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Use of common salt (NaCl) in the control *Epistylis* sp. in hybrid sorubim

Uso do sal comum (NaCl) no controle de Epistylis sp. em surubim hibrido

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Resumo: *Epistylis* sp. é um parasito emergente no Brasil e é comumente encontrado em surubim *Pseudoplatystoma* spp. durante os estágios iniciais de produção e, portanto, requer o estabelecimento de medidas profiláticas e terapêuticas, visando o seu controle. Portanto, o presente estudo teve como objetivo avaliar o uso de sal comum para controlar *Epistylis* sp. em juvenis de surubim. Os peixes (n=48) foram divididos aleatoriamente em três tratamentos, e um grupo de controle, com três repetições cada. Os tratamentos consistiram de três banhos terapêuticos realizados a cada 48 horas durante 20 minutos com diferentes concentrações de sal comum: 0,0%, 1,0%, 1,5% e 2,0%. O experimento teve duração de seis dias. Vinte e quatro horas após o último banho, os peixes foram amostrados para verificar a presença do parasito e colheita de sangue. Os tratamentos de sal de 1,5% e 2,0% foram eficazes na eliminação dos parasitos em 82% e 78% dos peixes tratados, respectivamente. Os peixes submetidos ao banho de sal a 2,0% apresentaram maiores valores de hematócrito e hemoglobina (P<0,05) em comparação aos peixes do grupo de controle, além de alterações nos valores dos leucócitos. O sal comum pode ser utilizado no controle do *Epistylis* sp. em juvenis de surubim naturalmente infectados.

Palavras-chave: ciliophora, juvenil, peritríquios sésseis, *Pseudoplatystoma* spp.

Abstract: Epistylis sp. is an emerging parasite in Brazil and is commonly found in surubim Pseudoplatystoma spp. during the initial stages of production and, therefore, requires the establishment of prophylactic and therapeutic measures, aiming at its control. Therefore, the present study aimed to evaluate the use of common salt to control Epistylis sp. in surubim juveniles. The fish (n = 48) were randomly divided into three treatments, and a control group, with three replicates each. The treatments consisted of three therapeutic baths performed every 48 hours for 20 minutes with different concentrations of common salt: 0.0%, 1.0%, 1.5% and 2.0%. The experiment lasted six days. Twenty-four hours after the last bath, the fish were sampled to check for the presence of the parasite and blood collection. Salt treatments of 1.5% and 2.0% were effective in eliminating parasites in 82% and 78% of treated fish, respectively. Fish subjected to the 2.0% salt bath showed higher hematocrit and hemoglobin values (P <0.05) compared to fish in the control group, in addition to changes in leukocyte values. Common salt can be used to control Epistylis sp. in naturally infected surubim juveniles.

Keywords: ciliophora, juvenile, sessile peritrichs, Pseudoplatystoma spp.

Introduction

Common salt is widely used in fish culture both for prophylaxis and treatment of various diseases, including those caused by ectoparasites (Andrade et al., 2005; Zuanon et al., 2009). Besides being considered a disinfectant and potential therapeutic agent, the common salt is widely available at low cost and is safe for fish, the environment and the handler. In this context, it has been tested to assess its effectiveness to control ectoparasites.

The antiparasitic effectiveness of common salt has already been reported against *Trichodina* sp. and *Gyrodactylus* sp. for Nile tilapia *Oreochromis niloticus* fingerlings, (Vargas et al., 2003); and, against *Ichthyophthirius multfiliis* for sorubim, *Steindachneridion* sp. (Klein et al., 2004) and catfish, *Rhamdia quelen* (Carneiro et al., 2005) and also against *Epistylis* sp. for channel catfish *Ictalurus punctatus* fingerlings (Hubert and Warner, 1975)

The occurrence of *Epistylis* sp. in the initial production phase of sorubim is a matter of concern for producers. Pádua et al. (2012) reported that the prevalence of *Epistylis* sp. in the carnivorous catfish produced in fish farms in the state of Mato Grosso do Sul ranged between 0.7% and 96.4%, whereas in hybrid sorubim, *Pseudoplatystoma* spp., the prevalence was 52.7%. Therefore, this protozoan has been considered as an emerging parasite in Brazil. In another study by Pádua et al. (2013), *Epistylis* sp. was considered as the second biggest problem in farmed brazilian catfish after *Ichthyophthirius multifiliis,* with a prevalence of 46%.

Hüseyin and Selcuk (2005) described *Epistylis* sp. as an important parasite since its association with the bacterium *Aeromonas hydrophila* can trigger the appearance of the red wound disease, which is characterized by hemorrhagic lesions in freshwater fish. The bacteria present in the *Epistylis* colonies are responsible for the lesions observed, since they secrete enzymes that eventually injure the tissues next to the colonies (Hazen et al., 1978), thus preventing fish commercialization.

Due to the economic losses caused by *Epistylis* sp. in the commercial sorubim production and the scarcity of information about the effectiveness of common salt as antiparasitic for native fish, this study proposes to evaluate the use of common salt to control *Epistylis* sp. of naturally infected juvenile *Pseudoplatystoma* spp.

Material and Methods

The experiment was conducted at the Ictioparasitology Laboratory of the Universidade Estadual de Mato Grosso do Sul, Aquidauana, MS. The procedures in this study were in accordance with the Ethical Principles in Animal Research and approved by the Committee for Ethics in Animal Experimentation at the UEMS protocol number 015/2013.

Juvenile catfish (n=48) with mean body weight 14.3 ± 2.2 g and mean total length 13.3 ± 0.7 cm showing visible colonies of the *Epistylis* sp. distributed in 12 polyethylene tanks 80L capacity (four fish per tank) with continuous water flow. During seven days, the fish were acclimatized and fed to apparent satiation twice a day with commercial feed for carnivorous fish (40% crude protein). Feeding management was the same during the acclimation and experimental periods, except on the days of the therapeutic baths when the fish were not fed.

The fish selected for the experiment had visible *Epistylis* sp. colonies on their body surface. Four fish were sampled for confirmation of parasitism by *Epistylis* sp. Mucus was scraped from fish body surface and placed between a blade and a coverslip to examination under a light microscope at 40x magnification. A score to parasitism intensity was assigned as described by Pádua et al. (2013).

Common salt in concentrations of 0.0% (control), 1.0%, 1.5% and 2.0% was administered as therapeutic bath. Treatment protocol consisted of three therapeutic baths performed every 48 hours during 20 min. During the bath, the water flow was stopped while aeration in the tanks was kept. After each bath, the juveniles were observed during two hours to estimate survival rate after the bath. At the end of six experimental days, the number of surviving fish was determined for each tank to final survival rate estimation. Twenty four hours after the last therapeutic bath, the remaining fish from all treatments were sampled for parasitological examination following the procedure described previously to evaluate the effectiveness of the therapeutic baths.

After parasitological examination, fish were anesthetized in a 50 $mg.L^{-1}$ eugenol solution and bled by caudal puncture using syringes and needles bathed in EDTA 3%. The following hematological parameters were determined: hematocrit (Htc) after blood centrifugation (12,000 g; 5 min) in micro-capillary tubes and subsequent reading in standard scale (Goldenfarb et al., 1971); hemoglobin concentration (Hb), according to the cianometahemoglobin method (Collier, 1944); and, erythrocyte counting (Er) in a Neubauer chamber after blood dilution in formalin citrate solution (1:200). The hematocrit and hemoglobin data were used to calculate the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of Wintrobe (1934). Blood smears were prepared in duplicate, air dried and stained with May Grünwald-Giemsa-Wright (Tavares-Dias and Moraes, 2003) and subsequently used for total and differential leukocytes counts and thrombocytes, as well.

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The following parameters were measured twice daily (08:00h and 16:30h) to determine water quality: pH (morning: 8.8 ± 0.1; afternoon: 8.8 ± 0.1) using a digital pH meter, dissolved oxygen (morning: 7.6 ± 0.2 mg L^{-1}; afternoon: 7.5 ± 0.2 mg L^{-1}) with a portable oxymeter, temperature (ºC) with mercury thermometer (morning: 24.9 ± 0.1 °C; afternoon: 25.9 ± 1.1 °C) and conductivity (morning: 316.7 ± 35.6 µS cm^{-1}; afternoon: 316.5 ± 45.1 µS cm^{-1}) with a portable conductivity meter.

The experimental design was completely randomized with four treatments and three replications (each tank containing four fish was considered with a repetitions). The survival, percentage of fish not parasitized and hematological parameters were analyzed by ANOVA and when significant, means were compared by Tukey test at 5% significance level.

Results

The initial parasitological examination revealed that 83.3% and 16.67% of the fish presented a parasite intensity score 3 (small focal colonies visible to the naked eye) and 4 (large multifocal colonies visible to the naked eye), respectively. Fish with intensity score 3 had small colonies located on one of the pectoral fins or just on the dorsal fin, while fish with score 4 had larger colonies simultaneously on the pectoral and dorsal fins.

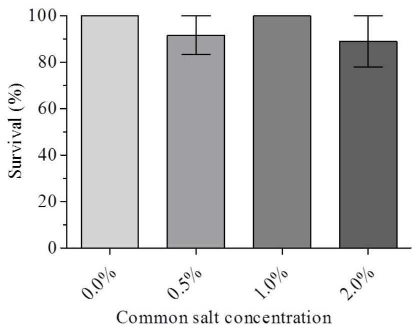

Figure 1. Survival (mean ± standard error) of hybrid sorubim naturally infected with *Epistylis* sp. after the therapeutic baths with common salt.

Fish tolerance to salt was satisfactory, given the high survival rate observed after the therapeutic baths and at end of the treatment protocol (Figure 1)

The treatments did not affect significantly the percentage of juvenile sorubim not parasitized at the end of treatment (Fig. 2). The salt baths containing 1.5% and 2.0% salt promoted the elimination of *Epistylis* sp. colonies in 82% and 78% of the treated fish, respectively.

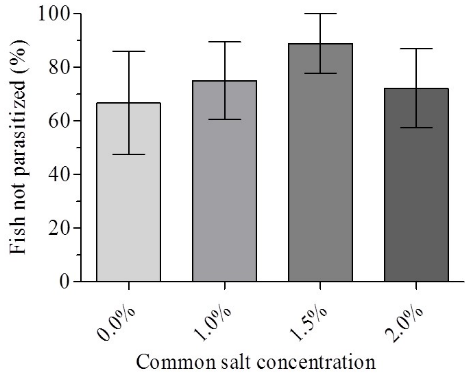

Figure 2. Percentage (mean ± standard error) of juvenile sorubim not parasitized for *Epistylis* sp. after the therapeutic baths with common salt.

Fish immersed in 2.0% salt bath showed higher hematocrit and red blood cells compared to the control group. The hemoglobin and erythrocyte values of these fish were also higher than those observed in fish treated with 1.5% salt bath (Table 1). Fish immersed in 1.0% salt bath had lower MCHC compared to the control group, but not significantly different from fish from the other treatments.

Table 1. Erythrogram (mean ± standard deviation) of juvenile sorubim naturally infected with *Epistylis* sp. after therapeutic baths with common salt.

Haematological parameters	Concentration common salt (%)			
	0.0	1.0	1.5	2.0
Hematocrit (%)	25.3 ± 1.2[B]	27.4 ± 3.1[AB]	24.7 ± 3.5[B]	30.9 ± 4.5[A]
Hemoglobin (g dL^{-1})	6.3 ± 1.1[AB]	5.5 ± 1.1[AB]	5.3 ± 0.8[B]	6.7 ± 0.9[A]
Erythrocyte ($x10^6$ μL^{-1})	1.3 ± 0.1[B]	1.7 ± 0.2[AB]	1.3 ± 0.2[B]	1.8 ± 0.4[A]
MCV (fL)	196.8 ± 32.0	165.3 ± 22.9	187.5 ± 29.0	177.6 ± 31.3
MCHC (g dL^{-1})	25.5 ± 6.3[A]	20.1 ± 2.6[B]	21.6 ± 2.5[AB]	21.8 ± 1.9[AB]

MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration.Means followed by different letters (A, B) in the row differ by Tukey (P<0.05).

The absolute monocyte, eosinophil and neutrophil values of fish exposed to 2.0% salt bath were higher compared to those from the 1.0% salt. The absolute values of basophils of fish treated with 1.0% and 1.5% salt were lower than the values observed in fish not exposed to salt (Table II). The absolute PAS-positive granular leukocyte (PAS-GL) values decreased in fish exposed to 1.5% salt compared to fish not exposed and exposed to salt (0.0%). The fish exposed to salt in concentration of 0.5% presented smaller total leukocytes compared the fish not exposed to salt (0.0%).

Table 2. Absolute values (mean ± standard error) of leukocytes and thrombocytes of juvenile sorubim naturally infected with *Epistylis* sp. after therapeutic baths with common salt.

Leucocytes ($x10^3$ μL^{-1})	Concentration common salt (%)			
	0.0	1.0	1.5	2.0
Monocyte	2.8 ±1.2[AB]	0.9 ±0.3[B]	2.0 ±0.5[AB]	3.9 ±0.8[A]
Lymphocyte	63.2 ±11.8[A]	42.6 ±6.5[AB]	34.6 ±6.2[B]	24.3 ±4.4[B]
Basophil	3.9 ±0.6[A]	1.4 ±0.4[B]	1.1 ±0.5[B]	2.3 ±0.6[AB]
Eosinophil	1.7 ±0.2[AB]	0.6 ±0.2[B]	1.7 ±0.4[AB]	2.0 ±0.5[A]
Neutrophil	12.1 ±3.0[AB]	7.4 ±1.5[B]	22.9 ±6.3[AB]	27.6 ±5.3[A]
PAS-GL[a]	3.6 ±0.8[A]	1.4 ±0.5[AB]	1.3 ±0.5[B]	2.6 ±0.7[AB]
Immature leucocyte	3.6 ±1.0	2.0 ±0.2	2.4 ±0.7	4.8 ±1.2
Total leucocytes	96.1 ±11.4[A]	56.0 ±6.3[B]	68.3 ±6.9[AB]	68.9 ±10.8[AB]
Total thrombocytes	60.5 ±8.1	63.3 ±9.8	66.2 ±4.6	61.4 ±12.3

[a]PAS-GL: PAS-positive granular leukocyte; Means followed by different letters (A, B) in the row differ by Tukey (P<0.05).

Discussion

The sorubim showed colonies of *Epistylis* sp. pectoral and dorsal fins. Overall the areas where the *Espitylis* sp. colonies attach to the body of the fish are characterized by the absence of skin, such as scales and dorsal fin (Gringnard et al., 1996). Therefore, the main infestation sites are the integument of the head and anal, pelvic, pectoral and dorsal fin rays as reported by Hubert and Warner (1975) for *Ictalurus punctatus* fingerlings, Buchmann and Bresciani (1997) for rainbow trout, *Oncorhynchus mykiss;* Abo-Esa (2008) for *I. punctatus*, Pádua et al. (2012) and Campos et al. (2014) for juvenile hybrid sorubim, *Pseudoplatystoma reticulatum* x *P. corruscans.*

According to Carneiro et al. (2006), several catfish species are sensitive to low salt concentrations. Corroborating this information, Beux and Zaniboni Filho (2007) explain that survival of pintado *Pseudoplatystoma corruscans* post larvae is dependent on the salinity. These authors observed higher survival rates in pintado post larvae exposed to salinities ranging 1.7 to 5.0%.

In this study, sorubim tolerance to salt was satisfactory, given the high survival observed. Sorubim tolerance to salt concentrations up to 2.0% indicates that the use of common salt with therapeutic product is possible due to the low risk for the animals, its low cost and easy use.

Channel catfish fingerlings, *I. punctatus*, parasitized by *Epistylis* sp. tolerated single bath with 1.5% common salt concentration for 60 min (Hubert and Warner, 1975) and Andrade et al. (2005) observed no mortality in larvae of guppies, *Poecilia reticulata*, exposed during 24 h to salt concentration of 10 g L^{-1}.

Although the results were not statistically significant, the percentage of infected fish at the end of treatment was satisfactory. The elimination of the colonies is due to the dehydration process caused by the product. Common salt causes dehydration of both parasites and fish. However, due to the size difference between parasites and fish, the dehydration process is more pronounced in the parasites. Although the salt concentration used did not exceed 2.0%, the exposure time was sufficient to cause dehydration of the parasites due to the osmotic pressure variation (Schelkle et al., 2009; Schelkle et al., 2011).

Satisfactory results have been reported for the use of common salt to control ciliate protozoans such as *Ichthyophthirius multifillis*. Klein et al. (2004) report that 3% salt concentration was effective to control *I. multifillis* in Iguaçu sorubim, *Steindachneridion* sp., when administered in a single 10-min bath, while Carneiro et al. (2005) recommended the use of 10 g.L^{-1} salt in three therapeutic baths every 48 hours during 60 min to control *I. multifillis* in the silver catfish *Rhamdia quelen*. Silva et al. (2009) found that treatment with common salt at a dose of 2.5 g L^{-1} gave eliminating ectoparasites (monogenea and ciliates protozoan) on larvae of Nile tilapia *Oreochromis niloticus*. Furthermore, larval survival rate was higher than 90%.

Although the control group (0.0% salt) was not exposed to therapeutic salt baths, it was observed that the number of infected fish decreased in 50%. It is believed that the water quality in the experimental boxes have contributed positively to reduce the infestation in this group since they were cleaned daily, to prevent the accumulation of uneaten feed and feces.

Protozoa occurrence and maintenance are related to environmental conditions of cultivation (Carneiro et al., 2006). Both the water temperature and the presence of organic material such as food scraps and/or feces contribute significantly to the proliferation of these parasites, especially for sessile ciliated protozoa which are filters of organic matter. According to Pritchett and Sanders (2007) and Jørgensen et al. (2009), the organic matter present in the culture environment is an important source of nutrients for *Epistylis* sp. favoring its reproduction and consequently contributing to the increased infestation.

The increase in hematocrit observed in fish exposed to common salt at a concentration of 2.0% can be related to the increased number of erythrocytes in the bloodstream, since the volume of the erythrocytes was changed by the treatments. The hematological parameters of the erythrocyte series of juvenile catfish, *Rhamdia quelen*, remained unchanged when exposed to salt concentrations of 2.0, 4.0, 6.0 and 8.0 g L^{-1} for 30 days. It is believed that the fish were able to acclimatize to changing salinity, after a certain time of exposure (Camargo et al., 2006). Brandão et al. (2008) found no changes in the hematocrit of pirarucus, *Arapaimas gigas*, exposed to salt concentrations of 3.0 and 6.0 g L^{-1} during a 3-hour transportation period. Similarly to this study, Fiúza et al. (2013) observed an increase in hematocrit and hemoglobin tambaquis *Colossoma macropomum* exposed to salt concentrations of 5 g L^{-1} and 10 g L^{-1}.

The present study suggests that the change of water salinity when fish were exposed to the salt baths have induced osmotic stress which was responsible for the changes in red blood cell counts, especially for hematocrit values and hemoglobin, according to Camargo et al. (2006). These same authors reported increasing neutrophils percentage and decreasing lymphocytes percentage with increasing salinity in juvenile silver catfish *Rhamdia quelem.*

The lymphopenia and neutrophilia observed in fish exposed to salt (Martins et al., 2004) are responses consistent with stress conditions. Lymphopenia is the most common response to stress, this process occurs reportedly due to a redistribution of these cells between the lymphopoietic organs or to the traffic of these leukocytes. Therefore, it can be inferred that a significant decrease of lymphocytes and increase of neutrophils values were a response to the stress caused by changing water salinity, which increases at the beginning of the treatment followed by a decrease at the end.

Both monocytes and neutrophils are leukocytes with phagocytic ability while neutrophilia is the most common response to infection. The role of eosinophils and PAS-GL, as well as other leukocytes is not well understood, but the occurrence of these leukocytes is related with the presence of parasites (Del Rio-Zaragoza et al., 2010).

The present study indicates that the common salt can be used as a therapeutic agent to control *Epistylis* sp. in naturally infected juvenile sorubim until the concentration of 2%. The results of parasitological examination of fish not exposed to salt, demonstrated that environmental conditions like absence or less amount of organic matter and good water quality, mainly temperature, have influence in the life cycle of *Epistylis* sp. Thus, adequate cleaning of the tanks, through the removal of organic matter and the temperature control may be important management strategies to control of *Epistylis* sp. fish farms nurseries of hybrid sorubim.

It is, therefore, recommended that further studies should be conducted to clarify the role the products used as therapeutic agents play in juvenile sorubim blood leukocytes changing.

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