CLOVE OIL AND MENTHOL AS ANESTHETIC FOR PLATY

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ABSTRACT

Studies with anesthetics in ornamental fish is still poor; so the aim of this study was to evaluate the induction and recovery times of platys (*Xiphophorus maculatus)* exposed to anesthetics clove oil and menthol. For the experiment, we used two groups of 40 juveniles fish, which were subjected to concentrations of 50, 100, 150 and 200 mg L^{-1} of the clove oil and 50, 100, 150, 200 and 250 mg L^{-1} of the menthol. In all concentrations evaluated of both anesthetics, animals reached deep anesthesia stage, without mortality observations after 96 h of the experiment. The concentrations 100 to 200 mg L^{-1} of clove oil and 100 to 250 mg L^{-1} of menthol provided a period of anesthetic induction and recovery considered within the ideal range. Thus, it is concluded that these anesthetic drugs, used in optimal concentrations, are effective for platys anesthesia.

Keywords: anesthesia; eugenol; induction; ornamental fish; recovery; *Xiphophorus maculatus*

ÓLEO DE CRAVO E MENTOL COMO ANESTÉSICOS PARA O PLATY

RESUMO

Estudos com anestésicos em peixes ornamentais ainda são incipientes; dessa forma, o objetivo deste trabalho foi avaliar os tempos de indução e recuperação de platys (*Xiphophorus maculatus)* expostos aos anestésicos óleo de cravo e mentol. Para a realização do experimento, foram utilizados dois grupos de 40 juvenis, os quais foram submetidos às concentrações de 50, 100, 150 e 200 mg L^{-1} de óleo de cravo e 50, 100, 150, 200 e 250 mg L^{-1} de mentol. Em todas as concentrações avaliadas de ambos os anestésicos os animais atingiram o estágio de anestesia profunda, sem observação de mortalidade após 96 h do experimento. As concentrações de 100 a 200 mg L^{-1} de óleo de cravo e 100 a 250 mg L^{-1} de mentol foram as que proporcionaram um período de indução e recuperação anestésica dentro da faixa considerada ideal. Dessa forma, conclui-se que os anestésicos, utilizados nas concentrações ideais, são eficazes para anestesia de platys.

Palavras chave: anestesia; eugenol; indução; peixes ornamentais; recuperação; *Xiphophorus maculatus*

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INTRODUCTION

In fish farming, practices performed during the management (biometrics, pathological analysis, hormonal applications and transport), often expose the fish to a variety of stressors, that may cause injuries and losses of scales and even losses on growth performance and death of the animals (BARTON, 2000).

One way to minimize this stress would be anesthetizing the animals, resulting in loss of sensitivity or insensitivity, which can occur at different levels: from a decrease, at safe levels, of specific physiological functions such as pain (local anesthesia) to the loss of consciousness, characterized by induced sleep and muscle relaxation (general anesthesia) (MASSONE, 2008).

The choice of anesthetic, however, should be related to several factors, such as its quick action on the nervous system (GONÇALVES *et al.*, 2008), economic feasibility, practicality, effectiveness and legal considerations (CHO and HEATH, 2000).

According to ROSS and ROSS (2008), is essential to know the optimal concentration of anesthetic that is required to induce the desired stage of anesthesia, since these concentrations vary depending on the species and size of fish and if used improperly are likely to cause death of fish.

Thus, many substances have been used for anesthesia of fish, among them natural anesthetics. Among the natural anesthetics produced in Brazil, those that are currently used are clove oil and menthol. The use of clove oil as an anesthetic for fish has been reported and pointed out as alternative to traditional anesthetics, used for handling and transport, because it is a natural oil inexpensive, safe to use, leaving no toxic residues in meat and environment (KEENE *et al.*, 1998; COOKE *et al.*, 2004). It is a phenolic substance obtained from dehydration of leaves, stems and flowers of dianthus (*Syzygium aromaticum*) (MAZZAFERA, 2003). The eugenol is the main component (70-95%) of clove oil, characterized as a yellow clear colorless liquid, volatile, with low water solubility. BARBOSA *et al.* (2007) evaluated the effect of eugenol on anesthetics baths in matrinxã (*Brycon amazonicus*) and concluded that it is suitable for anesthesia of this specie, and does not cause additional stress to the fish by its presence in concentrations of up to 60 mg L^{-1} for 10 minutes. Moreover, its effectiveness is greater than that of other substances which are used commercially (WAGNER *et al.*, 2003).

Menthol is an essential oil extracted from the genus Mentha plants (MARTINS, 2000). It has anesthetic and anti-inflammatory properties, and is of great economic importance in the pharmaceutical, cosmetic and food industry. Although proceeding from a medicinal plant, it is not known about the existence of waste in the carcass or alteration of flavor as well as on the physiological changes in fish resulting from the use of this product (GONÇALVES *et al.*, 2008).

In ornamental fish farming, studies that indicate better anesthetics, concentrations and responses in animals are still incipient; most of the works as mentioned above come from research on species of commercial purposes for consumption. Thus, this study is important because the objective is to evaluate the best concentration of clove oil and menthol anesthetics to platys (*Xiphophorus maculatus)*, verifying the anesthesia and recovery times of fish subjected to different concentrations of anesthetics.

MATERIAL AND METHODS

The experiment was conducted using two groups of 40 juvenile platys (*X. maculatus)* with average weight (± standard error) of 0.168 ± 0.03 g and 0.460 ± 0.04 g subjected to different concentrations of clove oil and menthol, respectively. The animals were acclimatized for 7 days in a system with tanks of 450 L with constant aeration and water recirculation. Feeding was carried out twice a day, *ad libidum*, at 9:00 h and 17:00 h, with commercial feed in flakes for ornamental fish. Daily siphonages were performed to remove the feces.

The experiment was conducted in a glass aquarium with a volume of 5 L, but containing 3 L of water. The clove oil (Biodinâmica Química e Farmacêutica Ltda.) and menthol crystals (Vetec Química Fina Ltda.) were diluted in ethanol (99.8%), resulting in a concentrated solution 1:10 for each anesthetic used. Preliminary tests were performed with the highest alcohol concentrations used in both experiments where it was observed that alcohol had no anesthetic influence on the evaluated animals.

Forty fish from each group were exposed to concentrations of 50, 100, 150 and 200 mg L^{-1} for clove oil and 50, 100, 150, 200 and 250 mg L^{-1} to menthol. The tests were carried out with each individual anesthetic, n = 10 for each group of clove oil and n = 8 for menthol group. After the induction period, recovery times were evaluated, so the animals were removed from anesthesia tank and transferred to another aquarium containing 10 L of anesthetic free water with constant aeration. The different stages of induction and recovery of anesthesia followed the criteria proposed by ROSS and ROSS (2008) adapted by SMALL (2003) (Chart 1). It was considered recovered the fish that restored the body balance and began to swim horizontally in the aquarium.

The time required for the appearance of the evaluated behavioral patterns was monitored with a digital stopwatch. The absence of reaction to external stimuli was observed by touching the fish lateral with a brush. After the reestablishment of the animals, they were put back in the recirculation system where remained for 96 hours with constant aeration and feeding for monitoring mortality.

Chart 1. Stages of anesthesia and recovery according ROSS and ROSS (2008), adapted by SMALL (2003).

Stage	Anesthesia	Recovery
I	Reduced swimming motion, but reaction to external stimuli and normal balance	Slight recovery of opercular movement and swimming movements
II	Loss of muscle movement and balance, decreased opercular movement and decreased reflexes to external stimuli	Balance recovery and mild reaction to external stimuli
III	Total loss of reflexes to external stimuli and opercular movement almost absent	Full recovery

The results were submitted to analysis of variance and means were compared by Tukey test at 5% probability, with statistical software SAS 9.0.

RESULTS

The physical and chemical parameters of water were (mean ± standard error): 8.23 ± 0.03 for pH, 6.23 ± 0.06 mg L^{-1} for dissolved oxygen and 27.33 ± 0.31 ºC for temperature.

In all clove oil concentrations fish reached stage 3, without mortality. The time of the three stages of anesthetic induction and recovery are shown in Table 1. The concentrations of 100 to 200 mg L^{-1} induced anesthesia below 3 min, therefore were considered optimal to induction of platys. There were significant differences (P<0.05) between concentrations for both induction and for recovery time. Observing the three stages of induction and recovery it can be noted that the concentration of 50 mg L^{-1} presented the highest induction and recovery time, differing significantly from other concentrations, except the 200 mg L^{-1} concentration on recovery. Clove oil was efficient in anesthesia of platys, because the product did not cause animal mortality and the animals recovered the movements securely.

As well as clove oil, menthol is an alternative to synthetic anesthetics used for anesthesia of fish. In the present study menthol was effective in the latency of the animals (Table 2), and did not cause mortality. Evaluating anesthesia with menthol, it can be observed that at all concentrations tested fish reached stage 3. The concentration of 50 mg L^{-1} had the highest induction time to stage 3 of anesthesia, significantly differing from the other concentrations (P<0.05). Just as clove oil, significant differences (P<0.05) between the concentrations in the stages of induction were found for menthol, but this did not occur with recovery times. Concentrations 100 to 250 mg L^{-1} induced fish anesthesia more quickly, with times shorter than three minutes.

Table 1. Induction and recovery time (minutes) of platys (*Xiphophorus maculatus*) subjected to different concentrations of clove oil.

Clove oil concentration	Induction time (min)			Recovery time (min)		
(mg L^{-1})	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
50	0.33 ± 0.03^{a}	0.52 ± 0.05^{a}	3.92 ± 0.70^{a}	1.38 ± 0.28^{a}	3.98 ± 0.58^{a}	5.68 ± 0.80^{a}
100	0.25 ± 0.02^{ab}	0.47 ± 0.05^{a}	1.07 ± 0.17^{b}	1.00 ± 0.13^{a}	2.10 ± 0.28^{b}	3.40 ± 0.35^{b}
150	0.15 ± 0.02^{b}	0.35 ± 0.03^{ab}	0.65 ± 0.08^{b}	1.03 ± 0.12^{a}	1.58 ± 0.17^{b}	3.20 ± 0.17^{b}
200	0.17 ± 0.02^{b}	0.25 ± 0.02^{b}	0.47 ± 0.03^{b}	1.57 ± 0.20^{a}	2.67 ± 0.35^{ab}	4.10 ± 0.32^{ab}

Data are presented as mean ± standard error. Means followed by the same letter in the column do not differ at 5% significance by Tukey test.

Table 2. Induction and recovery time (minutes) of platys (*Xiphophorus maculatus*) subjected to different concentrations of menthol.

Menthol concentration	Induction time (min)			Recovery time (min)		
(mg L^{-1})	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
50	0.58 ± 0.10^{ab}	3.10 ± 0.13^{a}	5.38 ± 0.39^{a}	1.92 ± 0.56^{a}	2.77 ± 0.60^{a}	5.45 ± 0.83^{a}
100	0.74 ± 0.12^{a}	1.31 ± 0.16^{b}	1.82 ± 0.12^{b}	2.06 ± 0.37^{a}	3.28 ± 0.45^{a}	5.71 ± 0.80^{a}
150	0.38 ± 0.03^{b}	0.81 ± 0.07^{c}	1.32 ± 0.12^{bc}	1.75 ± 0.37^{a}	2.64 ± 0.53^{a}	4.79 ± 0.64^{a}
200	0.34 ± 0.03^{b}	0.87 ± 0.08^{bc}	1.35 ± 0.13^{bc}	2.51 ± 0.61^{a}	3.67 ± 0.75^{a}	4.22 ± 0.45^{a}
250	0.34 ± 0.03^{b}	0.72 ± 0.11^{c}	1.15 ± 0.15^{c}	1.46 ± 0.22^{a}	2.72 ± 0.35^{a}	4.62 ± 0.86^{a}

Data are presented as mean ± standard error. Means followed by the same letter in the column do not differ at 5% significance by Tukey test.

DISCUSSION

Analyzing the experimental data with clove oil we can observe that the fish recovery time is influenced by the time of exposure to anesthetic, and as the concentration increased, the time of exposure to the anesthetic decreased, according to OSTRENSKY (2000). However, the results obtained with the use of menthol, indicate that only the induction time, i.e., exposure of the fish was affected by the anesthetic concentrations; thus there is no significant difference in recovery times. MELLO *et al*. (2012), by anesthetizing Nile tilapia (*Oreochromis niloticus*) with different concentrations of menthol also observed that with increased concentrations of anesthetic there is a reduction in the induction and recovery times of animals.

The time to achieve the stage 3 of anesthesia for concentrations of 100, 150 and 200 mg L^{-1} clove oil remained within the time limit considered safe for inducing anesthesia of animals (≤ 180 seconds) (KEENE *et al*., 1998). The recovery of the animals induced with the same concentrations was less than 10 min, considered safe by PARK *et al*. (2009). The same occurred with the concentrations of 100 to 250 mg L^{-1} menthol.

The eugenol (active ingredient found in clove oil) proved to be very effective in immobilizing numerous species of fish in the fish farming. Eugenol is characterized by presenting the fast induction of anesthesia and prolonged recovery, which is ideal to be used in biometrics, surgery or spawns management, because in these manipulations, it is necessary that the fish remains under anesthesia for a long period of time after being removed from anesthetic (PRINCE and POWELL, 2000). These data agree with the data presented in this study, where the animals anesthetized with clove oil obtained low induction times, considering the concentrations of 100 to 200 mg L^{-1}, as well as longer recovery times.

Eugenol has been proposed by INOUE *et al*. (2003) as an alternative anesthetic because it was an affordable natural product and no present apparent poisoning risk to anesthetized animals. In this sense, several studies also have reported the efficacy and safety of the use of menthol for anesthesia fish. FAÇANHA and GOMES (2005), anesthetizing tambaquis (*Colossoma macropomum*) reported that exposure of animals within 30 min to menthol, in proper concentration, does not cause fish mortality. SIMÕES and GOMES (2009), submitting tilapia (*O. niloticus*) to twice the concentration considered ideal for species, also did not obtain mortality of animals. Furthermore, the reduction in stress responses was also reported by PEREIRA-DA-SILVA *et al.* (2009) when anesthetizing lambaris (*Astyanax altiparanae*), showing that menthol has a good safety margin for anesthesia of fish. Likewise, in this study anesthetics tested showed the efficiency and safety of anesthesia platys, in which even the highest concentrations tested did not cause mortality of animals.

The induction and recovery times found in this study to anesthesia with clove oil are similar to those of OKAMOTO *et al*. (2009) with juveniles of *Trachinotus marginatus*. In this work, intervals were found between two and 11 min in the induction and 3-14 min for the fish recovery. GOMES *et al*. (2001) reported the results of the induction and loss of equilibrium in less than two minutes when placed in concentrations of 50 and 100 mg L^{-1} and times superior than seven minutes to the recovery. In all cases, the authors report efficiency in any of the tested concentrations, which can also vary depending on the size of fish.

Similar results to the present study were found in the anesthetic concentration of menthol used in tambaqui (*C. macropomum*), tilapia (*O. niloticus*), pacu (*Piaractus mesopotamicus*) and cachara (*Pseudoplatystoma reticulatum*) (FAÇANHA and GOMES, 2005; SIMÕES and GOMES, 2009; MELLO *et al*., 2012; SANCHEZ *et al*., 2014a,b).

All presented results support the idea that each fish species has a specific time for induction, according to their physiology. Thus, knowing that the managements are inevitable in all stages of development, reproduction, transportation and surgery, it is necessary to study the efficiency of different types of anesthetic products for ornamental fish that are less stressful and can reduce any mortality in routine practices to the animals.

CONCLUSIONS

The results indicate that anesthetics clove oil and menthol may be used for anesthesia of platys. The best results were obtained at concentrations of 100 to 200 and 100 to 250 mg L^{-1} clove oil and menthol, respectively.

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