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Histological Changes in the Gills and Digestive System of Tilapias (*Oreochromis niloticus*) Raised in Eutrophic Environments

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ABSTRACT

According to experts in environmental pollution and ichthyology, the study of the liver and other structures such as gills and intestines are recognized as valid and fast methods for evaluating the consequences of water resources contamination in fish. Since the high levels of N and P increase the depletion of oxygen in farming systems, causing changes in the fish gills followed by episodes of massive death and liver changes, this study aims to evaluate the histological changes caused by direct fish exposure in eutrophic environments. The results have indicated that higher fertilization (of the mesocosms leads to a bigger total weight and liver weight in the Nile tilapia, the lower hepatocyte vacuolization, higher energy allocation to growth, increased intestinal lumen with larger villi and goblet cells number, as well as higher intestinal absorption. Since fish from fertilized mesocosms have not shown parasitic infestation on gills, it may indicate that a mesocosms fertilization can also decrease the parasitic action in the Nile tilapia. The histological changes, as well as pathological lesions observed in this study indicates that fish have responded to episodes of stress, regarding the eutrophic environments, with low oxygen levels, lack of renewal of the water flow and organic pollution.

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INTRODUCTION

The tilapias are native fish from Africa, introduced in different continents and currently are found in commercial breeding in almost 100 countries. Due to its features and physiology, reproductive biology, genetic plasticity, development of domesticated lineage and commercialization facility are located in the forefront of the world aquaculture (Fitzsimmons, 2000).

With the fish-farming expansion in Brazil from the 80 decade, it has been observed a growing interest by breeders regarding the economic losses caused by fish mortality. In the culture tanks there must be balance among the host health, the proliferation of pathogen agents and the water environment conditions. This way, the bad water quality, the reduction of dissolved oxygen, sudden temperature changes, high fish density, inappropriate handling or unbalanced nutrition are factors able to cause stress to animals, predisposing them to different bacteria, fungi and parasitic infections (Lim *et al*., 2005).

According to Plumb (2001) and Lima and Leite (2006), parasitic diseases are among the most frequent problems in the aquaculture, jeopardizing the fish performance, besides spreading pathogenic agents to the environment, causing losses to producers and risks to public health.

According to experts in environmental pollution and ichthyology, the study of the liver and other structures (intestine and gills) have been recognized as fast methods and valid to verify the consequences of the water resources contamination in fish (Santos, 2003). Since the high levels of N and P increase the oxygen depletion in farming systems, causing changes in the fish gills followed by episodes of massive death and liver changes, this study aims to evaluate the histological changes caused by direct fish exposure in eutrophic environments.

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MATERIAL AND METHODS

Animals and tanks:

This study has been performed with the Nile tilapia (*Oreochromis niloticus*) in the fingerlings stage (30 days) and developed in the fish-farming department of the Agricultural Science Center of the Federal University of Paraíba State (6° 57' 46" S, 35° 41' 31" O), 618 m. height.

The experiment was carried out during 5 months (January to May 2010) in fish nurseries tanks excavated in natural terrain measuring 10 x 50 m^2 and approximately 1.5 m deep, using mesocosms built with transparent polyethylene bags (0.2 mm thick) and aluminum round frames (1.0 m diameter), besides support structures to fix the buoys (plastic bottles), following the recommendations of Arcifa and Guagnoni (2003). The meso-environments remained open at the top 30 cm above the water surface and bottom (1 m deep) was closed, so there was no contact with the sediment.

The fish specimens were randomly separated in groups according to Table 1. After the growth period (150 days) and death due the environmental conditions (120 days), the animals have been threaded and liver, gills and intestine samples have been taken. The samples for light microscopy have been dissected with maximum diameter of 5 mm. The material fixation was made with Metacarn (60% methanol; 30% chloroform and 10% acetic acid) during 12 hours. The inclusion of the material was made in paraplast for morphology through pattern histological coloration.

To verify the morphological aspects were used the hematoxylin-eosin (HE), Schiff periodic acid (PAS) and trichrome of Masson stain. The analysis of the slides was performed through microscope Olympus BX 60, coupled to camera Axio CAMHRc with Software Zeiss KS 400.

Statistical analysis:

In the statistical analysis, it has been compared the average of the 3 groups (treatments 1, 2 and 3) applying ANOVA and after the Tukey test. The results were considered as significant to the level of 5% (p = 0.05) (GraphPad Prism 5).

Experimental design and light microscopy:

Table 1: Description of the treatments performed in the mesocosms in which were allocated the Nile tilapias (Areia, Paraíba – Brazil).

TREATMENT	DESCRIPTION
Treatment 1 (T_1)	Control (5 fish specimens)
Treatment 2 (T_2)	15,32g of Simple Superphosphate + 5,11g of Ammonium sulfate (5 fish specimens)
Treatment 3 (T_3)	15,62g of Simple Superphosphate + 5,21g of Ammonium sulfate (5 fish specimens)

Results:

The animals weight, as well as the liver weight in T_3 was higher significantly than T_1, being T_2 equal ($p<0.05$) to other groups. While the relation liver/total weight and the weight of the gutted animals, despite higher in T_3, was not significantly different among the three groups (Figure 1).

In the animals from the control treatment (witness) (T_1) the hepatocytes have presented themselves as big cells, with big and round nucleus with evident nucleolus and vacuolated cytoplasm. The vacuoles have shown lipid droplets or reservation energetic material. In the animals' livers in treatments T_2 and T_3, the hepatocytes were lower and less vacuolated when compared to the hepatocytes of the tilapias in T_1 (Figure 2).

The histological analysis of the animal's liver in T_3 have demonstrated that the liver of these animals were less positive to PAS (less glycogen in the liver) than the animals in T_1 and T_2. It has been verified, also, higher positivity in the areas of hepatic veins and door veins than the ones in the bile duct (Figure 3). In the animals which had died, due the oxygen deletion, after 120 days of experiment, in the environments with treatments T_1, T_2 and T_3 was verified that the liver of these ones also was positive to PAS in the same level of the animals from T_1 and T_2 after fishery. In the liver of these animals which had died, was observed dilated and congested sinusoids, hepatocytes with irregular shape and massive hepatocyte death, denoting hepatic failure.

The intestine of the animals in T_2 and T_3 had bigger lumen than those from T_1, being those from T_2 intermediate. The animals from the meso-environments with T_3 had presented more villus and villi and had more goblet cells in their extension. Moreover, the copies submitted to this treatment had presented bigger cells and with bigger amount of secretion (mucin) in each cell, evidenced by the biggest positivity of these cells to the PAS, which color glycoproteins.

In some intestine samples of the copies of T_1 (witness), it has been found epithelial vacuoles. These vacuoles are cysts of parasites (macro-gametes of nematodes).

In T_1, when analyzed the intestines with the coloration of trichrome of Masson, it has been verified that these ones were less colored, the animals intestine in T_3 were the most positives to this coloration (Figure 4).

The histological analysis of the intestines of animals which had died in the different groups, have shown that these have presented less crimps, villus and villi, what would indicate low absorption of nutrients. These intestines also contained few goblet cells, villi with injured epithelium and parasite cysts (Figure 5).

The animals' gills have shown secondary lamellae individualized in the 3 treatments (T_1, T_2 and T_3), however some animals from T_1 have shown parasitic infestation and some animals from T_3 have shown hyperplasia of the secondary lamellae with partial fusion of themselves (Figure 6).

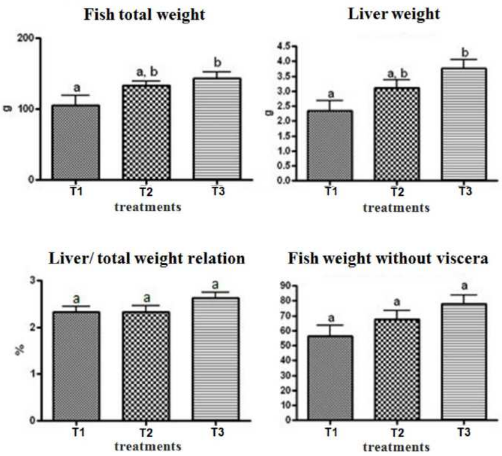

Fig. 1: Graphics of weight, liver weight, relation liver/total weight and gutted weight of the Nile tilapias in T_1, T_2 and T_3.

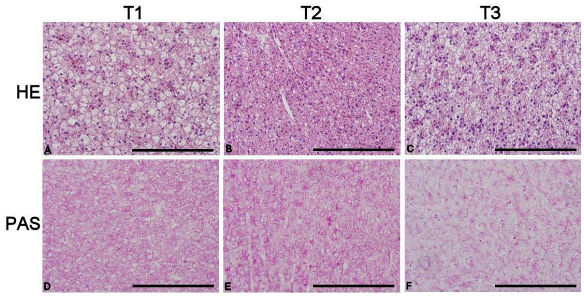

Fig. 2: Photomicrographs of liver of the tilapias in T_1, T_2 and T_3. A, B and C) Visualization of hepatocytes more vacuolated in T_1 than in T_2 and T_3. Hematoxylin-eosin (HE) stain. D, E and F) Visualization of the liver parenchyma demonstrating higher positivity to Schiff periodic acid (PAS) in the animals from T_1 and T_2 when compared to T_3. Schiff periodic acid (PAS) stain. Bar: 200 µm.

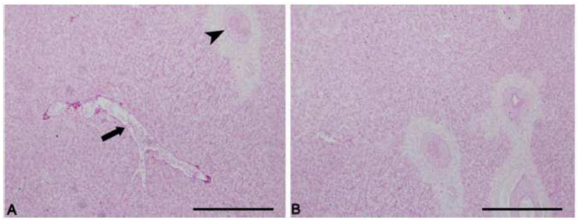

Fig. 3: Photomicrography of liver of the tilapias in meso-environments T_3. A) Visualization of the regions occupied by the bile ducts (less positive to PAS - arrowhead) and portal vein (arrow). B) Visualization of the bile ducts with their adjacent regions less positive to PAS. Schiff periodic acid stain. Bar: 500 µm.

Discussion:

According to environmental pollution and ichtyology experts, the study of the liver and other structures (gills and intestines) may be used to verify the consequences of the water resources contamination in fish (Santos, 2003). This organ is susceptible to pathological processes, target of parasites and neoplasia (Okhiro and Hintol, 2000) specially in fish which live in polluted water (Couch and Harshbager, 1985).

In the liver of the animals in T_2 and T_3, the hepatocytes have shown themselves smaller and less vacuolated than the hepatocytes from T_1. In an experiment involving the partial hepatectomy of the liver of African catfish (*Clarias gariepinus*), these features were observed after the hepatectomy, the moment in which there was a peak of cell proliferation (Santos, 2003). This way, it has been understood that in the liver of the animals from T_2

and T_3, there is greater cell proliferation than those in the control treatment.

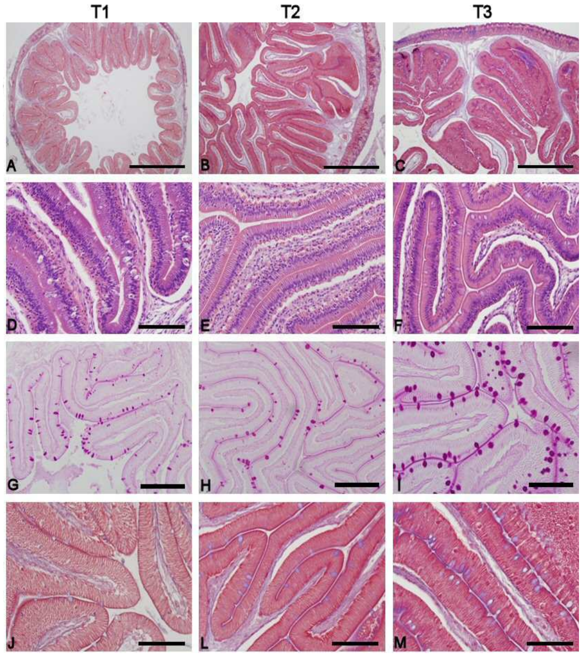

Fig. 4: Photomicrography of the intestine of tilapias in T_1, T_2 and T_3. A, D, G and J) Photomicrography of the intestine of animals submitted to T_1 (Control). B, E, H and L) Photomicrography of the intestine of animals submitted to T_2. C, F, I and M) Photomicrography of the intestine of animals submitted to T_3. Observe that animals from T_1 have the smallest intestinal lumen and present more intestinal cysts. Observe that animals from T_3 have the highest amount of goblet cells in the intestine and have more positive intestine to the thricrome of Masson stain, which may indicate greater absorptive activity. A, B, C, J, L and M) Thricrome of Masson stain. D, E, F) Hematoxylin-eosin stain. G, H and I) Schiff periodic acid stain. A, B, C) Bar: 500 µm. D, E, F, G, H, I, J, K, L, M). Bar: 200 µm.

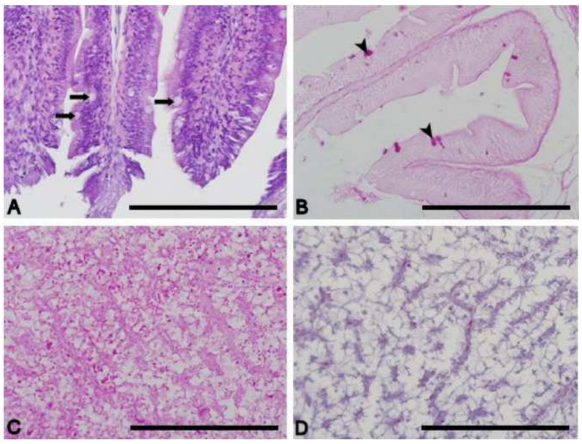

Fig. 5: Photomicrography of representative intestine and liver of tilapias which had died in T_1, T_2 and T_3. A) Visualization of intestine with epithelial injuries and cysts of parasites (arrows). Hematoxylin-eosin (HE) staining. Bar: 200 µm. B) Visualization of the intestine with few goblet cells (arrow head). Periodic acid Schiff (PAS) stain. Bar: 200 µm. C) Visualization of the liver (positive to PAS). PAS stain. Bar: 200 µm. D) Visualization of the vacuolated hepatocytes and massive necrosis. HE stain. Bar: 200 µm.

In the T_3, the liver of the animals is less positive for PAS (less glycogen in the liver). The healthy liver is less positive to the PAS, when compared with a liver with some pathology (Guerra *et al*., 2009). Also in the T_3, it has been verified higher positivity in the areas of hepatic veins and portal veins than the ones in the bile duct. It is noteworthy that, the blood which comes through the hepatic vein to the liver has traveled throughout the digestive tract, so, is a blood richer in nutrients, what would explain the biggest positivity to the PAS next to these regions. Since the positivity to the PAS represents greater accumulation of glycogen, the lower positivity to the PAS in the liver of the animals from T_3 may indicate a destination of the hepatic glycogen supply for the growth of the animals. This result would subsidize the biggest weight reached for the animals in T_3.

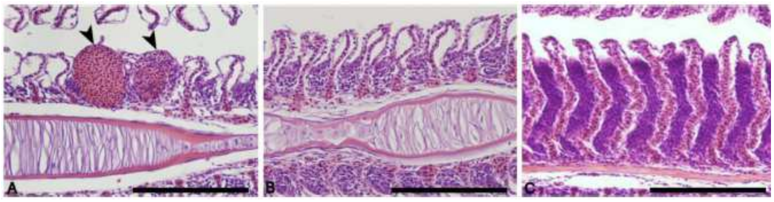

Fig. 6: Photomicrography of the gills of tilapias in T_1, T_2 and T_3. A) Gill of tilapia with parasites (arrow head) among the secondary lamellae. B) Primary and secondary lamellae. C) Secondary lamellae of the gill with lamellar hyperplasia causing partial fusion of the lamellae. Hematoxylin-eosin stain. Bar: 200 µm.

The results achieved also demonstrate a bigger positivity of the goblet cells in the intestine to the PAS, which colors glycoproteins, in the T_3, regarding other groups. This bigger amount of goblet cells and the biggest secretion content makes possible that the alimentary cake pass through the intestinal tract easily avoiding constipations and protecting the intestinal mucosa of damages caused by fasting or pathogenic agents (Gomide-Júnior, 2004).

The fact that animals from T_2 and T_3 present more villi makes these animals have a bigger surface for absorption of nutrients and gain more weight. This bigger absorption surface, mainly found in T_3, may contribute with a higher hepatic metabolism, taking to an increased liver and greater weight gain of the copies, what happened during the observation time (Figure 1).

In some intestines of the animals from the meso-environments T_1 have been found parasites cysts (macro-gametes of nematodes). These findings may support the findings of Gomide-Júnior (2004), which suggest that the biggest intestinal production of mucus (found in T_3) may protect the intestinal mucosa from damages caused by pathogenic agents.

The histological analysis of the animals intestines which had died in different groups have demonstrated intestines with little crimps, villus and villi, which would indicate low nutrients absorption and the presence of parasites, related to the crude protein levels in the ration (Braccini *et al*., 2007). Another factor that favors the reproduction of these parasites is the eggs incubation and the larvae protection of the Nile tilapia which is made in the mouth (El-Sayed, 2006).

According to Garcia-Santos *et al*. (2007), the morphological changes of the gills, in response to environmental changes, can represent adaptive strategies for the conservation of some physiological functions. Thus, the types of histopathological injuries observed in this study, indicate that, fish are responding to the effect of toxic agents present in the water and sediment.

When analyzing the gills of the animals in the different experimental treatments, it has been evidenced that the gills had individualized secondary lamellae, however some animals from T_1 had presented parasitic infestation themselves; and some animals from T_3 had shown hyperplasia of secondary lamellae with partial fusion themselves, a fact that may be related to the presence of parasites, favored by storage and the deterioration in the water quality of the meso-environments (MacPhee, 2001).

The epithelial rise of lamellae may have occurred once this characteristic is one of the first alterations that occur when the animal is under some type of stress. According to studies of Garcia-Santos *et al*. (2007) the lamellar epithelium separates itself from the lamellae, creating a space that may be filled by water, being able to lead to an edema formation. The changes of epithelial rise type may serve as a defense, with the increase of the distance of diffusion between the water and the blood intervening, however, in the efficiency of the gaseous exchanges and ionic transport.

Regarding the parasitic infestations, studies of Zanolo and Yamamura (2006) reports that these infestations occur more frequently in intensive tilapia fish-farming systems. These processes are related to eutrophic environments, with bad water quality, low oxygen contents, lack of renewal and organic pollution in the tanks with high cultivation densities (Arana, 2004; Martins, 2004).

The severity of the injuries caused by parasites depends on several factors like the group of the mentioned parasite, its location and the particular way how they act on the hosts, being the gills injuries particularly important, once this organ reacts substantially to the presence of parasites causing, thus, a marked cell proliferation implying in the reduction or loss of the respective respiratory activity, which, in the most serious cases, it may cause the death of the host by asphyxia (Zanollo and Yamamura, 2006), fact occurred in some units after the 76^{th} day of experiment. However, the fact that animals from T_2 and T_3 do not show parasitic infestation may indicate that a fertilization of the mesocosms can diminish the parasitic action in the Nile tilapias.

Conclusions:

The histological changes occurred in the fish had happened in response to the environmental changes, it may be related with the adaptive strategies for conservation of some physiological functions. The analysis of the histopathological injuries observed in this study indicates that fish had responded to the episodes of stress, regarding the eutrophic environments, with low oxygen levels, lack of renewal of the water flow and the organic pollution.

The experimental studies with mesocosms have shown themselves as relevant in the evaluation of the artificial eutrophication on the phytoplankton growth and histological changes in fish, providing subsidies for the conservation of aquatic ecosystems, predominant in the semi-arid landscape and vulnerable to the anthropogenic action.

REFERENCES

Arana, L.V., 2004. Fundamentos de aqüicultura. USFC.

Arcifa, M.S., W. Guagnoni, 2003. A new model of enclosure for experiments in lentic water. Acta Limnol. Brasil., 15(1): 75-79.

Braccini, G.L., L. Vargas, R.P. Ribeiro, R.M. Takemoto, M.A.P. Lizama, V.M. Fülber, 2007. Ectoparasitos de tilápia do Nilo (*Oreochromis niloticus*), das linhagens Chitralada e GIFT, em diferentes densidades e alimentadas com dois níveis de proteína. Acta Scientiarum: Animal Sciences, 29(4): 441-448.

El-Sayed, A.F.M., 2006. Tilapia culture. CABI Publishing.

Fitzsimmons, K., 2000. Tilapia: the most important aquaculture species of the 21st century. In: International Symposium on Tilapia Aquaculture (Ista). ISTA, 1: 3-8.

Garcia-Santos, S., S.M. Monteiro, J. Carrola, A. Fontainhas-Fernandes, 2007. Alterações histológicas em brânquias de tilápia nilótica *Oreochromis niloticus* causadas pelo cádmio. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 59(2): 376-381.

Gomide-Junior, M.H., E.V. Sterzo, M. Macari, I.C. Boleli, 2004. Revista Brasileira de Zootecnia, 33: 1500-1505.

Guerra, R.R., M.R. Trotta, O.M. Parra, J.L. Avanzo, A. Bateman, T.P.A. Aloia, M.L.Z. Dagli, F.J. Hermandez-Blazquez, 2009. Modulation of extracellular matrix by nutritional hepatotrophic factors in thioacetamide-induced liver cirrhosis in the rat. Brazilian Journal of Medical and Biological Research, 42: 1-8.

Lima, L.C., R.C. Leite, 2006. Boas coletas garantem bons diagnósticos. Panorama da Aqüicultura, 16(96): 24-29.

Lim, C., M.Y. Aksoy, P.H. Klesius, 2005. Nutrition, immune response and disease resistance in fish. In: Simpósio de Nutrição e Saúde de Peixes. UNESP, 1:46.

MacPhee, D., 2001. Monogenean (fluke) infestations of the gills of farmed salmon in Maine and New Brunswick. In: Annual New England farmed fish health management workshop. New England Farmed fish health management.

Martins, M.L., 2004. Cuidados básicos e alternativas no tratamento de enfermidades de peixes na aqüicultura brasileira. In: Sanidade de organismos aquáticos. Liv. Varela.

Plumb, J.A., 2001. Overview of warm-water fish diseases. In: Lim, C., C.D. Webster (Ed.). Nutrition and fish health. Food Product Press.

Santos, N.P., 2003. Regeneração hepática em bagre africano (*Clarias gariepinus*) após hepactectomia parcial. M. S. thesis, Universidade de São Paulo, Pirassununga, Brazil.

Zanolo, R., M.H. Yamamura, 2006. Parasitas em tilápias do nilo criadas em sistema de tanques-rede. Semina: Ciências Agrárias, 27(2): 281-288.