

## ORIGINAL PAPER

# Effects of a homeopathic complex in Nile tilapia (*Oreochromis niloticus* L.) on performance, sexual proportion and histology

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**This study aimed to evaluate the effects of the homeopathic complex *Homeopatila RS*<sup>®</sup> (REAL Homeopathy, Brazil), in the performance, sexual proportion and gills and liver histology of the Nile tilapia fish (*Oreochromis niloticus* L.). 4,800 post-larvae were treated for 45 days, in 12 tanks (500 L), in a closed environment, with density of 400 larvae per tank. Three treatments were given: alcohol 30%, negative control (C); hormonal, positive control, 17 $\alpha$ -methyltestosterone (H); homeopathic, *Homeopatila RS*<sup>®</sup>, in ethanol (HH), with four replications. Mean values for length, weight, liver somatic index, condition factor, survival rate, average values of histological alterations and sexual proportion were determined. Analysis revealed significant differences ( $p < 0.05$ ) in growth, survival rate, liver somatic index and average values of hepatic histological alterations between treatments. It was concluded that the addition of *Homeopatila RS*<sup>®</sup> to the diet of Nile tilapias, during the phase of gonadal differentiation, did not induce any alteration in the sexual proportion. Homeopathically treated fish were significantly smaller, but had significantly greater survival than the other two groups, there was no significant difference in final total biomass. The homeopathically treated fish had a lower liver/somatic index with less hepatic lipid inclusions than the other groups. *Homeopathy* (2008) 97, 190–195.**

**Keywords:** Nile tilapia; homeopathy; liver somatic index; survival; histology; sexual proportion

## Introduction

The fish species Nile tilapia (*Oreochromis niloticus* L.) has potential for fish farming. In recent years, several techniques have been used to produce totally male populations since they have a superior growth rate compared to females and fewer present reproductive problems, for instance, precocious sexual maturity with periodic asynchronic

spawning, causing overpopulation in the cultivation tanks and reduction in growth rate.<sup>1</sup>

The incorporation of steroid hormones in fish feeds is widely practised, with the aim of establishing monosex male populations. But the method is inconvenient, it is labour intensive and involves direct manipulation of hormones by employees, environmental residues, and reduced fish survival rates.<sup>1,2</sup>

A world appeal for environment conservation and growing consciousness on toxic residues, has driven the search for new approaches using less artificial chemical products.<sup>3</sup>

Homeopathy stimulates the vital energy of the organism.<sup>4</sup> Animal homeopathy, is now being used in the treatment of herds, using a principle that considers the herd as an animal unit, therefore, subjected to the same laws that govern individual homeopathy. Homeopathy has been used in animals, not only to treat infections, but also as

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Received 4 July 2007; revised 27 June 2008; accepted 5 August 2008

prevention against diseases and for the modulation of organic responses, mainly in those related to stress.<sup>5</sup>

There are few papers published using homeopathy in aquatic organisms. For instance, Guedes *et al.*<sup>6</sup> using a biotherapy derived from frog's thyroid gland (1:10<sup>25</sup>), slowed alteration in metamorphosis rates.

Our hypothesis was that the addition of the complex containing a homeopathic preparation of iodine, carp hypophysis extract and extract from tilapia testes would induce alteration in the sexual proportion (masculinization), of the Nile tilapias. Regarding to the other parameters investigated no hypotheses were formulated.

The aim of the present experiment was to evaluate the performance, the gonadal response, gills and liver histology of the Nile tilapias, during the phase of gonadal differentiation, with the use of the homeopathic complex *Homeopatila RS*<sup>®</sup> (REAL Homeopathy, Brazil), compared to 17 $\alpha$ -methyltestosterone (positive control) and alcohol 30% (negative control), added to the fish feed.

## Material and methods

### Animals and experimental conditions

4800 Nile tilapias post-larvae (*Oreochromis niloticus*) were used. The fish originated from spawning in earth ponds, the initial total length average was 0.82  $\pm$  0.04 cm. They were distributed at random in 12 blue polyethylene tanks containing 500 L of water (400 post-larvae/tank). The tanks were heated, with daily individual of 20% water renewal and constant aeration. The tanks were checked twice daily, to monitor temperature; environment and water. To analyze the water quality, the pH parameters were checked weekly, through digital phmeter, dissolved oxygen (mg/L), ammonia (mg/L) and nitrite (ppm) using ALFAKIT (Alfa Techno Chemistry Company).

### Treatments and diets

Three treatments, shown in Table 1, were used; each with four replications, giving a total of 12 experimental units.

The homeopathic complex was specially prepared for the present experiment by REAL Homeopatia (Brazil) laboratory, with solutions of *Iodum* 12C (10<sup>-24</sup>); carp hypophysis extract 12C (10<sup>-24</sup>) and extract from tilapia testes 30C (10<sup>-60</sup>).

The complex was added to the fish food as a hydroalcoholic solution in ethanol 30% which was sprinkled on the food, then homogenized and air-dried. The food was revolved every 30 minutes, for 24 h, and protected against solar light and against any contact with chemical products, until losing the odor of alcohol. The same process was

performed for the negative control treatment, alcohol 30% was added. For the hormonal treatment 17 $\alpha$ -methyltestosterone was used (positive control); the product was added weekly to the fish food, in the dosage of 60 mg/kg.<sup>1</sup> The commercial compound Anhambi Pisces 40%<sup>®</sup> was the fish food.

The feeding rate was readjusted weekly, according to the total length of the fish sampled.<sup>1</sup> The daily diet was distributed five times/day, at 7:00 am, 10:00 am, 13:00 pm, 16:00 pm, and 19:00 pm. Treatments were given for 28 days, then for a further 16 days, the post-larvae received non-medicated fish food.

### Sanitary condition evaluation

Before the separation of the post-larvae into tanks, 100 post-larvae were collected, weighed, measured and submitted to an external parasitic exam, following the method described by Eiras *et al.*<sup>7</sup> to identify ectoparasites. The occurrence was calculated as being the number of infested hosts/number of examined hosts.<sup>8</sup>

Three fish per tank were collected weekly, 12 fish per treatment, anesthetized with 0.1% benzocaine. The weight and the total length were measured to adjust the feeding rate. The fish were then euthanased, by sectioning the cervical spinal and for the external parasitic exam conducted.

### Biometry

At the end of the experimental period (45 days) the fish were fasted for 24 h then anesthetized, measured, weighed and sacrificed. Then the specimens were immersed in formalin (5%), for gonadal analysis, or buffered formalin (10%) for histological analysis. Samples were measured with a pachymeter and 40 fish were weighed in each replication (160 fish/treatment). Four samples of each replication were collected (16 fish/treatment), to determine the liver somatic index was calculated by using the formula: LSI=(liver weight/total live weight)  $\times$  100.

The final survival rate was evaluated by the difference between the number of animals at the beginning and end, taking into account the fish used for parasite analysis during the period.

### Histological analysis

Tissue specimens such as liver and gill were removed from four fish per replication (16 fish/treatment), treated with buffered formalin (10%), then immersed in paraffin wax for light microscopy.

Sections were cut at 3  $\mu$ m and stained with hematoxylin and eosins (HE). Periodic Acid Schiff reaction (PAS) and hematoxylin were used to detect glycogen deposits within hepatocytes. The sections were analyzed using an image analysis system.

For gill analyses, all filament of the second right gill arch, of each animal were observed. For liver analyses, ten areas close to the center lobular vein were observed using histological cuts. Histological changes were appraised semi-quantitatively,<sup>9</sup> and the severity of lesions classified as: class 1 = no histological alteration; class 2 = moderate and

**Table 1** Treatments used during the period of gonadal differentiation in post-larvae of Nile tilapias (*Oreochromis niloticus* L.)

Treatment	Doses/Kg ration
(C) Alcohol 30% (negative control)	50 mL
(H) 17 $\alpha$ -methyltestosterone (positive control)	60 mg
(HH) <i>Homeopatila RS</i> (homeopathic complex)	50 mL

restricted alteration; class 3 = severe and extensive alteration. On the basis of these data, a Mean Assessment Values (MAV) of histological alterations was given to each animal. The average of MAV was calculated for each treatment based on individual data.

**Gonadal analysis**

Sixty fish from each replication (240 fish/treatment), were sacrificed by sectioning the cervical spinal marrow and preserved in formalin (5%). The removal procedure and gonads identification was done by the aceto-carmin technique (Wassermann and Afonso).<sup>10</sup>

**Statistical evaluation**

Data were submitted to variance analysis and, when significant ( $p < 0.05$ ), were compared by using Tukey's test. Data related to sexual proportion were submitted to Chi-square analysis ( $\chi^2$ ). Values are presented as mean  $\pm$  SEM. The program Statistical Analysis System (SAS)<sup>11</sup> was used. For histological alteration values, the average and the standard error of the mean were calculated, for each group, and were analyzed by Mann-Whitney's test for independent samples. The significance level considered was 5% ( $p < 0.05$ ).

**Results**

**Physiochemical parameters**

The average room temperature and water temperature were, respectively,  $30.53 \pm 4.16^\circ\text{C}$  and  $26.77 \pm 2.81^\circ\text{C}$ . Table 2 shows the water physiochemical parameters during the experimental period.

**Occurrence of ectoparasites**

Before being distributed by treatments, 100% of larvae were parasitized. The ciliate ectoparasite *Trichodina* sp. had 80% occurrence and monogenetic *Gyrodactilus* sp. + *Trichodina* sp. (mixed infection), represented 20% of occurrence. Analyses at one week and up to the end of the experimental showed no ectoparasites in any sampled fish.

**Fish performance**

Data shown in Table 3 demonstrated that, for the group treated with *Homeopatila RS*, the parameters regarding final total length, standard length and final weight were significantly lower compared to other treatments. However, the

**Table 3** Medium values and standard error of the mean regarding the performance of Nile tilapias (*Oreochromis niloticus* L.), submitted to treatments such as: negative control, alcohol 30% (C); positive control, 17 $\alpha$ -methyltestosterone (H); homeopathic complex, *Homeopatila RS*, hydroalcoholic solution (HH), in the gonadal differentiation period

Variable	Treatments		
	C (n = 160)	H (n = 160)	HH (n = 160)
Initial TL (cm)	0.82 $\pm$ 0.04	0.82 $\pm$ 0.04	0.82 $\pm$ 0.04
Final TL (cm)	4.57 $\pm$ 0.69a	4.70 $\pm$ 0.44a	3.82 $\pm$ 0.60b
Final PL (cm)	3.65 $\pm$ 0.57a	3.81 $\pm$ 0.35a	3.03 $\pm$ 0.51b
Final weight (g)	1.81 $\pm$ 0.84a	1.93 $\pm$ 0.61a	1.07 $\pm$ 0.75b
Condition factor	1.75 $\pm$ 0.22a	1.78 $\pm$ 0.26a	1.70 $\pm$ 0.22a
LSI (%)	7.80 $\pm$ 1.63a	7.54 $\pm$ 1.32a	5.48 $\pm$ 1.42b
Survival (%)	54.1a	50.3a	87.8b
*Biomass (g)	1495.06	1484.17	1434.87

TL: total length; PL: pattern length; Condition factor = (Final weight/TL final<sup>3</sup>)x100; (LSI) Liver Somatic Index = (liver weight/liver weight)  $\times$  100; Survival = 100 - Final N (initial N - collected N); \*Total Biomass estimated = [(initial N - collected N)  $\times$  survival (%)]  $\times$  final average weight (g); values followed by different letters indicate significant differences between the groups, survival values by using Chi-square test and others by using Tukey's test, for a 5% significance level.

survival rate was significantly superior to the other groups. The liver somatic index of the *Homeopatila RS* group was significantly lower than the other groups treated. No other significant difference between treatments was observed.

**Histological analysis**

Table 4 shows MAV of the gill histological alterations. The histological analysis of gills demonstrated no significant difference between the treatments. Table 5 shows MAV of the liver histological alterations. The average values, for lipid inclusion in the liver, were significantly lower in the *Homeopatila RS*, group compared to other groups. Figure 1 shows the histological appearances.

**Sexual proportion**

Table 6 shows the results of gonadal analysis. In the group treated with *Homeopatila RS*, the proportion of male gonads was not significantly different from control groups. But both groups show significantly fewer male gonads than the hormonally treated group.

**Discussion**

The average values for temperature, pH, dissolved oxygen, ammonia and nitrite in the tanks remained within

**Table 2** Mean values and standard error of the mean of water physiochemical parameters in the treatments

Parameter	Treatments		
	C	H	HH
pH	6.76 $\pm$ 0.43	6.57 $\pm$ 0.41	6.63 $\pm$ 0.29
Oxygen (mg/L)	6.71 $\pm$ 0.33	6.76 $\pm$ 0.40	6.53 $\pm$ 0.35
Nitrite (ppm)	0.029 $\pm$ 0.009	0.030 $\pm$ 0.010	0.030 $\pm$ 0.010
Ammonia (mg/L)	0.85 $\pm$ 0.37	0.81 $\pm$ 0.32	0.79 $\pm$ 0.29

C = negative control, alcohol 30%; H = positive control, 17 $\alpha$ -methyltestosterone; HH = homeopathic complex, *Homeopatila RS*.

**Table 4** Medium values and standard error of the mean of histological alteration in the gills of Nile tilapias (*Oreochromis niloticus* L.) submitted to treatments, such as: negative control, alcohol 30% (C); positive control, 17 $\alpha$ -methyltestosterone (H); homeopathic complex, *Homeopatila RS*, hydroalcoholic solution (HH), during the period of gonadal differentiation, appraised in MAV

Treatments	Hiperplasy	Lamelar fusion	Telangiectasy
C (n = 16)	1.68 ( $\pm$ 0.60)a	1.25 ( $\pm$ 0.44)a	1.25 ( $\pm$ 0.44)a
H (n = 16)	1.62 ( $\pm$ 0.80)a	1.18 ( $\pm$ 0.40)a	1.18 ( $\pm$ 0.40)a
HH (n = 16)	1.56 ( $\pm$ 0.72)a	1.18 ( $\pm$ 0.55)a	1.00 ( $\pm$ 0.0)a

MAV measured semi-quantitatively, varying from class 1 to 3: class 1 = no histological alteration; class 2 = moderate and punctual alteration; class 3 = severe and extensive alteration; values followed by different letters, indicate significant differences between the groups, according to Mann-Whitney's test, for a 5% significance level.

the range of relative comfort for the species studied, according to Ribeiro,<sup>1</sup> throughout the experimental period. Thus avoiding a possible confounding influence of water quality on the developing and health of the animals. The parasites found at the initial exam are normal in larvae from earth ponds and might cause injury when the environmental conditions favour their reproduction, as in the case of: high stocking density, low quality feeding and/or palatability, as well as in environments with low quality of water, mainly, in relation to temperature, dissolved oxygen and toxic compounds.<sup>12-15</sup> The absence of ectoparasites, in subsequent exams can be attributed to the good quality of the water, evidenced by the analyses of the physiochemical parameters, which were used in the breeding of experimental animals.

There was a significant difference between the treatments ( $p < 0.05$ ), in terms of average final total length, final pattern length and final weight. The fish treated with *Homeopatila RS* showed lower values than other treatments, however, the survival rate of fish treated with *Homeopatila RS* was superior to the other groups. The final density in the homeopathy group might have caused inferior growth. When studying the density effects, in Nile tilapia Vera Cruz and Mair<sup>16</sup> observed that increased stocking density resulted in a reduction of growth rates, weight and survival of animals. According to Popma and Lovshin,<sup>17</sup> 70% to 80% of final survival index in larvae is normal during the phase of sexual reversion of tilapias. The main factor, which affects the

**Table 5** Medium values and standard of the mean of histological discoveries in the liver of Nile tilapias (*Oreochromis niloticus* L.) submitted to treatments such as: negative control, alcohol 30% (C); positive control, 17 $\alpha$ -methyltestosterone (H); homeopathic complex, *Homeopatila RS*, hydroalcoholic solution (HH), during the period of gonadal differentiation, appraised in MAV

Treatments	Lipid inclusion	Leukocyary infiltrated
C (n = 160)	2.12 ( $\pm$ 0.80)a	1.12 ( $\pm$ 0.34)a
H (n = 160)	2.25 ( $\pm$ 0.68)a	1.12 ( $\pm$ 0.50)a
HH (n = 160)	1.43 ( $\pm$ 0.72)b	1.06 ( $\pm$ 0.25)a

MAV measured semi-quantitatively, varying from class 1 to 3: class 1 = no histological alteration; class 2 = moderate and punctual alteration; class 3 = severe and extensive alteration; values followed by different letters, indicate significant differences between the groups, according to Mann-Whitney's test, for a 5% significance level.

survival index of the species, is physiologic stress, especially when the reversion is stimulated by steroid hormones.

According to Martins,<sup>18</sup> the quality of organic response can vary because fish and other animals, when less stressed have superior immune condition and better survival. Adams<sup>19</sup> reported that fish submitted to environmental stress, show an acute response including mortality.

The low quality of the post-larvae in the initial phase, when 100% were parasitized, is an important factor. Different physiologic responses that might have happened in larvae treated with homeopathy might explain the better survival rate.

The homeopathic complex *Homeopatila RS* consists of *Iodum* 12C, carp hypophysis 12C and tilapia testis 30C. An action on the metabolism of the thyroid and its hormones is attributed to *Iodum*.<sup>3</sup> In fish it is present in form of follicles associated to the ventral aorta, it plays an important role influencing the growing of bones, cartilages and muscles, and carbohydrate metabolism, proteins and lipids, activating the sexual cycle events and the maturation of gonads.<sup>20</sup>

Alteration in the frog metamorphosis rates was observed with homeopathically prepared thyroxine (Endler *et al.*,<sup>21</sup> Welles *et al.*,<sup>22</sup> and Weber *et al.*,<sup>23</sup>). Guedes *et al.*<sup>6</sup> observed that the metamorphosis rates were significantly inhibited compared to control.

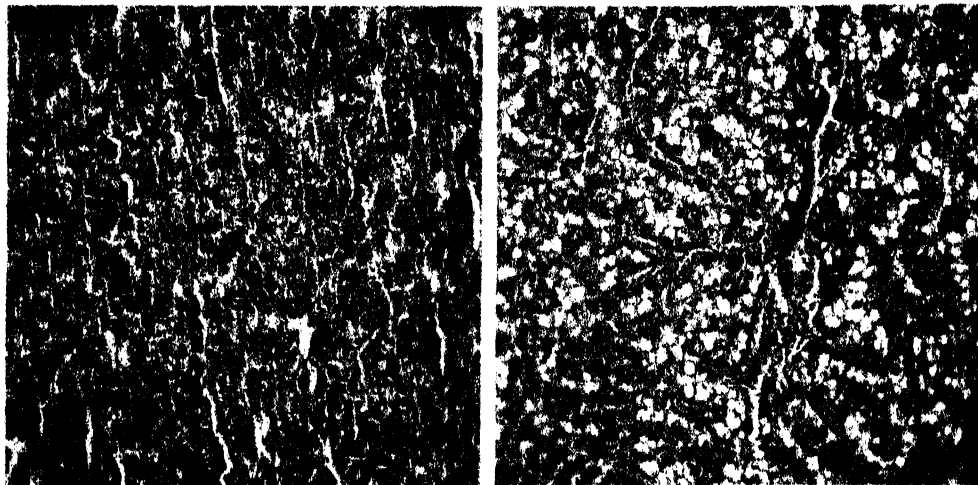
Soares *et al.*<sup>24</sup> studying the reproduction induced in *Brycon cephalus*, observed that the larvae originating from females were treated with carp's pituitary extract and Triiodothyronine (T<sub>3</sub>), showed lower time of birth, initial growth but superior survival indexes, when compared to control.

Studies using tilapia larvae (*Oreochromis mossambicus*), *Chanos chanos* and European eel (*Anguilla anguilla*) immersed in Thyroxine reported increased growth and survival which was dose and species dependent.<sup>2</sup>

Animals treated with *Homeopatila RS*, also showed a liver somatic index significantly lower than other groups (Table 3).

Experiments in tilapias have shown differences in liver somatic indexes, correlated to lipid or glycogen deposition in the organ, depending on the quality and amount of feeding, as well as the phase of development.<sup>25-27</sup> Differences in hepatic metabolism, in animals treated with homeopathy, can be considered as factors that could alter the liver proportion in relation to the corporal weight.

The presence of lipid inclusion and leukocyte infiltrate (Table 5) was observed in the animals' liver, in all studied groups, however, there was a significant reduction in the average values of lipid inclusion in the group treated with *Homeopatila RS* in relation to the control and 17 $\alpha$ -methyltestosterone groups. Studies of the effects of thyroid hormones in the lipid metabolism regulation of *Anabas testudineus* teleost, concluded that such hormones cause a significant reduction of hepatic lipidgenesis, lowering triglycerides, free fatty acids and cholesterol rates.<sup>28</sup> The histological charges found in gills of animals analyzed in this study (Table 4), were cell filament hyperplasia, lamellar fusion and telangiectasia. Although telangiectasiae were not



**Figure 1** Hepatic tissue of Nile tilapia (*Oreochromis niloticus* L.), PAS, 400×. **A:** absence of lipid inclusion, intense glycogen deposition, treated with *Homeopatila RS*; **B:** intense lipid inclusion, larger glycogen deposition in the periphery of the lobular vein center, treated with 17α-methyltestosterone.

observed in the gills of fish treated with *Homeopatila RS*, statistical analysis revealed that there was no significant difference between the groups.

The relatively low frequency of gill lesions in the different groups, in the present work, can be explained by factors such as the physicochemical parameters of the water, which were within values considered ideal for Nile tilapia species, and the good sanitary condition of animals during the treatment period, as revealed by the parasite exams. Lesions found in gills maybe the result of earlier parasitism.

The gill lesions observed in the present study were also described by Leonardo *et al.*<sup>29</sup> in Nile tilapia larvae, treated with vitamin C, during sexual reversion, and the frequency in alterations seen in the present experiment, was similar to the ratio in fish which received vitamin C supplementation compared to the group which did not.

There was no significant effect resulting from homeopathic treatment, on proportions of male and female gonads (Table 5); the percentage of males in this group (52.5%) was lower than those observed by other workers (61.5% and 64.3%)<sup>30,31</sup> when no products were used for performing sexual reversion. With 17α-methyltestosterone treatment, significant differences were observed; the rate of male gonads (95%) was smaller than other published results.<sup>30,31</sup>

**Table 6** Gonadal proportion of Nile tilapia (*Oreochromis niloticus* L.), submitted to treatments such as: negative control, alcohol 30% (C); positive control, 17α-methyltestosterone (H); homeopathic complex, *Homeopatila RS*, hydroalcoholic solution (HH), in the gonadal differentiation period

Treatments	Gonads		
	Male (%)	Female (%)	Intersex (%)
C (n=240)	52.5 b	47.5	0.0
H (n=240)	95.0 a	0.0	5.0
HH (n=240)	52.5 b	45.0	2.5

Values followed by different letters, indicate significant differences between the groups, by Chi-square test, for a level of significance of 5%.

In summary, the addition of the homeopathic complex *Homeopatila RS* to fish food to Nile tilapia (*Oreochromis niloticus* L.), in the phase of gonad differentiation did not cause alteration in the sexual proportion, but was associated with positive effects on the survival of larvae and lower average value for hepatic lipid inclusion compared to the other groups. The mean size of fish in the homeopathic treated group was significantly smaller, but survival significantly higher. These two factors may be inversely correlated. There was no significant difference in final total biomass.

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