

## ORIGINAL PAPER

# Morpho-functional response of Nile tilapia (*Oreochromis niloticus*) to a homeopathic complex

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**Background:** This study evaluated the performance, prevalence of ectoparasites and morpho-functional response of the liver and the branchiae of Nile tilapia (*Oreochromis niloticus*) raised on fish meal with added of the homeopathic complex *Homeopatia 100*<sup>®</sup> at different concentrations.

**Methods:** Post-reversed juvenile Nile tilapia (*O. niloticus*) of the GIFT (Genetic Improvement of Farmed Tilapia) strain were used in this study. The performance, ectoparasite prevalence and parasite load in the branchiae and skin as well as the liver and branchial histology. Fish were randomly assigned to receive one of four treatments: control, 20 mL hydroalcoholic solution (alcohol 30° GL); 20 mL *Homeopatia 100*<sup>®</sup> per kg of meal; 40 mL *Homeopatia 100*<sup>®</sup> per kg of meal; or 60 mL of *Homeopatia 100*<sup>®</sup> per kg of meal, compared to control with out the addition of the complex. There were four replications per treatment type (16 experimental units total) at a density of 40 fish per m<sup>3</sup> over a period of 57 days. The Kruskal–Wallis *H* test ( $p < 0.05$ ) was employed to analyse the physical and chemical parameters of water as well as for parasite prevalence; whereas analysis of variance was used for liver performance. If the values were significant ( $p < 0.05$ ), they were compared by Tukey's test. Multiple comparisons of averages were performed using Student's *t* test ( $p < 0.05$ ).

**Results:** There were no significant between the physical and chemical parameters of the water between the different groups at the end of the experiment. Significant differences ( $p < 0.05$ ) in the mixed parasite conditions were found within the different *Homeopatia 100*<sup>®</sup> treatments. The hepatosomatic ratio of fish treated with *Homeopatia 100*<sup>®</sup> was significantly lower than that of fish from the control group. The best results in the liver and branchiae occurred in fish receiving *Homeopatia 100*<sup>®</sup> at 40 mL/kg in terms of the number of hepatocytes/mm<sup>2</sup>, the intercellular glycogenic behaviour, the rates of histological changes (hyperplasia, lamella fusion and telangiectasia) and the percentage of neutral and acidic mucin-producing cells.

**Conclusion:** The addition of *Homeopatia 100*<sup>®</sup> at a concentration 40 mL per kg/meal to the diet of juvenile Nile tilapias resulted in improved hepatocytes and intracellular glycogen levels as well as the lowest mean rate of branchial histological changes with an increase in acidic mucin-producing cells compared to neutral mucin-producing cells, compared to control. *Homeopathy* (2013) 102, 233–241.

**Keywords:** Homeopathy; Population homeopathy; Aquaculture; Tilapia farming; Performance; Histopathology

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## Introduction

During the last twenty years, aquaculture in Brazil increased rapidly primarily through tilapia breeding. Tilapia represent approximately 50% of fish production in the country. Brazil has enormous aquaculture potential due to temperature conditions and its excellent hydrographic resources.<sup>1</sup>

The environment is a recurring theme in discussions related to water resources, with special references to fish. The latter are highlighted due to their economical, breeding and ecological aspects.<sup>2</sup> The sustainable use of underground water is important, since surface water has been degraded by numerous agents, including agricultural poisons, chemical fertilisers and veterinary drugs. These agents may have unknown consequences on human and animal health.<sup>3</sup>

*Oreochromis niloticus*, the Nile tilapia, is affectionately dubbed 'the aquatic chicken' for its rapid and efficient growth. This species fish grows rapidly and is undemanding with respect to habitat and diet. They occupy a low position on the food chain such that there is little opportunity for mercury to build up in their flesh – as is the case for certain predatory species – and their flavour is good.<sup>4–6</sup>

The intensification of fish breeding brought about complications related to management stress,<sup>7</sup> inefficient health and producers' lack of information with regard to health risks.<sup>8</sup> Fish became liable to several illnesses, especially parasitic diseases, due to either the lack or negligence of prophylactic measures, with the consequent propagation of microorganisms that can cause stress, host–parasite–environment imbalance, performance decrease and high mortality rates.<sup>9</sup>

Research in the histopathology of organs that process fish metabolism such as the branchiae<sup>10</sup> and the liver<sup>11</sup> is highly relevant in investigations on the Nile tilapia. Because research has already reported on the pathologies caused by vitamin deficiencies, structural and cellular changes are excellent biomarkers after exposure to chemical agents that may cause a biochemical response and modifications in enzyme kinetics.<sup>11</sup> These changes can also be used in the evaluation of morphological lesions that may interfere in fish health and performance.<sup>12</sup>

## Material and methods

### Study site and period

The experiment was performed at the fish breeding station of the State University of Maringá (UEM–CODAPAR) in the district of Floriano, Maringá, PR, Brazil (23°31'25" S; 52°03'12" W) for 57 days. Experiments performed with animals were approved by the Committee for Ethical Conduct Animal Experimentation of the State University of Maringá (Protocol no. 037/2008).

### Animals, installations and feeding

Six hundred and forty sex-reversed juvenile Nile tilapia (*O. niloticus*) of the GIFT (Genetic Improvement of Farmed Tilapia) strain were used, having a mean initial

weight and length 44.0 g ( $\pm 7.9$ ) and 13.1 cm ( $\pm 0.8$ ), respectively. They were provided by a commercial fish breeding farm. The experimental installation was comprised of a chamber with 16 randomly distributed 1000 L glass-fibre boxes with 40 animals each and 160 animals per treatment. Water renewal rate was 30% vol./day with a constant aeration system and water siphon per box that was cleaned three times per week to remove accumulated organic matter.

The control treatment as well as three concentrations of the homeopathic complex *Homeopatila 100*<sup>®</sup> were administered as follows: T1 (control), 20 mL hydroalcoholic solution (alcohol 30° GL); T2, 20 mL; T3, 40 mL; and T4, 60 mL of *Homeopatila 100*<sup>®</sup> per kg of meal, with four repetitions for each treatment. The homeopathic complex was prepared by Real H Homeopatia (RealH Homeopatia, Campo Grande, MS, Brazil). Table 1 shows its composition. The complex was mixed into the meal, and left to dry in the open air for 24 h until it lacked any smell of alcohol. The meal was stored in a well-ventilated area in the shade away from chemical products and magnetic field equipment.

The meal was provided daily in three portions (9 h, 13 h, 17 h) corresponding to the nutritional requirements for the species.<sup>13</sup> Fish juveniles were fed on a commercial extruded meal (2.5 mm diameter, 42% crude protein (CP)) during the first 28 days followed by the same commercial meal (5 mm diameter, 32% CP) until the end of the experiment.

### Physical and chemical parameters of water

The mean values of the physical and chemical parameters of water, temperature, pH, dissolved oxygen and electrical conductivity were measured three times per week and verified twice per day at 9 h and 16 h prior to siphoning and after feeding. The means were then calculated.

### Parasite analysis

The first calculation of the presence of ectoparasites was undertaken upon initiation of the experiment by sample collection from 100 fish specimens. The specimens were first anaesthetised with benzocaine (1 g/10 mL alcohol/10 L water),<sup>14</sup> and a scraping test was performed on the first branchial arch and the dorsal region of the left side of each specimen. After 30 days and at the end of the experiment on the 57th day, parasite samples of eight fish specimens were repeatedly collected (32 per treatment). The specimens' biometric data were also recorded.

**Table 1** Composition of the complex *Homeopatila 100*<sup>®</sup>

Compound	/1000 mL
<i>Iodum 12cH</i>	250 mL
<i>Sulphur 30cH</i>	250 mL
<i>Natrum muriaticum 200cH</i>	250 mL
<i>Streptococcinum 30cH</i>	250 mL
Medium (ethyl alcohol 30° GL)	Q.s.p

Source: REALH Homeopatia – Brazil.

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