabolism, thus, inducing the mobilization of energy between osmoregulatory organs (e.g., gills and intestines) and a non-osmoregulatory organ (e.g., liver) (Arjona *et al.*, 2009; Tseng & Hwang, 2008; Fiess *et al.*, 2007).

During stress, neurohormonal stimulation occurs as the primary response, resulting in increased levels of corticosteroids and catecholamines (Karsi & Yildiz, 2005). After this, a series of physiological changes occur (secondary responses) which are characterized, according Braun *et al.* (2010) and Baltzegar *et al.* (2014), as the increasing demand of energy supplied by hyperglycemia through activating glycogenolysis / gluconeogenesis, inhibiting glycolysis, and increasing hepatic glycogenesis; processes that result in the production of glucose (Montero *et al.*, 1999).

Corroborating this study, Sarma *et al.* (2013) observed hyperglycemia in *Clarias batrachus* exposed to salinity 4 when compared to the control group. Jeanette *et al.* (2007) reported a significant increase in glucose plasma levels in *Oreochromis mossambicus* with the increase of salinity. These authors attributed the hyperglycemic response to stress and / or increased energy demand to the maintenance of the hydro-mineral balance.

The hematocrit value analyzed is a parameter that reflects the percentage of erythrocytes in the blood relative to the amount of white blood cells (leukocytes), platelets and blood plasma (Ranzani-Paiva *et al.*, 2013). A decreasing tendency in the hematocrit percentage, as observed in this project in all salinities in relation to the control group, may be related to stress, which could lead to osmotic inflow of water and consequent hemodilution (Martinez *et al.*, 2006). Farrell & Robertson (2002) also associated a reduction in hematocrit caused by increased salinity, with changes in metabolism and cardiac depression, leading to decreased blood volume.

Table I. Compilation of data of hemograms in *Oreochromis niloticus* submitted to different environments and treatment. Hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), total count of erythrocytes (TCE).

Treatment	Glucose (mg/dL)	Hb (g/dL)	Hct (%)	MCHC (g/dL)	TCE ($x10^3$ mm^3)	Author
Salinity 0	48.4 ± 2.7	3.9 ± 0.16	48.4 ± 2.70		2.65 ± 0.11	Present work
Salinity 15	49.8 ± 2.58	3.76 ± 0.24	49.8 ± 2.56		2.62 ± 0.16	Present work
Salinity 25	54.2 ± 3.96	3.56 ± 0.15	54.2 ± 3.96		2.54 ± 0.08	Present work
Salinity 35	61.4 ± 5.59	3.46 ± 0.28	61.4 ± 5.59		2.34 ± 0.09	Present work
Salinity 0		8.5 ± 1.9	30.6 ± 5.0	28.9 ± 9.7		Tavares-Dias & Faustino (1998)
Salinity 0		7.0 ± 0.4	27.8 ± 1.6	27.8 ± 1.7		Ueda *et al.* (1997)

The decreasing tendency in hematocrit percentage was accompanied, in the present project, while the total count of erythrocytes decreased in the Nile Tilapia of the group exposed to salinity 35. This occurred because the fish passively lose water during their initial exposure to a hyperosmotic environment, and, subsequently, there is a compensatory increase in water intake resulting in a transient dilution of blood parameters (Martinez-Alvarez *et al.*, 2002; Emelike *et al.*, 2008; Pereira *et al.*, 2016).

This possible compensation of water intake was not observed by Plaut (1998), in the species *Salaria fluviatilis* and *Salaria pavo*, where an increase in hematocrit was observed with increasing salinity. The author attributed the alteration of the environmental salinity with changes in the water content in the blood, since the fish in hypersaline environment tends to lose water.

In our study, an increase in leukocyte count was observed. Since leukocytes are sensitive to stress, they both may be related to the stress hormone levels (Davis & Maerz, 2008) and may reflect the overall immune state, thus, leukocyte profiles are particularly useful in the field of conversation physiology (Ranzani-Paiva & Silva-Souza, 2004; Barbieri & Bondioli, 2013).

The data from this study show a possible stimulation of the immune system before the stress. This is related to the activation of the sympathetic nervous system and release of catecholamines in which the blood cells, including leukocytes, are deployed as part of the acute response. Changes in the number of leukocytes in the blood are characterized, probably, by increasing the number of neutrophils (Tort, 2011).

The number of white blood cells may also be affected by a variety of physiological and environmental factors (Witeska, 2005). Studies show that stimulating the immune system causes an increase in the number of white blood cells in fish exposed to Roundup (Modesto & Martinez, 2010) and mercury (Maheswaran *et al.*, 2008).

In addition to this, there was a decrease in the percentage of hemoglobin in the Nile Tilapia exposed to salinity 35, suggesting a loss of oxygen transport capacity in the face of stress. These results corroborate Rani *et al.*'s (2016), which reported a decrease of hemoglobin in *Labeo rohita* exposed to higher salinities (6 and 8), consequentially reducing feeding due to physiological stress.

Similar results were also observed after juvenile and adult *T. guineans* were acclimated to 15 salinity (Akinrotimi *et al.*, 2012).

Whereas the present study was carried out at a temperature of 20°C due to ambient cooling and knowing that fish metabolism is directly related to temperature, further studies evaluating the correlation of salinity with the tested parameters at different temperatures are recommended.

Conclusion

Hematological parameters alter the extent to which salinity is increased, thus indicating physiological changes during salinity adaptation. The mean values of blood glucose, of hemoglobin percentage, of erythrocyte and leukocyte total counts were different only between zero salinity and the salinity of 35. The percentage of total hematocrit showed no statistical difference in the treatments.

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