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Performance, parasitic infections, hematology and hepatic histology of *Colossoma macropomum* (tambaqui) fed on homeopathic product

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Homeopathic products may act in an organism by stimulating the immune system, allowing the restoration of balance and encouraging organic response under stress. This study investigated the performance, blood, morphological and parasitological parameters in *Colossoma macropomum* (tambaqui) fed diets containing different concentrations of Homeopatila 100®. Juvenile tambaqui underwent four treatments with three replicates: 0, 20, 40 and 60 ml Homeopatila 100®/kg of extruded feed with 32% crude protein for 60 days. At the end of 60 days the growth performance, blood parameters, gill parasites and hepatic histology in fish fed homeopathic product were evaluated. Treatment with Homeopatila 100® did not improve the growth performance of fish. There was no difference in the prevalence and abundance of monogeneans and protozoans in the gills of fish, except in those fed with 60 ml homeopathic product. The plasma glucose levels were higher in fish fed diet containing 40 and 60 ml homeopathic product. The mean corpuscular volume and hematocrit levels in fish fed 20 and 60 ml were higher than in controls. In fish with fed 40 ml increased number of neutrophils and reduced number of lymphocytes was found. However, 60 ml of the product caused increase in the number of monocytes and reduced the number of lymphocytes, eosinophils and PAS-positive granular leukocytes. Under conditions in this study, the Homeopatila 100® did not improve fish performance or reduce parasitic infections, but showed a relative improvement in blood response of fish fed on 40 ml of this homeopathic complex.

Key words: Fish farming, homeopathy, monogenea, parasites, protozoa, blood.

INTRODUCTION

Currently, almost half of the fish production comes from aquaculture in Brazil, but due to demand for products

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based on fish, the production will increase in the coming decades, mainly by socioeconomic and health reasons. The country has a huge potential for expansion of this activity, such as favorable climate, possibility of use of Union's water for fish farming, both reservoirs and estuary, plus a large number of biodiversity species with livestock potential (Rocha et al., 2013). Moreover, it produces native species of great economic interest as *Colossoma macropomum* (tambaqui), a Serrasalmidae from the Amazon River basin.

Tambaqui is the most cultivated native fish in Brazil; its production was 111,084.1 tons in 2011, accounting for 20.4% of total domestic production. This production represents an increase of over 100% compared to 2010 (MPA, 2013). This increase in domestic production is mainly due to its zootechnical characteristics that favor the production of this Amazonian fish, such as rapid growth, relative resistance to diseases and good tolerance to high temperature and to low levels of dissolved oxygen in the water (Araujo-Lima and Gomes, 2005; Santos et al., 2013). It has regular supply of fingerlings and good yield of fillet without skin, its flesh is of good nutritional quality and its production cycle in cages is short, six to eight months (Oliveira et al., 2013).

Growth in fish farming is one of the most important and commonly used criteria to measure the fish response to the diet or ingredients used in the feed. Because growth is the measure of greatest applicability in the production systems to assess the growth performance of cultured fish, once it is closely related to productivity and profitability (Fracalossi et al., 2013), being assessed in different ways. With the intensification of the tambaqui fish farming, the disease problems have increased mainly by the infections of protozoan *Ichthyophthirius multifiliis* and monogenean helminthes, which may impair the production and productivity (Tavares-Dias et al., 2013). Such problems of parasitic diseases require constant need of treatment to reduce and control the parasites during intensive production of this fish. Prophylactic care should be permanent in fish farming of tambaqui, due to the difficulty of treating infectious and parasitic diseases when installed. Homeopathic products may act in the body of animals by stimulating the immune system, allowing the restoration of balance and encouraging organic responses in reducing stress. The use of such products, besides contributing to the prophylaxis by reducing the management stress, can reduce the use of chemotherapy and antibiotics, avoiding risks to the environment, animals and consumers (Siena et al., 2010). However, the use of homeopathic products and their potential benefits are virtually unknown in fish farming.

In *Oreochromis niloticus* (Nile tilapia), 40 ml Homeopatila 100®/kg diet improved the survival of fingerlings, feed conversion, hepatosomatic index, increased the number of muscle fibers, number of hepatocytes and hepatic glycogen (Vargas and Ribeiro, 2009; Braccini et al., 2013). However, there are few studies on the effects of homeopathic products in fish. This study evaluated the growth performance, blood and morphological parameters and gill infections in *C. macropomum* fed diets containing different concentrations of Homeopatila 100®.

MATERIALS AND METHODS

Experimental design

In this experiment, 300 fingerlings of *C. macropomum* (12.0 ± 0.5 cm and 42.7 ± 3.1 g) obtained from commercial fish farming (Macapá, AP, Brazil) were acclimated for seven days in water tanks. Fish were randomly distributed in water tanks (500 L), containing 400 L useful volume, maintained at a density of 25 fish per tank. The design was completely randomized with four treatments (20 ml hydroalcoholic solution - controls, 20, 40 and 60 ml Homeopatila 100®/kg diet) and three replications. The homeopathic product was added to the extruded commercial diet containing 32% crude protein and fish fed for 60 days.

Preparation of diets with homeopathic product

We used commercial diet containing 32% crude protein, 65 g ether extract, 70 g crude fiber, 100 g mineral matter, 12 g calcium, 6000 mg phosphorus,16000 IU vitamin A, 250 IU vitamin E, 4500 IU vitamin D3, 30 mg vitamin K3, 325 mg vitamin C and 32 mg thymine (B1) for each kg of diet. Weekly, Homeopatila 100® in the form of hydroalcoholic solution was incorporated to the commercial feed using a hand sprayer. Composition of the complex Homeopatila 100®, for 1000 ml: 250 ml of iodum 12 cH, 250 ml of sulphur 30 cH, 250 ml of natrummuriaticum 200 cH, 250 ml of streptococcinum 30 cH and q.s medium (ethylalcohol 30%). Subsequently, the feed was homogenized and dried at room temperature, removing it periodically for 24 h. The feed was stored in a cool dry place without any incidence of sunlight, chemicals and equipment that emitted magnetic field until being loose and without alcohol odor (Siena et al., 2010). The same inclusion process was conducted to the control treatment using 20 ml of 30% alcohol per kg feed. The amount of feed provided to fish was *ad libitum*, and three times a day (9:00, 13:00 and 17:00 pm).

Parameters of growth performance

In the initial and final experiment, all fish were weighed (g) in a digital scale and measured in total length (cm) using caliper to determine the following parameters of the body growth performance:

1. Weight gain (g) = WG = $(W_1 - W_2)$
2. Daily weight gain (DWG) = WG/t
3. Specific growth rate (%) = (SGR) = 100 × $(LnW_1 - LnW_2) / t$
4. Feed conversion rate (FCR) = Amount / weight gain

Where W_1 = mean weight (g) in the final experiment; W_2 = mean weight (g) in the initial experiment; t = time (days) of experiment

5. Relative condition factor (Kn) (Le-Cren, 1951).

Procedures for collection and analysis of blood parameters

After 60 days of feeding with 20 ml of hydroalcoholic solution

Table 1. Physical and chemical parameters of the farming water of *Colossoma macropomum* fed on different concentrations of Homeopatila 100®.

Treatments (mg/kg)	Oxygen (mg/L)	pH	Temperature (ºC)	Conductivity (µs/cm)	Total ammonia (mg/L)
Control	5.57±0.58[a]	6.05±0.41[a]	29.82±0.41[a]	0.036±0.003[a]	0.85±0.57[a]
20	5.43±0.64[a]	6.12±0.38[a]	29.90±0.42[a]	0.037±0.003[a]	0.66±0.37[a]
40	5.49±0.61[a]	6.09±0.39[a]	29.93±0.42[a]	0.038±0.005[a]	0.71±0.54[a]
60	5.46±0.63[a]	6.09±0.38[a]	29.98±0.43[a]	0.036±0.003[a]	0.69±0.37[a]

Means followed by different letters in the same column indicate differences between treatments by the Tukey test ($p < 0.05$). Values expressed as mean ± standard deviation.

(control), 20, 40 and 60 ml Homeopatila100®/per kg feed, five fish per replicate of the different treatments were anesthetized with eugenol (15 mg/L water) (Inoue et al., 2011) for blood collection. An aliquot of blood collected by puncture of the caudal vessel was collected from the 60 fish with the aid of syringes containing Ethylenediaminetetraacetic acid (10%). The blood was used to determine the total number of erythrocytes in a Neubauer chamber, concentration of hemoglobin using Drabkin's reagent and reading on a spectrophotometer at 540 nm absorbance and hematocrit by the microhematocrit method. With this data, the Wintrobe erythrocytic indices were calculated: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Blood smears were confectioned and stained with a combination for differential leukocyte counts in up to 100 cells of interest, in each extension and also for determining the total number of leukocytes and total thrombocytes (Ranzan-Paiva et al., 2013). Leukocytes were identified and classified in lymphocytes, monocytes, neutrophils, eosinophils and PAS-positive granular leukocytes (LG-PAS), following the recommendations of Tavares-Dias et al. (1999). A portion of blood was centrifuged to obtain the plasma and determination of the concentrations of glucose and total protein using kits from Doles (GO, Brazil) and spectrophotometric reading.

Parasitological analysis

After 60 days of feeding on 20 ml hydroalcoholic solution (control), 20, 40 and 60 ml Homeopatila100® per kg diet, 10 fish of each repetition of the different treatments were anesthetized with eugenol (15 mg/water) (Inoue et al., 2011), then the gills were collected for parasitological analysis. The gills of 120 fish were collected, fixed in 5% formalin and used for collection, fixation, quantification and preparation for the identification of parasites (Eiras et al., 2006). The prevalence and mean abundance were calculated (Bush et al., 1997) for each treatment.

Biometric and histological analyses of the liver

After 60 days of feeding on 20 ml of hydroalcoholic solution (control), 20, 40 and 60 ml Homeopatila100® per kg diet, 10 fish of each replication of the different treatments were anesthetized with 15 mg/L of eugenol (Inoue et al., 2011) for liver collection. The liver of 120 fish was removed, weighed on a precision scale used to determine the Hepatosomatic index (HSI) (Tavares-Dias et al., 2000). For each treatment, liver of two other fish of each repetition was collected, totaling 24 fish, washed in sodium chloride (0.85%) and fixed in Bouin solution for 12 h. Then, the fragments were washed in 70% ethanol for removal of picric acid, dehydrated and embedded in paraffin, following routine procedures. After making the slides (in duplicate) for morphological analysis and determination of the number of hepatocytes per area, the staining was performed with hematoxylin and eosin (Behmer et al., 1976) and for histochemical staining of hepatic glycogen (Beçak and Paulete, 1976); in order to quantify the percentage occupied by this inclusion/area, the periodic acid-Schiff (PAS) + hematoxylin was used. The morphometric analyses of liver tissue were performed from images captured on Olympus BX41 optical microscope coupled with camera Olympus Q-Color 3, using a 40× magnification objective. Fifty images of each fish were analyzed, totaling 300 images/treatment; the standardized floor area was 20914.7 μm^2. Such measurements were performed with the aid of image analysis of software Image Pro Plus® (Version 4.5, Media Cybernetics, USA).

Physical and chemical parameters of water in the tanks

In the experimental tanks, water renovation was constant and the accumulated debris was siphoned every two days. The water temperature, pH, dissolved oxygen levels, ammonia and electrical conductivity were measured daily, using digital devices (YSI, USA) for each purpose.

Statistical analyses

All data have been previously evaluated under assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett tests, respectively. For data with normal distribution the analysis of variance (ANOVA - one way) was used, followed by Tukey test for means comparison. For data that did not follow this pattern of distribution the Kruskal-Wallis test was used, followed by Dunn's test for means comparison.

RESULTS

The physical and chemical parameters of the water in the farming tanks of tambaqui fed on different concentrations of Homeopatila 100® showed no differences between them (Table 1). Initial and final values of length and weight of tambaqui are in Figure 1. There was no significant difference ($p > 0.05$) among treatments after 60 days of feeding on homeopathic product. After 60 days of fish feeding, the weight gain, feed conversion ratio and specific growth rate were different only in fish kept at 60 ml Homeopatila 100®/kg diet compared to control and other treatments. However, the Kn of fish was not influenced by treatments with homeopathic product (Table 2). The gills of the fish were parasitized by

Table 2. Parameters of production performance of *Colossoma macropomum* fed on different concentrations of Homeopatila 100® for 60 days.

Treatments (ml/kg)	WG (g)	DWG(g/day)	FCR	SGR (%)	Kn	S (%)
Control	154.4 ± 34.3[ab]	2.6 ± 0.57[a]	1.52	2.51 ± 0.33[ab]	0.99 ± 0.01[a]	100
20	167.1 ± 41.3[ab]	2.8 ± 0.68[a]	1.53	2.63 ± 0.34[a]	1.00 ± 0.01[a]	100
40	169.8 ± 47.69[b]	2.8 ± 0.79[a]	1.57	2.65 ± 0.40[a]	1.00 ± 0.03[a]	100
60	150.57 ± 42.03[a]	2.5 ± 0.70[b]	1.72	2.47 ± 0.35[b]	0.99 ± 0.01[a]	100

WG: Weight gain; DWG: Daily weight gain; FCR: Feed conversion rate; SGR: specific growth rate; Kn: relative condition factor; S: Survival. Means followed by different letters in the different rows indicate differences between treatments by the Tukey test (p <0.05). Values expressed as mean ± standard deviation.

Table 3. Prevalence (P%) and mean abundance (MA) of parasites in the gills of *Colossoma macropomum* fed different concentrations of Homeopatila 100®/kg feed for 60 days.

Parasite species	Control		20 ml/kg		40 ml/kg		60 ml/kg	
	P (%)	MA	P (%)	MA	P (%)	MA	P (%)	MA
Ichthyophthirius multifiliis	100	7.144.3 ± 4033.6[a]	100	13.310.9 ± 4997.0[b]	100	10.401.8 ± 6853.3[ab]	100	54.108.3 ± 70851.9[c]
Piscinoodinium pillulare	0	0[a]	0	0[a]	0	0[a]	30	362.5 ± 615.43[b]
Anacanthorus spatulathus	100	24.1 ± 18.0[a]	100	40.6 ± 59.5[ab]	100	24.9 ± 14.2[a]	100	53.2 ± 33.1[b]
Notozothecium janauachensis	100	27.1 ±15.3[a]	100	30.5 ± 30.60[a]	100	36.0 ± 26.2[ab]	100	51.9 ± 31.0[b]
Mymarothecium boegeri	100	15.3 ± 4.8[a]	100	14.7 ± 10.0[a]	100	22.8 ± 21.0[a]	100	43.4 ± 27.7[b]
Linguadactyloides brinkimanni	23.3	0.96 ± 2.8[a]	13.3	0.63 ± 2.3[a]	3.3	0.1 ± 0.5[a]	10	54.108.3 ± 70851.9[c]

Values expressed as mean ± standard deviation. Means followed by different letters in the same row indicate difference between treatments by the Dunn test (p <0.05), for mean abundance.

Ichthyophthirius multifiliis Fouquet, 1876 (Ciliophora), *Piscinoodinium pillulare* (Schäperclaus, 1954) Lom 1981 (Dinoflagellida), *Anacanthorus spathulatus* Kritsky, Thatcher and Kayton, 1979, *Notozothecium janauachensis* Belmont-Jegu, 2004, *Mymarothecium boegeri* Cohen and Kohn, 2005 and *Linguadactyloides brinkimanni* Thatcher and Kritsky, 1983 (Dactylogyridae). There was no difference in the prevalence of these parasites among groups of fish fed on different concentrations of homeopathic product. However, *P. pillulare* occurred only in fish maintained on diets containing 60 ml of Homeopatila100® (Table 3).

In hosts, the highest parasite abundance was of *I. multifiliis* and the lowest abundance was of *L. brinkimanni*. The abundance of *I. multifiliis* was higher in fish fed on diets containing 20 and 60 ml Homeopatila 100® compared to control fish. The abundance of *A. spatulathus*, *N. janauachensis* and *M. boegeri* was higher in fish fed diets containing 60 ml of homeopathic product when compared with controls (Table 3). Plasma glucose levels were higher in fish fed on diets containing 40 and 60 ml Homeopatila 100® when compared to controls. Hematocrit and Mean corpuscular volume (MCV) of fish fed on diets containing 20 and 60 ml of homeopathic product were higher than in controls. In fish fed diet contaning 20 ml of homeopathic product, the total number of thrombocytes and monocytes were higher than in controls. However, the number of lymphocytes was reduced in all groups fed on different concentrations of homeopathic products. The number of neutrophils was increased only in fish with 40 ml Homeopatila 100® when compared to controls, while the number of PAS-LG and eosinophils were smaller in fish fed on this homeopathic product 100® (Table 4).

The percentage of glycogen did not differ among treatments with homeopathic product. However, the number of hepatocytes was lower in

Table 4. Blood parameters of *Colossoma macropomum* fed on different concentrations of Homeopatila 100®/kg feed for 60 days.

Parameters	Control	20 mL/kg	40 ml/kg	60 ml/kg
Glucose (mg dl^{-1})	93.4 ± 5.4[a]	99.9 ± 8.6[a]	110.9 ± 11.5[b]	113.6 ± 12.4[b]
Protein (mg dl^{-1})	3.6 ± 0.5[a]	3.6 ± 0.3[a]	3.6 ± 0.4[a]	3.6 ± 0.4[a]
Hematocrit (%)	19.7 ± 1.5[a]	22.5 ± 3.7[b]	20.7 ± 2.3[ab]	22.9 ± 1.8[b]
Hemoglobin (g/dl^{-1})	7.2 ± 1.3[a]	7.25 ± 0.97[a]	7.0 ± 0.7[a]	7.5 ± 0.9[a]
RBC (number 10^6/μl^{-1})	0.99 ± 0.17[a]	0.87 ± 0.20[a]	0.95 ± 0.2[a]	0.96 ± 0.26[a]
MCV (fL^{-1})	204.5 ± 33.9[a]	263.9 ± 55.6[b]	224.5 ± 39.3[ab]	251.3 ± 55.8[b]
MCHC (g/dl^{-1})	36.8 ± 6.1[a]	32.8 ± 4.7[a]	33.9 ± 3.6[a]	32.8 ± 2.3[a]
Thrombocytes (number μl^{-1})	19.000 ± 6577[a]	12.682 ± 3856[b]	30.8747 ± 8073[c]	27.230 ± 14599[ac]
Leukocytes (number µl-[1])	48.877 ± 9706[a]	30.683 ± 9667[b]	43.235 ± 12115[a]	42.062 ± 12675[a]
Lymphocytes (number μl^{-1})	20.938 ± 5151[a]	11.762 ± 5059[b]	13.209 ± 6887[b]	11.193 ± 4164[b]
Monocytes (number μl^{-1})	18.397 ± 6083[ac]	11.040 ± 3980[b]	15.359 ± 4826[a]	22.330 ± 8524[c]
Neutrophils (number μl^{-1})	4589 ± 2927[a]	4611 ± 3330[a]	9646 ± 5586[b]	6.565 ± 3654[ab]
Eosinophils (number μl^{-1})	3269 ± 2494[a]	2.029 ± 943[ab]	3349 ± 2225[a]	1.380 ± 721[b]
PAS-LG (number μl^{-1})	1802 ± 9618[a]	1431 ± 1042[ab]	1671 ± 1163[a]	743 ± 334[b]

Means followed by different letters in the different rows indicate differences between treatments by the Dunn test ($p < 0.05$). Values expressed as mean ± standard deviation. PAS-LG: PAS-positive granular leukocytes.

Table 5. Number of hepatocytes, hepatic glycogen and hepatosomatic index (HSI) of *Colossoma macropomum* fed on different concentrations of Homeopatila 100® for 60 days.

Treatments (ml/kg)	No. hepatocytes	Glycogen (%)	HSI (%)
Control	247.8 ± 38.1[a]	19.5 ± 2.7[a]	2.17 ± 0.15[ab]
20	251.8 ± 38.1[a]	19.8 ± 2.6[a]	2.17 ± 0.20[a]
40	223.3 ± 34.1[b]	19.4 ± 1.6[a]	1.99 ± 0.34[b]
60	246.9 ± 25.7[a]	19.0 ± 2.2[a]	2.05 ± 0.29[ab]

Means followed by different letters in the same column indicate differences by the Dunn test ($p < 0.05$). Values expressed as mean ± standard deviation.

fish treated with 40 ml of Homeopatila 100®/kg of feed when compared to the other treatments, but the hepatosomatic index was lower than that in fish treated with 20 ml of homeopathic product (Table 5).

There was no morphological change in the liver of fish fed on diets containing homeopathic product and controls. Fish treated with 20 ml Homeopatila 100®/kg diet showed diffuse melanomacrophage centers in the hepatocytes and blood vessels, while in fish kept in 40 ml homeopathic product, such structures were only observed in blood vessels. However, in control fish and with 60 ml homeopathic product melanomacrophage centers were observed (Figure 2).

DISCUSSION

In Tambaqui fed control diet 20 ml of hydroalcoholic solution, 20, 40 and 60 ml of Homeopatila100® there was no mortality for 60 days. Such concentrations of Homeopatila 100® when added to the diet of Nile tilapia increased the survival of fingerlings for 60 days (Siena et al., 2010; Braccini et al., 2013). 20 and 40 ml Homeopatila 100®/kg did not affect the performance parameters of tambaqui similarly to that described for Nile tilapia fed the same concentrations of that homeopathic product in commercial feed (Siena et al., 2010). This homeopathic product had no benefic effect on feed conversion of tambaqui, since the best results were observed in the control group, unlike the expected, as it improved feed conversion in Nile tilapia fed on diets containing 40 ml of Homeopatila 100®/kg feed (Siena et al., 2010; Braccini et al., 2013). In addition, feeding on 60 ml Homeopatila 100® reduced weight gain, mean daily gain, specific growth rate and increased feed conversion of tambaqui. Moreover, this concentration did not affect apparent feed conversion, weight and length of Nile tilapia (Siena et al., 2010; Braccini et al., 2013) kept under similar conditions to the present study.

The condition factor, a quantitative indicator of fish's

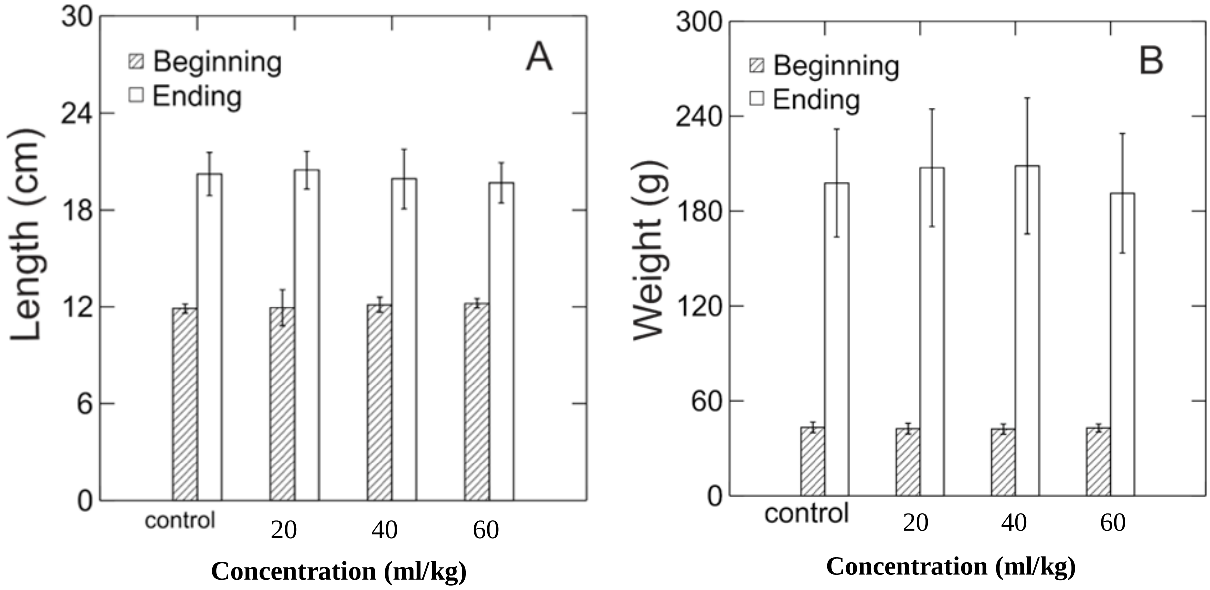

Figure 1. Initial and final length (**A**) and initial and final body weight (**B**) of *Colossoma macropomum* fed during 60 days with different concentrations of Homeopatila 100®. Values expressed as mean ± standard deviation.

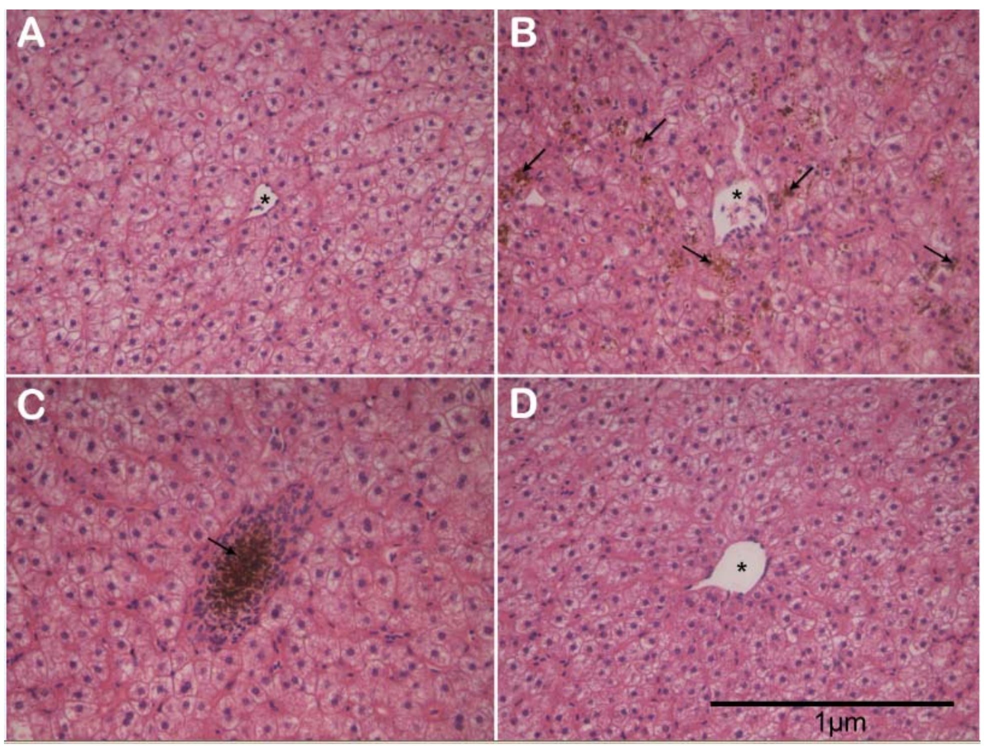

Figure 2. Liver morphology of *Colossoma macropomum* fed 0 mL of Homeopatila 100®/kg. (A) 20 ml, (B) 40 ml, (C) and 60 ml (D), highlighting the presence of melanomacrophage centers (arrows). *Indicate blood vessel.

body condition used to evaluate the different feeding conditions, interference of population density and other environmental conditions (Le-Cren, 1951; Guidelli et al., 2011) showed no difference among treatments of Homeopatila 100® in tambaqui which was similar to that found in Nile tilapia fed this same homeopathic product and at the same concentrations (Valentim-Zabott et al., 2008).

No difference in mean daily gain and specific growth rate was observed in tambaqui (42.7 ± 3.1 g) fed diet containing 32% crude protein and 20 ml of hydroalcoholic solution (control), 20 and 40 ml of Homeopatila 100®. Such growth performance parameters were higher than those described by Lemos et al. (2012) for the same fish (7.7 ± 0.2 g) when fed diets containing 26% crude protein; but the feed conversion was better. Moreover, the weight gain was similar to that reported by Pereira-Junior et al. (2013), for tambaqui (6.6 ± 0.1 g) fed on diets containing 38.3% crude protein, but apparent feed conversion was also lower. However, such differences are attributed to the initial fish size, which were larger in this study, and also to the different levels of protein used in the diet. Therefore, these results indicate that, during growth, tambaqui seems to have better feed conversion than at the beginning of fattening.

The gills of tambaqui fed diets containing 20 ml of hydroalcoholic solution (controls), 20, 40 and 60 ml Homeopatila 100®/kg diet were found infected by *I. multifiliis, P. pillulare* and four monogenean species (*A. spathulatus*, *N. janauachensis, M. boegeri* and *L. brinkimanni),* but *I. multifiliis* was the most abundant parasite and *P. pillulare* was only found in fish treated with 60 ml of this product. However, with the use of homeopathic product only *L. brinkimanni* showed reduction in prevalence, because regardless of treatment, all fish were parasitized by *I. multifiliis* and monogenean species, but the abundance of these parasites was higher in fish treated with 60 ml of product homeopathic. In addition, there was no difference in the prevalence of *Trichodina* sp. and Gyrodactylidae species as well as the mean intensity of parasites for Nile tilapia maintained for 60 days with diets containing these same concentrations of Homeopatila 100® (Braccini et al., 2013).

Tambaqui fed 60 ml Homeopatila 100® had lower growth performance and hence were the most parasitized by monogeneans and *I. multifiliis*, since the latter is an opportunistic protozoan. Moreover, infection with *P. pillulare*, another opportunistic protozoan, occurred only in those fish. However, for goats using homeopathic products, the number of eggs of gastrointestinal helminthes was reduced (Neves et al., 2012), but for sheep these drugs had no antiparasitic efficacy (Cavalcante et al., 2007; Signoretti et al., 2008), as has occurred in fish in the present study. Although the use of homeopathic products do not always have efficacy against helminthes, homeopathy can help to reduce the effects of parasitic infections, balancing the host-parasite relationship (Cavalcante et al., 2007; Signoretti et al., 2008).

In this study, only fish fed diets containing 20 and 60 ml Homeopatila 100® had higher hematocrit and higher MCV, while the levels of plasma protein, hemoglobin concentration, hematocrit, red blood cell count and MCHC were not affected by treatment with the homeopathic product. However, Nile tilapia also fed with Homeopatila 100® showed reduction in plasma levels of cortisol, glucose, hematocrit, hemoglobin, red blood cell count and MCHC (Vargas and Ribeiro, 2009), then featuring a macrocytic-hypochromic anemia. On the other hand, no sign of anemia occurred in all fish of this study, but there was a higher concentration of glucose in fish of the highest concentrations of Homeopatila 100®, showing signs of stress. However, fish treated with 60 ml of Homeopatila 100® had higher parasitism, caused possibly by stress (Tavares-Dias et al., 2009b).

The thrombocytes are multifunctional cells of fish, since they primarily participate in the coagulation process and secondly, assist the defense mechanism (Ranzani-Paiva et al., 2013; Santos and Tavares-Dias, 2011), thus being in constant movement between the hematopoietic organs and circulation. Reduction in the number of these blood cells may indicate a hemostatic disorder. In this study, fish fed 20 ml Homeopatila 100® showed reduced number of thrombocytes, while those fed 40 ml showed an increase. Vargas and Ribeiro (2009) reported increased percentage of thrombocytes for Nile tilapia fed with this same homeopathic product.

In fish, the leukocyte count is an important tool to infer the state of health and immune system because of the many functions of these cells. Lymphocytes are white blood cells involved in a variety of immune functions such as immunoglobulin production and modulation of defense. Neutrophils are the first phagocytic leukocytes in response to infection. Monocytes and PAS-LG are phagocytes that perform migration to the inflammatory site during infectious processes. Eosinophils are white blood cells that may participate in the defense process against parasites (Ranzani-Paiva et al., 2013; Santos and Tavares-Dias, 2011). In *C. macropomum*, 20 ml of Homeopatila100® caused leukopenia due to lymphocytopenia and monocytopenia, while 40 ml of this product led to a Neutrophilia accompanied by lymphocytopenia. However, treatment with 60 ml of Homeopatila 100® resulted in monocytophilia accompanied by lymphocytopenia, eosinopenia and reduced number of LG-PAS. Similarly, the use of Homeopatila 100® also caused a reduction in the percentage of lymphocytes and eosinophils in Nile tilapia (Vargas and Ribeiro, 2009). Neutrophilia has been reported to fish with parasitic infections and sometimes can be accompanied by lymphocytopenia, depending on the stage of infection and effects of stress caused by parasitism (Santos et al., 2011).

In fish, the liver is a hematopoietic organ, but also stores large amount of glycogen and fat, then such reserves can influence its weight, also interfering with the hepatosomatic index (Tavares-Dias et al., 2000; Barbosa et al., 2011). In *C. macropomum*, 60 days of feeding on diets containing different concentrations of Homeopatila 100® did not affect the Hepatosomatic index and percentage of hepatic glycogen, but 40 mL reduced the amount of hepatocytes. However, in *O. niloticus* treated with Homeopatila 100®, there was decreased amount of lipid inclusion in the liver and consequently in the HSI, but it has been reported to increase on hepatocytes number (Siena et al., 2010; Vargas and Ribeiro, 2009).

The liver also controls many vital functions as it has important role in physiology and immunity. It has perisinusoidal cells of the reticulum-endothelial system that, when present, have a phagocytic function, because they are a type of macrophage known as melanomacrophages (Bombonato et al., 2007). The melanomacrophages have several functions in fish, such as phagocytosis or pathogens, antigen processing in the immune response, destruction, detoxication or recycling of endogenous and exogenous materials, deposits of metabolites of dead cells, including red blood cells, as well as response to different antigens (Campos et al., 2008; Manrique et al., 2014). *Colossoma macropomum* maintained with 20 ml Homeopatila 100®/kg diet showed diffuse melanomacrophages centers in hepatocytes, but in fish kept with 40 ml of this homeopathic product, such structures were restricted to vascular channels (Campos et al., 2008).

Conclusion

For *C. macropomum* the Homeopatila 100® did not reduce the infections of parasites in the gills, but showed a relative improvement in blood parameters of fish fed on 40 ml. As the Homeopatila 100® has not improved the performance of the fish; therefore, the use of this homeopathic product is an additional cost in fish production for the fish farm. However, this homeopathic product was prepared in principle aiming at requirements of Nile tilapia, African fish with different biology from *C. macropomum*, an Amazonian fish.

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Conflict of interest

Authors declare that there are no conflicts of interest

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