ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 10, Issue, 01, pp. 33262-33265, January, 2020

RESEARCH ARTICLE OPEN ACCESS

EFFICACY OF SALT TO CONTROL LEECH INFESTATION IN ORNAMENTAL PLECO FISH

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ARTICLE INFO

Article History:
Received 03rd October, 2019
Received in revised form
04th November, 2019
Accepted 16th December, 2019
Published online 31st January, 2020

Key Words:
Blood, Hematological Response, Hematophagous parasites, Control

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ABSTRACT

This study evaluated the efficacy of formalin and sodium chloride bathing to control leech in freshwater ornamental *Peckoltia oligospila* loricariid fish through blood parameters. This experiment was conducted by using a completely randomized design in three aspects (control, 250mg/L formalin short bath, and 15g/L NaCl short bath for 15 minutes) and four replicates. Water quality, blood parameters and glucose were evaluated. NaCl showed the best results to control parasites. There was no significant difference related to blood parameters. NaCl treatment promoted parasites control over *Peckoltia oligospila* without changes in hematological parameters.

Citation: Rudã F. B. Santos, Mikaelle S. Neves, Natalino C. Souza, Henrique M. Dias *et al.* 2020. "Efficacy to treat leech parasite of ornamental pleco fish", *International Journal of Development Research*, 10, (01), 33262-33265.

INTRODUCTION

Amazon ornamental armored catfish provide economic importance to commercial balance in Brazil with high market values for exportation. These fish are captured by fisherman who maintain them until commercialization. However, as result of ectoparasite infestation, external lesions can reduce market values and provoke the animal rejection, which causes economical losses. Leeches frequently infest many plecos species. Those parasites usually do not cause major damage, but they can transmit several diseases such as trypanosomiasis (D'agosto and Serra-Freire 1993) and some viruses (Faisal and Schulz, 2009). However, in high infestations, due to hematophagous habit, they cause anemia (Pavanelli *et al*., 1998), which provokes the host death (Thatcher, 2006).

Furthermore, the importance of this parasite and the diseases associated to it must be considered before fish exportation. Chemicals as formalin at a concentration of 250mg/L in short baths for 15 minutes have controlled leeches, but this procedure was not recommended for tropical plecos. The formaldehyde is a substance widely used in fish treatment against protozoa and monogenean parasites (Pavanelli et al., 1998, Thatcher, 2006, Wangen, 2012). Another product frequently used in fish culture is sodium chloride (NaCl), commonly used for fish transport since it increases the survival timing and fish stress reduction (Oliveira et al. 2009, Silva et al. 2009). The main advantage of NaCl is the low hazard to fish and environment (Wangen, 2012). That safety use can be evaluated by blood parameters. This study aimed at evaluating the use of NaCl and formalin to control leeches on Peckoltia oligospila (Günther 1864).

MATERIALS AND METHODS

Artisanal fisheries in Guamá River Basin-PA-BR aid to capture twelve infected *P. oligospila* (Fig.1A) fish. The fish were measured (13.15±1.82cm total length; 10.4±1.43cm standard length and 34.18±15.98g weight) and the leeches *Batracobdela* sp. Blanchard, 1900 (Hirudinida: Glossiphoniidae) were counted (Fig.1B). Afterward, twelve polyethylene containers (3 L) received one infested fish each. A completely randomized design which evaluated three groups (2 treatments, 1 control) and 4 replicates was used. Each fish was considered a replicate (ethics committee 004/14CEUA). The treatments consisted in 15 minutes of 250mg formalin/L and 15g NaCl/L short baths. After bathing, we captured the fish and the parasites counted in order to determine the efficacy by the equation: Efficacy (%) = INL-FNL/INL X 100; INL= initial number of leeches and FNL=Final number of leeches.

During the experiment, water parameters, such as pH (Quimis® Q-400BC/BD), dissolved oxygen and temperature (Oxygen Meter LT Lutron DO-5519) and electric conductivity (Hanna HI 8733) were measured.

After parasite counting, blood was collected from the caudal vein puncture by using EDTA–rinsed syringes. Glucose (Gl, mg/dL), hematocrit (Ht, %), total plasmatic protein (TPP, g/dL) were determined according to Bicudo *et al.* (2009). The total number of erythrocytes (Er= cel/mm³) were obtained in a Neubauer chamber. The total hemoglobin (Hb, g/dL) was determined in an automatic cell counter (Celm 550). Another sample was used to differential counting of leucocytes by blood smears stained with Giemsa/May Grunwald and Rosenfeld (1947). Total leucocytes count, differential leucocytes count and total trombocytes count were determined according to Ishikawa *et al*. (2008). The mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated according to Wintrobe (1934). The data were submitted to Shapiro-Wilk normality test and then submitted to ANOVA (p=0.01) and Tukey (p=0.05) test to compare the means.

RESULTS

The water electric conductivity and pH presented differences among the treatments (Table 1). The oxygen concentrations showed values above 5 mg/L and the temperature was constant at 27.47 ± 0.39 °C. Sodium chloride was the most efficient to control leech (80.35 ± 24.31%, P < 0.05) when compared to formalin-treated fish (64.28 ± 24.04%) (Fig. 2). The hematology parameters have no statistical difference between the treatments (p > 0.01).

Table 1. Water parameters during NaCl and formalin treatments to control *Batracobdella* sp. on *Peckoltia oligospila*

Treatment	Conductivity (µS/cm)	pH	Oxygen (mg/L)
Control	58.94 ±30.97[b]	5.61 ±0.63[b]	5.8 ± 0.60[a]
NaCl	25,240.00±1,415.27[a]	5.01 ±0.30[b]	6.16 ±0.40[a]
Formalin	44.00±23.44[b]	6.27 ±0.66[a]	6.16 ±0.32[a]

Different letters means significant difference between the treatments (p<0.05).

Table 2. Blood parameters of *Peckoltia oligospila* after formalin and NaCl short baths treatments

Hematological parameters*	Control	NaCl	Formalin
Glucose (mg/dL)	83.75±20.64	65.33±17.67	72.75±29.54
Hematocrit (%)	16.12±6.99	23.62±10.68	28.12±12.05
TPP (TPP, g/dL)	7.27±3.20	7.38±1.09	7.18±2.8
Hemoglobin (g/dL)	6.55±3.07	10.25±5.21	10.2±5.31
MCV (fL)	139.43±26.1	191.34±28.45	187.05±51.41
MCH (g/dL)	57.22±16.54	82.82±8.7	66.19±19.91
MCHC	40.54±6.46	43.15±5.38	35.83±8.3
Erythrocytes (x10^5/µL)	1.2±0.5	1.2±0.4	1.4±0.4
Lymphocytes (%)	51±12.7	56±6.92	70.±8.1
Neutrophils (%)	37.5±12.02	32.6±2	26±9.5
Monocytes (%)	11.5±0.7	11.3±4.9	6±4.6
Leukocytes (x10^6/µL)	1445.4±424,	1280±1140.5	700±504.7
Trombocytes (cells/100 leucocytes)	984.0±169,7	720±120	746.6±291.43

Total plasma protein (TPP) Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH, picog), mean corpuscular hemoglobin concentration (MCHC). * No significant difference between the treatments (p>0.05).

A

B

Fig. 1. (a) *Peckoltia oligospila*. (b) *Batracobdella* sp. parasitizing *Peckoltia oligospila* (arrow)

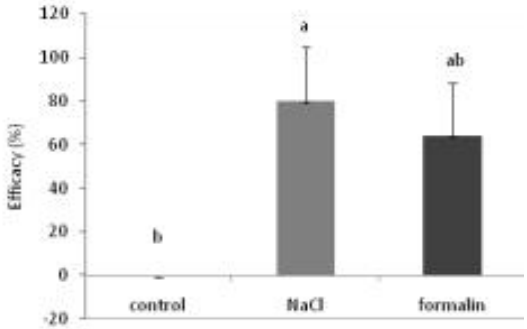

Fig. 2. Efficacy of NaCl and formalin treatments to control *Batracobdella* sp. infestation on *Peckoltia oligospila*. Different letters reported significant difference between the treatments (p<0.05)

DISCUSSION

The best results from NaCl treatment probably occurred due to the loss function of secretory cells presented in leech´s epithelium, which is responsible for osmoregulation. In freshwater leeches, when the osmotic pressures exceed a critical threshold, there were increases in energetic demands for maintaining the osmotic regulatory equilibrium, which compromised other organism functions, mainly neural functions (Singhal et.al. 1989). Further, the use of salt at 6 g/L for *Brycon cephalus* transportation reduced stress and parasitism by dinoflagellates (*Piscinoodinium pillulare*) (Carneiro *et al*., 2002). Salt also used to prevent injuries, as well as fungal and *Flavobacterium columnare* infections in handling situations that promote loss scales and skin lesions (Kubitza, 2007). The blood parameters have been recognized as a tool to measure the physiological state of fish (Carneiro *et al*., 2002). There are no effects of salt on hematological parameters probably due to the short time of exposure. Moreover, while studying *Rhamdia quelen,* Camargo et al. (2006) managed NaCl (0 to 8g /L) salt bath exposures for 30 days. However, they have not observed influence on erythrocyte count. Similarly, Brandão et al. (2006) evaluated the transport stress response of Pirarucu (*Arapaima gigas*) by using salt in closed system, nonetheless the authors have not observed influence on hematological parameters.

The products recommended for use in fish treatment must not cause any damage to animal tissues, as well as accumulate in tissue, water or substrate. On the contrary, they must be safe for animals and humans, must have low cost and easy application (Martins 2004). Considering these facts, formalin must be avoided because it causes gill damage, and in high concentration, produces hepatic changes (Cruz *et al*., 2005). Intense alterations in both gills and liver can be responsible for compromising fish welfare and facilitating secondary infections. Besides, the use of formalin to control fish parasites provides controversial results. The control of monogeneans in 250 mg/L formalin baths concentrations was not effective and in 400 mg/L baths over 1 h showed moderate efficacy (Katharios *et al*. 2006). Currently, still missing studies about to leech control, probably due to its low pathogenicity and low intensity of infestation (Pavanelli *et al*., 1998; Tavares-Dias *et al*., 2010). Nevertheless, it is noteworthy that leeches are vectors of many diseases, such as viruses and trypanosomes (D'Agosto and Serra-Freire 1993). For exported ornamental fish, leech control might be highly recommended to avoid disease dissemination in other countries. Hence, the 15g NaCl/L short bath is recommended to control leech infestation in *Peckoltia oligospila*, due to its effectiveness and environmental safety, as well as for not being a fish stressor. Further studies should be carried out to evaluate the effect of salt on fish after transporting, as a prophylactic measure to avoid stressful conditions and later mortalities.

Acknowledgments: The authors thank CNPq (National Council for Scientific and Technological Development) by financial support, Martins M.L for grant, (CNPq 302493/2010-7) and Fujimoto, R.Y. (305195/2016-6) for scholarship. The authors also thank Santos, R.F.B., CAPES (Coordination for the Improvement of Higher Education Personnel) and Neves, M.S.

Ethics committee *CEUA*-004/14CEUA

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