Antiparasitic efficacy and blood effects of formalin on *Arapaima gigas* (Pisces: Arapaimidae)

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**ABSTRACT**

This study evaluated the in vitro and in vivo antiparasitic efficacy of formalin against *Dawestrema cycloancistrium*, the effects on the physiological response of *Arapaima gigas* and the residual action on fish muscle after 96 h of exposure. As regards the in vitro assay, 0, 22, 44, 66, 88, 110, 330, 660 and 880 mg L$^{-1}$ formalin were tested. After 1 h of exposure to 660 and 880 mg L$^{-1}$ formalin, there was a 100% mortality of *D. cycloancistrium* as well as after 2 h of exposure to 330 and 110 mg L$^{-1}$ and 3 h of exposure at 44, 66 and 88 mg L$^{-1}$. Concerning the in vivo test, when fish were exposed to formalin at 0, 220, 330, 440 and 550 mg L$^{-1}$, there was 100% survival at all concentrations and exposure times evaluated. Baths of 1 h with 440 and 550 mg L$^{-1}$ formalin showed 93.3% and 99.3% efficacy respectively. However, the baths of 12 h with 55 and 66 mg L$^{-1}$ formalin had the efficacy of 44.5% and 55.5% respectively. In 1 h baths with 220, 330, 440 and 550 mg L$^{-1}$ formalin, hematocrit, hemoglobin, number of total erythrocytes, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, plasma glucose levels, cortisol, total proteins, chloride, calcium, sodium, potassium and magnesium of the fish presented no differences in relation to the control values. However, in baths of 12 h with 33, 44, 55 and 66 mg L$^{-1}$ formalin, there was a decrease in hematocrit, plasma levels of calcium and chloride, and increased levels of glucose and cortisol, depending on the concentration of formalin used. In the fish muscle, the formalin residue decreased after 96 h in all concentrations and periods evaluated, returning to values close to the control ones. The results indicate that formalin had its efficacy successfully proved in the treatment against *D. cycloancistrium* at higher concentrations such as 440 and 550 mg L$^{-1}$ formalin and shorter exposure time (1 h) without compromising fish homeostasis and consumer food safety.

**Statement of relevance:** The manuscript represents original research on use of formalin in vitro and in vivo for treating infection by monogenean *Dawestrema cycloancistrium* in *Arapaima gigas*, the giant fish from Amazon. In the fish, muscle the residue levels of formalin after exposure was also investigated. Formalin have efficacy in the treatment against *D. cycloancistrium* at higher concentrations (440 and 550 mg L$^{-1}$) of formalin and shorter exposure time (1 h) and without compromising *A. gigas* homeostasis and consumer food safety.

1. Introduction

With the intensification of *Arapaima gigas* farming (pirarucu), diseases caused by ectoparasites are limiting factors for the development of the aquaculture activity of this fish, once it has caused epizootic outbreaks with losses of 80% during the nursery stage (Araújo et al., 2009a, 2009b; Marinho et al., 2013). Studies have shown that monogeneans *Dawestrema cycloancistrium* Price & Nowlin, 1967 and *Dawestrema cycloancistrioides* Kritsky, Boeger & Thatcher, 1985 are the main ones that cause diseases in *A. gigas* fish farmed in the Amazon (Araújo et al., 2009a, 2009b; Marinho et al., 2013). In addition to that, monogeneans can directly affect the zootechnical performance of the fish during the productive cycle, causing anemia, reduced growth and inappetence (Araújo et al., 2009a, 2009b; Marinho et al., 2013).
In aquaculture, one of the ways to control and treat diseases so as to minimize economic losses has been the use of chemical products such as formalin (Yildiz and Pulatsu, 1999; Fajer-Ávila et al., 2003; Stephens et al., 2003; Sharp et al., 2004; Siţâ-Bobadilla et al., 2006; Fujimoto et al., 2006; Pahor-Filho et al., 2012; Paixão et al., 2013; Andrade-Porto et al., 2017). Nevertheless, formaldehyde has been classified by the International Agency for Research on Cancer (IARC) as a human carcinogen. Recently, epidemiological studies have suggested that the exposure to this chemical can lead to the development of human hematopoietic cancers, such as leukemia (Lai et al., 2016). Safe concentrations of formalin recommended as a chemotherapeutic drug in baths for A. gigas have not been established (Andrade-Porto et al., 2017), and it has thus been used incorrectly and empirically in the aquaculture of this important Amazonian fish. Beyond that, therapeutic concentrations are used without taking the adequate exposure time, toxicity, tissue changes and accumulated residue in the muscle of treated fish into consideration (Jung et al., 2001; Andrade-Porto et al., 2017), as well as changes in homeostasis of animals exposed to it. Therefore, studies on the efficacy of formalin for A. gigas as well as their physiological and residual effects on the muscle are required. With this in view, the present study is aimed at evaluating the efficacy of formalin against monogeneans, as well as its effects on the physiology and the residual action on A. gigas muscle.

2. Materials and methods

2.1. Fish

Juveniles of Arapaima gigas (n = 300), with an average weight of 38.9 ± 9.3 g and an average standard length of 15.8 ± 1.4 cm, were obtained from a commercial fish farm in the municipality of Nova Olinda do Norte, state of Amazonas. The fish were transported to the Laboratory of Physiology Applied to Fish Farming (LAFAP), the Experimental Aquaculture Station of the Coordination of Technology and Innovation (COTI) of the National Institute for Research in the Amazon (INPA), Manaus, state of Amazonas. These fish were acclimated for 15 days. This study was approved by the Ethics Committee on Animal Research (CEUA), of Nilton Lins University (Protocol No 001/2012).

2.2. In vitro test with formalin and D. cycloancistrium in the gills of A. gigas

To test the sensitivity of D. cycloancistrium to formalin (Sigma-Aldrich 37%), five naturally parasitized A. gigas fingerlings were used. The experimental design consisted of 9 treatments: 0, 22, 44, 66, 88, 110, 330, 660 and 880 mg L⁻¹ formalin, with three replicates, totaling 27 branchial arches. Each branchial arch containing D. cycloancistrium was placed in Petri dishes with different concentrations of formalin. A visual field with 20 specimens of D. cycloancistrium was chosen for the analysis of the immobilization of the parasites in each branchial arch, using a stereomicroscope (Zeiss). Mortality and time of immobilization of the parasites were recorded every 15 min. The parasites were considered dead when they remained totally immobilized. The in vitro test was terminated when there was 100% mortality in the controls (Fajer-Ávila et al., 2003).

The in vitro test results were used to determine the average effective concentration or mean effective concentration (EC_{50}), characterized by the nominal concentration of the toxic agent causing an acute effect (lethality or immobility) at 50% of test organisms during the exposure period (Zagatto and Bertoletti, 2006), which was estimated by the Trimmed Spearmann-Karber method (Hamilton et al., 1977).

2.3. Prior analyses of infection by D. cycloancistrium in the gills of A. gigas

Ten A. gigas juveniles were euthanized by cerebral commotion for prior evaluation of parasitic rates by monogeneans before the therapeutic baths. Branchial arches were fixed in 5% formalin, used for parasite counting and, subsequently, for the determination of prevalence, mean intensity and mean abundance (Bush et al., 1997). The identification of monogeneans followed Thatcher’s recommendations (Thatcher, 2006). A total of 2.377 D. cycloancistrium were found in the gill of the examined A. gigas, with a prevalence of 100%, mean intensity of 237.7 ± 37.6 and mean abundance of 237.7 ± 37.6. The remaining fish were subjected to food deprivation for 24 h prior to the start of therapeutic baths for gastrointestinal emptying.

2.4. In vivo tests with A. gigas exposed to formalin

The experimental design was a completely randomized block with five treatments and three replicates with five fish each. In vivo tests consisted of therapeutic baths of 1 h with 0, 220, 330, 440 and 550 mg L⁻¹ formalin and therapeutic baths of 2 h with 0, 33, 44, 55 and 66 mg L⁻¹ formalin. Seventy-five A. gigas fingerlings were used for the 1 h bath and 75 for the 2 h bath. Therapeutic baths were performed in 60 L glass aquaria, with a static water system and constant aeration. These concentrations were based on the lethal concentration (CL_{50-96h}) of 36.4 mg L⁻¹ (Andrade-Porto et al., 2017).

After the short and long baths, in order to collect the gills, three fish of each replicate were used to evaluate the efficacy of the treatments. The branchial arches of the fish from each treatment were collected and fixed in 5% formalin for the parasite count. The prevalence and mean abundance were determined (Bush et al., 1997) and the efficacy of treatments in vitro and in vivo was calculated (Martins et al., 2001).

The water from each aquarium was filtered in qualitative Whatman filter papers n° 1, in order to visualize possible monogenean specimens that might have been detached from the gills. The filtered water and the filter itself were examined separately in Petri dishes under a stereoscopic microscope.

After the therapeutic baths, the remaining fish were kept for a recovery period of 96 h in 60 L aquaria, with clean water and constant aeration. At the end of this period, the parasitic load of the fish, the occurrence of mortality and the formalin residue in the fish muscle were evaluated.

2.5. Blood collection and determination of blood parameters in A. gigas exposed to formalin

Blood samples were collected from 3 fish within each replicate of the treatments, by puncture of the caudal vessel using EDTA-containing syringes (10%) and divided into two aliquots. The first aliquot was used to count total erythrocytes with the help of a Neubauer chamber, determination of the hematocrit by the microhematocrit method and hemoglobin concentration by means of the cyanometahemoglobin method. Based on these methods, the hematometric indexes were calculated: Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC).

The second aliquot was centrifuged at 75G in order to obtain plasma. It was used in the analysis of total plasma proteins, glucose, cortisol and ions Na⁺, K⁺, Ca²⁺ and Cl⁻. The total protein levels were determined by the modified biuret method and glucose by means of the enzymatic colorimetric method (glucose oxidase). Commercial kits were used for both of them (Humam, Brazil). Plasma ions such as Na⁺, K⁺ and Ca²⁺ were determined with an atomic absorption spectrophotometer (Perclin Elmer, Mod. 1100B). The plasma levels of Cl⁻ were determined by the colorimetric method using a commercial kit (Lactest®). Cortisol was determined by means of the ELISA method, using a commercial kit (EIA, Kit 55,050, Human®) and spectrophotometer reading with Espectra Max®, at a wavelength of 450 nm.
2.6. Water quality parameters

The levels of dissolved oxygen, pH, electrical conductivity, total ammonia, total alkalinity, nitrite and carbon dioxide in the aquaria water were determined by means of appropriate appliances for each purpose before and after the formalin therapeutic baths. The concentrations of Na+, K+, Mg²⁺ and Ca²⁺ ions were determined by means of an atomic absorption spectrophotometer (Perclin Elmer (AAS), Mod. 1100B).

2.7. Determination of formalin concentration in the water of aquaria and in the muscles of A. gigas

The determination of the levels of formalin in the water and in the A. gigas muscle exposed to formalin was performed. The concentration of formalin in the water and tissue was determined by NaOH and ZnSO₄·7H₂O and further reading by the method of 4-amino-3-hydrazine-5-mecapto-12,4-triazole (Jung et al., 2001). For the determination of formalin accumulation in the exposed fish muscle, a total of eight fish from each concentration of therapeutic baths were used.

2.8. Statistical analyses

All data were previously evaluated on the assumptions of normality and homoscedasticity using Shapiro-Wilk and Bartlett respectively. For the data with normal distribution, the analysis of variance was used (ANOVA - One Way), followed by the Tukey test for comparison between means. For data that did not follow this distribution pattern, Kruskal-Wallis was used, followed by the Dunn’s test for comparison between means (Zar, 2010).

3. Results

After 1 h of formalin exposure, none of the physical and chemical variables in aquaria water presented differences (p > 0.05) when compared to controls (Table 1). After 12 h of formalin exposure, there was an increase in electrical conductivity and ammonia levels in the aquaria with 44, 55 and 66 mg L⁻¹ formalin in relation to the controls (Table 2).

After 1 h of exposure, in vitro, to 660 and 880 mg L⁻¹ formalin, there was 100% efficacy against D. cycloancistrium. After 2 h of exposure, 100% efficacy was observed against D. cycloancistrium when using 110 and 330 mg L⁻¹ formalin, but at the lowest concentrations of formalin, 100% of monogeneans mortality occurred only within 3 to 4 h of exposure. In the control group, mortality started after 4 h of the assay (Fig. 1). The mean effective concentration (50CE) for D. cycloancistrium at 1 h and 2 h of exposure to formalin was of 115.3 (91.7–144.9) mg L⁻¹ and 54.2 (46.9–62.7) mg L⁻¹, respectively.

There was a high reduction in the abundance of D. cycloancistrium after 1 h of exposure to 440 and 550 mg L⁻¹ formalin. There was no efficacy against D. cycloancistrium at low concentrations of formalin during 12 h baths (Table 3). After the 96 h recovery period of the fish exposed to formalin, there was a reduction in the abundance of D. cycloancistrium for the ones in the 1 h baths with 440 and 550 mg L⁻¹, which presented higher efficacy (Table 4).

One-hour baths with different concentrations of formalin did not cause changes in fish blood parameters (Table 5). In fish from the 12 h baths with 55 and 66 mg L⁻¹ formalin, glucose levels increased and chloride levels decreased, cortisol levels also increased in those exposed to 44, 55 and 66 mg L⁻¹ formalin and calcium levels decreased when compared to controls. Furthermore, there was a reduction in the hematocrit of fish exposed to 33, 44 and 55 mg L⁻¹ formalin (Table 6).

Formalin concentrations in the A. gigas muscle were higher in fish exposed to 440 and 550 mg L⁻¹ formalin, after 1 h baths. In the 12 h baths, formalin concentrations in the A. gigas muscle were higher in fish exposed to 44, 55 and 66 mg L⁻¹ formalin. In addition to that, there was a reduction in formalin concentrations in the fish muscle after the 96 h recovery period, in the 1 h and 12 h formalin baths (Table 7).

The concentrations of formalin residue in aquaria water after the 1 and 12 h therapeutic baths showed no alteration between the different concentrations used (Table 8).

4. Discussion

The aquaria water quality where A. gigas were exposed to formalin remained within the limits considered acceptable for them when they are farmed in the Amazon (Rebelatto-Junior et al., 2015). However, during the 12 h formalin baths, electrical conductivity and total ammonia levels increased in fish exposed to 44, 55 and 66 mg L⁻¹. In contrast, other studies reported that the use of formalin caused increased pH and reduced concentration of dissolved O₂ in the water (Treves-Brown, 2000; Martins, 2004). These changes were not found here, corroborating the findings of other studies (Fajer-Ávila et al., 2003; Sitjà-Bobadilla et al., 2006; Pahor-Filho et al., 2012; Paixão et al., 2013).

In the in vitro tests with D. cycloancistrium, from the gills of A.gigas, there was 100% efficacy with 660 and 880 mg L⁻¹ formalin after 1 h of exposure and 90% efficacy with 330 mg L⁻¹ formalin after 2 h of exposure. The mean effective concentration of D. cycloancistrium was 115.3 mg L⁻¹ formalin after 1 h of exposure and 54.2 mg L⁻¹ after 2 h

**Table 1** Physical and chemical parameters of water after 1 h of exposure of *Arapaima gigas* to different concentrations of formalin.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentrations of formalin (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>OD (mg L⁻¹)</td>
<td>7.5 ± 0.04³</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25.9 ± 0.3³</td>
</tr>
<tr>
<td>pH</td>
<td>6.4 ± 0.04³</td>
</tr>
<tr>
<td>CE (μS/cm)</td>
<td>31.2 ± 1.8³</td>
</tr>
<tr>
<td>AT (mg L⁻¹)</td>
<td>0.04 ± 0.8³</td>
</tr>
<tr>
<td>Nitrite (mg L⁻¹)</td>
<td>0.02 ± 0.03³</td>
</tr>
<tr>
<td>Alc (CaCO₃ mg L⁻¹)</td>
<td>6.0 ± 0.0³</td>
</tr>
<tr>
<td>DT (CaCO₃ mg L⁻¹)</td>
<td>2.5 ± 0⁰</td>
</tr>
<tr>
<td>CO₂ (mg L⁻¹)</td>
<td>10.7 ± 1.4³</td>
</tr>
<tr>
<td>Ca²⁺ (mīg L⁻¹)</td>
<td>0.8 ± 0⁰</td>
</tr>
<tr>
<td>Mg²⁺ (mīg L⁻¹)</td>
<td>0.08 ± 0.00²</td>
</tr>
<tr>
<td>Na⁰ (mīg L⁻¹)</td>
<td>1.8 ± 0.002²</td>
</tr>
<tr>
<td>K⁺ (mīg L⁻¹)</td>
<td>1.6 ± 2.2³</td>
</tr>
</tbody>
</table>

Values express means ± standard deviation. Different letters in the same line indicate significant differences by Tukey test (p < 0.05). OD = Dissolved oxygen; CE = Electrical conductivity; AT = Total ammonia; Alc = Total alkalinity; DT = Total hardness; CO₂ = Carbon dioxide.
Therefore, formalin should be previously tested for it to be used in therapeutic fish baths (Fajer-Ávila et al., 2003).

The different development phases of monogeneans (e.g., oncomiracidium and adults) may or may not be affected by formalin (Yildiz and Pulatsu, 1999; Sitjà-Bobadilla et al., 2006). Larvae and adults of Sparicystole chrysophrii in gills of Sparus aurata exposed to 300 mg L\(^{-1}\) formalin for 30 min showed 100% efficacy in vitro and prevented the hatching of eggs (Sitjà-Bobadilla et al., 2006). In this study, during in vitro tests, it was observed that larvae of D. cycloancistrium in gills of A. gigas were more sensitive to formalin than adults (data not shown).

For D. cycloancistrium in the gills of A. gigas, the efficacy of the short baths (1 h) was 93.6 and 99.3% when 444 and 550 mg L\(^{-1}\) formalin were used, respectively, whereas in the baths of 12 h the highest efficacy was lower. Similarly, in therapeutic baths with 250 mg L\(^{-1}\) formalin during 1 h for the control of Benedenia seriolae and Zeuxapta seriolae in Seriola lalandi lalandi, the efficacy was of 80% (Sharp et al., 2004). For Hemigrimus sp. exposed to 27.5 mg L\(^{-1}\) formalin during 1 h, the efficacy was 77.7% (Paixão et al., 2013). In contrast, for Halotrema abdon in the gills of Glaucusoma hebraicum with 25 mg L\(^{-1}\) formalin during 10 and 24 h, there was no antiparasitic efficacy (Stephens et al., 2003).

Arapaima gigas exposed to 22, 44, 66, 88 and 110 mg L\(^{-1}\) formalin presented clinical signs and behavioral changes such as erratic swimming, lethargy, crowding on the water surface, loss of hydrodynamic equilibrium, spasms and agonistic confrontation, which were observed only at 88 and 110 mg L\(^{-1}\) (Andrade-Porto et al., 2017). No mortality of A. gigas was observed whatsoever during the 1 h and 12 h therapeutic baths.
Table 5
Blood parameters of Arapaima gigas exposed to different concentrations of formalin during 1 h.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Concentrations of formalin (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Hemoglobin (mg dL(^{-1}))</td>
<td>7.0 ± 0.9(^a)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>27.9 ± 13.3(^a)</td>
</tr>
<tr>
<td>RBC (x 10(^8) µL(^{-1}))</td>
<td>1.90 ± 0.29(^a)</td>
</tr>
<tr>
<td>CHCM (g dL(^{-1}))</td>
<td>27.2 ± 2.8(^a)</td>
</tr>
<tr>
<td>HCM (pg)</td>
<td>37.7 ± 7.7(^a)</td>
</tr>
<tr>
<td>VCM (fL)</td>
<td>144.5 ± 26.5(^a)</td>
</tr>
<tr>
<td>Glucose (mg dL(^{-1}))</td>
<td>45.2 ± 13.7(^a)</td>
</tr>
<tr>
<td>Total protein (g L(^{-1}))</td>
<td>1.6 ± 0.6(^a)</td>
</tr>
<tr>
<td>Cortisol (ng mL(^{-1}))</td>
<td>93.9 ± 51.6(^a)</td>
</tr>
<tr>
<td>Cl(^−) (mmol L(^{-1}))</td>
<td>51.5 ± 10.1(^a)</td>
</tr>
<tr>
<td>Ca(^++) (mmol L(^{-1}))</td>
<td>0.5 ± 0.1(^a)</td>
</tr>
<tr>
<td>Mg(^++) (mmol L(^{-1}))</td>
<td>0.1 ± 0.02(^a)</td>
</tr>
<tr>
<td>Na(^+) (mmol L(^{-1}))</td>
<td>1.2 ± 0.2(^a)</td>
</tr>
<tr>
<td>K(^+) (mmol L(^{-1}))</td>
<td>2.2 ± 0.7(^a)</td>
</tr>
</tbody>
</table>

Values express means ± standard deviation. Different letters in the same line indicate significant differences by Tukey test. RBC = Total red blood cells, CHCM = Mean corpuscular hemoglobin concentration, HCM = Mean corpuscular hemoglobin, VCM = Mean corpuscular volume, Cl = Chloride, Ca\(^++\) = Calcium; Mg\(^++\) = Magnesium; Na\(^+\) = Sodium; K\(^+\) = Potassium.
ectoparasites in long-term baths is less effective than in short baths, as it is rapidly consumed within the water column (Treves-Brown, 2000; Stephens et al., 2003); similar to what occurred in the present study. Thus, the formalin residue in the aquaria water of A. gigas, after the short (1 h) and long (12h) baths ranged from 25 to 40%, in relation to the concentration added at the beginning of the experiment. Twenty-four hour baths with 27.5 mg L$^{-1}$ formalin for Pterophyllum scalare, in six alternate-day applications, showed 71% efficacy in controlling monogeneans (Fujimoto et al., 2006). In contrast, for G. hebraicum, prolonged 27.5 mg L$^{-1}$ formalin baths during 24 h were not effective in controlling monogeneans (Stephens et al., 2003). Nevertheless, as formaldehyde is a human carcinogen (Noordiana et al., 2011; Lai et al., 2016) and a toxic product, herbal medicines such as Mentha piperita have been assayed in order to control monogeneans in A. gigas (Malheiros et al., 2016). Therefore, formalin should be handled carefully during antiparasitic treatments in fish farming (Andrade-Porto et al., 2017).

One of the effects of formalin is to cause tissue changes in fish gills, which can lead to osmoregulatory disorders (Treves-Brown, 2000; Jung et al., 2003; Andrade-Porto et al., 2017). In general, formalin may affect the gills, acting on the ionic regulation that induces a rapid decrease in conductivity and ammonia levels in aquaria water increased. Such changes may also have contributed to the increase in cortisol levels in fish.

Although fish from the controls of this study were not exposed to formalin, 0.52 μg/g (1 h baths) and 0.60 μg/g formalin (2 h baths), formalin was detected in them. These levels were lower in comparison to the ones found in fish exposed to formalin. In Nile tilapia, formalin residue in the muscle of control fish was 1.34 μg/g, similar to the one found in fish exposed to 125 mg L$^{-1}$ formalin (Xu and Rogers, 1995). Formaldehyde concentration in the muscle of Paralichthys olivaceus and Sebastes schlegeli exposed to different concentrations of formalin was also similar to the one in control fish (Jung et al., 2001). Besides being a natural product in the metabolism of animals and plants, formaldehyde is essential for the biosynthesis of amino acids, thus, naturally occurring in many kinds of food (Owen et al., 1990). It can also be produced in fish tissues during the period in which muscles are stored for analyses (Xu and Rogers, 1995; Noordiana et al., 2011). High levels of formalin do not accumulate in the fish tissues due to subsequent conversion of formaldehyde into other chemical compounds such as through the oxidation of lipids because of microorganism activities (Noordiana et al., 2011). However, the concentrations of formalin found in fish used for our study were not influenced by postmortem changes because formalin residues in the muscle were assayed immediately after fish were killed.

In Brazil, the National Plan for the Control of Residues in Products of Animal Origin (PNCR) by the Ministry of Agriculture, Livestock and Supply (MAPA) has not yet included the determination of formaldehyde residues in fish. According to the United States Environmental Protection Agency (EPA), acceptable daily intake of formaldehyde is 0.2 μg/g body weight per day (Noordiana et al., 2011). Xu and Rogers (1995) cite that endogenous levels of 3.0 to 12.8 μg/g formaldehyde have been found in fish. Noordiana et al. (2011) reported levels of formaldehyde residues varying from 0.38 to 15.7 μg/g per weight of fresh fish commercialized in wet markets. The formalin residue in the A. gigas muscle, in this study, after 96 h of recovery was low, presenting no risk of intoxication for consumers in the concentrations tested. Xu and Rogers (1993) demonstrated that in Morone saxatilis exposed to 25 and 250 mg L$^{-1}$ formalin for 1 h and 24 h respectively, formalin was no longer detected in the fish muscle after the 4th day of exposure. In Nile tilapia, after baths of 96 h, 1.30 and 1.57 μg/g formalin were reported for fish exposed to 125 and 250 mg L$^{-1}$ formalin respectively, indicating that the concentrations and persistence of formalin in the water affected the absorption of formalin on the part of the fish (Xu and Rogers, 1995).

5. Conclusions
The results, in vitro and in vivo, showed that the antiparasitic efficacy of formalin against D. cycloancistrium was dose-dependent. Short baths (1 h) with concentrations of 440 and 550 mg L$^{-1}$ formalin are recommended for the control of D. cycloancistrium in the gills of A. gigas without compromising fish homeostasis. However, caution is advised regarding the use of such concentrations directly into farming tanks, since there may be synergistic and antagonistic effects with variables in different environments.

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