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ARTIGO CIENTÍFICO

Clove oil, benzocaine and sodium chloride concentrations during the transport simulation of Nile tilapia

Concentrações de óleo de cravo, benzocaína e cloreto de sódio durante a simulação de transporte de Tilápia do Nilo

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Abstract: The objective of this study was to evaluate the effect of different concentrations of clove oil (eugenol), benzocaine and sodium chloride on the induction, return time and survival rate of Nile Tilapia (*Oreochromis niloticus*) during the transport simulation of 5 hours. The experimental design was completely randomized, with 9 treatments in three replicates: Treatment 1: control, Treatment 2: benzocaine 20 mg / L, Treatment 3: benzocaine 40 mg / L, Treatment 4: eugenol 3 ml / L, Treatment 5: eugenol, Treatment 6: eugenol 3 ml + 5 g salt, Treatment 7: eugenol 6 ml + 10 g salt, Treatment 8: 5 g / L salt, Treatment 9: 10 g / L salt. No significant difference was observed for immediate survival rate, survival rate between 24 and 48 hours. In relation to the induction times, those were lower and significant for benzocaine 40 mg / L, eugenol 6 ml / L and the association between eugenol and salt (6 ml / L + 10 g) in comparison with other treatments. The return times of the anesthetics benzocaine 40mg / L, eugenol 6 ml / L and conjugation between eugenol and salt (6ml / L + 10g) were lower in relation to the treatments with lower concentration. The survival rate demonstrated satisfactory results in all treatments. However, benzocaine at 40 mg / L concentration was considered the most effective anesthetic for tilapia because it presented shorter induction and recovery times.

Key words: Anesthetic; Fish; Induction time; Recovery time.

Resumo: Objetivou-se avaliar o efeito de diferentes concentrações de óleo-de-cravo (eugenol), benzocaína e cloreto de sódio no tempo de indução e retorno e na taxa de sobrevivência de Tilápia do Nilo *(Oreochromis niloticus)*, durante a simulação de transporte de 5 horas. O delineamento experimental foi inteiramente casualizado, com 9 tratamentos em três repetições: Tratamento 1: controle, Tratamento 2: benzocaína 20 mg/L, Tratamento 3: benzocaína 40 mg/L, Tratamento 4: eugenol 3 ml/L, Tratamento 5: eugenol 6 ml/L, Tratamento 6: 3 ml/L de eugenol + 5 g/L de sal, Tratamento 7: 6 ml de eugenol + 10 g/L de sal, Tratamento 8: sal 5 g/L, Tratamento 9: sal 10 g/L. Não foi observada diferença significativa para taxa de sobrevivência imediata, taxa de sobrevivência entre 24 e 48 horas. Em relação ao tempo de indução, este foi inferior e significativo para os tratamentos de benzocaína 40 mg/L, eugenol 6 ml/L e a associação entre 6 ml/L de eugenol mais 10g/L de sal em relação aos demais tratamentos. O tempo de retorno dos anestésicos benzocaína 40mg/L, eugenol 6 ml/L e conjugação entre 6ml/L de eugenol e 10g/L sal foi menor em relação aos tratamentos com menor concentração. A taxa de sobrevivência demonstrou resultados satisfatórios em todos os tratamentos. No entanto, a benzocaína na concentração 40 mg/L foi considerado o anestésico mais eficiente para tilápia por apresentar menores tempos de indução e recuperação.

Palavras-chave: Anestésico; Peixe; Tempo de indução; Tempo de recuperação.

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INTRODUCTION

The increase in fish production is due to the growing demand for fish in the domestic market and the country's immense potential for the activity, which could lead Brazil to become one of the largest fish producers in the world. The production of fish in Brazil, verified by IBGE, grew by 452% in the period from 2006 to 2013, from 104,888 tons to 474,329 tons (FAO, 2014; SILVA, 2017). In this scenario fish farming becomes an alternative for increases in this fish production. Among the species of national importance highlighting the tilapia that has quality in the production, good zootechnical performance low cost of production mainly in the initial stages (BOSCOLO et al., 2001; OLIVEIRA et al., 2010).

The intensification of fish production in addition to environmental factors, capture management, light intensity and transport leads to stressful situations such as reduced growth, survival and low immune system (LUZ; PORTELA, 2005; ABREU et al., 2009; DINIZ; HONORATO, 2012; NAVARRO et al., 2017).

Fish anesthesia can be applied during routine and necessary practices within fish culture, such as biometrics, pathological analysis and surgical procedures, to immobilize fish during handling or spawning and for transportation. All of these procedures can lead to a variety of stressors that have the potential to affect fish performance and sanity (GOMES, 2001; MELLO et al., 2012; NAVARRO et al., 2017). The importance of the knowledge and performance of methods that allow the of such activities with the minimum of interference in the vital and physiological functions of the fish is paramount for the improvement of the welfare of these animals during the several stages of its production process.

The main transport success factor is to enclose the highest fish density in the lowest possible water volume, with no mortality, deterioration of water quality (temperature, oxygen concentration and ionic constitution) and stress (TAKAHASHI et al., 2006; DELBON et al., 2012; NAVARRO et al., 2017). In order to minimize stress and reduce the mortality rate of fish exposed to long periods of transport and handling, the development of efficient high density transport methods is essential to reduce expenses and avoid losses during this stage of fish farming (UETA et al., 2007; ZAHL et al., 2009; LUZ et al., 2013).

Increasingly, the production of new equipment and transport procedures has led to a positive effect on the transposition of live fish (PARK et al., 2009). Anesthetics have often been used during fish transport in order to reduce agitation and stress (PARK et al., 2009). In addition, the positive effect of sodium chloride on fish transport has been reported by several researchers (ADAMANTE et al., 2008; BRANDÃO et al., 2008).

Several stages of induction and recovery have been described in the literature and these differ from one another according to behavioral characteristics that the animals present (WOODY et al., 2002). The determination of the adequate time for the animal to reach a certain anesthetic stage is of fundamental importance in the management planning that will be used in fish farming (ROUBACH; GOMES, 2001). The duration of each anesthetic stage varies according to the drug, method used, species and biological and environmental conditions (ROSS; ROSS, 2008).

An effective anesthetic should be easy to use, bearing low risks to animals and men; Short induction (three minutes) and recovery time (five minutes); Do not leave persistent effects on fish behavior when anesthetized successively; To present biocompatibility and good penetration properties in the action sites; Should not trigger allergic reactions; should be stable in solutions and undergo rapid biotransformation inside the body (NAVARRO et al., 2017).

Clove oil is a phenolic substance obtained from the distillation of clove leaves, stems and flowers, which has, as its main component, Eugenol (70 to 95%), that has been used for handling fish for short periods (ROTILI et al., 2012). Benzocaine is an anesthetic substance of the chemical ester group and in its original form is crystalline and white (Ethyl 4-aminobenzoate) (BARBOSA et al., 2007; ROSS; ROSS, 2008; NAVARRO et al., 2016).

Although sodium chloride (NaCl) is not an anesthetic substance, this is an important mitigating medium for the effects of stress in freshwater fish~~,~~ since it tends to equalize the osmotic gradient between the water and plasma of the fish, so that there is a reduction in the diffusion of ions into the water; It helps in the control of some parasites along the transport and stimulates the active excretion of the ammonia through the exchange of the ammonium ion in the blood of the fish by the sodium ions present in the water (CARNEIRO; URBINATI, 2001).

The objective of this study was to evaluate the effect of different concentrations of clove oil (eugenol), benzocaine and sodium chloride on the induction, return time and survival rate of Nile Tilapia (*Oreochromis niloticus*) during transport simulation.

MATERIAL AND METHODS

The experiment was conducted at the SEAPA-DF Fish Technology Center, located at BR 450, Km 30, Park Way, Brasília-DF.

A total of 1,800 fingerlings of Nile Tilapia (*Oreocromis niloticus*) of the variety G.I.F.T. (Genetically Improved Farm Tilapia) from the same fish farm were employed. Males and females of tilapia present age of 50 days with total average length of 5 ± 0.86 cm and final mean weight 6 ± 1.64 g. This selection was carried out with the help of the Bernauer model fish catcher model D6002.

The experimental design was completely randomized, with 9 treatments in three replicates: Treatment 1: control, Treatment 2: benzocaine 20 mg / L, Treatment 3: benzocaine 40 mg / L, Treatment 4: eugenol 3 ml / L, Treatment 5: eugenol 6 mg/L, Treatment 6: eugenol 3 ml + 5 g salt, Treatment 7: eugenol 6 ml + 10 g salt, Treatment 8: 5 g / L salt, Treatment 9: 10 g / L salt. Each treatment consisted of 50 fish. The test solutions were previously prepared prior to the experiment. The benzocaine was manipulated at the tested concentrations of 20mg / L and 40mg / L in a manipulation pharmacy, eugenol was diluted previously in 70% alcohol based on the studies of Mylonas et al. (2005). The salt was weighed on a precision scale in the amount required.

The fish were fasted for 24 hours and were packed in transparent plastic bags with a capacity of 20L, containing 5L of water, adding pure oxygen and the substances under study. Each experimental unit consisted of a plastic bag containing 50 fingerlings. The fish were kept in the bags in the laboratory for 5 hours, the average time used to transport fish in the day to day fish farming. The interference degree of anesthetics in fish behavior (Table 1) was registered according to (ROUBACH; GOMES, 2001, WOODY et al., 2002).

Table 1. Behavioral characteristics of Nile tilapia fingerlings.

Stage	Behavior	
	Roubach and Gomes (2001)	Woody et al. (2002)
1	Light sedation. Reaction loss to visual movements and touch.	Visible imbalance of opercular movements
2	Mild anesthesia. Partial equilibrium loss.	Partial equilibrium loss in maintaining normal swimming position when standing still
3	Deep anesthesia. Total loss of balance	Total loss of balance and inability to recover the vertical swimming position
4	Surgical anesthesia I. Decreased opercular movements	Absence of reaction to any stimulus
5	Surgical anesthesia II. Minimum opercular movement	-
6	Marrow collapse. Overdose	-

To calculate the time of return, after 24 and 48 hours of each experimental cycle, the animals were placed in boxes of water of 1000 liters with flow rate of 3L / s and with constant aeration. For the liberation in tanks, all the still closed bags were placed inside the reservoirs, until these reach the temperature equilibrium between the transport bags water and the reservoir water. After the acclimation and release step, the return time of the anesthetic to the normalization of fish reactions was timed based on the data from the anesthetic return table of Hikasa et al. (1986). The notation of this data started from stage II, since no opercular movements disappear, and followed up to stage V, observed in all animals of the treatments tested.

Table 2. Return of anesthetic to fish behavior.

Stage	Behavioral response
I	Reappearance of opercular movements
II	Partial return of balance and swimming capacity
III	Full balance recovery
IV	Swimming and reaction to external stimuli still faltering
V	Total balance recovery and normal swimming capacity

*Modified from Hikasa et al. (1986).

The temperature, dissolved oxygen and pH of the water were measured before and after the transport simulation procedure, using a calibrated thermometer, oximeter (YSI, USA) and commercial kit (Alfakit, Brazil).

The data obtained in the analyzes of this experiment were submitted to the Tukey test at 5% of probability using SAS software.

RESULTS AND DISCUSSION

The mean values obtained were: 21.74 ± 0.43 ° C for initial temperature, 21.33 ± 0.03 ° C for final temperature, 5.5 for pH; 6.64 ± 0.36 mg.L^{-1} for initial and final dissolved oxygen of 5.90 ± 0.1 mg. L^{-1} according to (NAVARRO et al., 2012).

The measured temperature and the pH in the experiment were below the values recommended as ideal for tilapia cultivation (Table 3) (KUBITZA, 1998). The lowest temperatures in all treatments are suitable for transport according to Kubitza (1998) because fish excrete less ammonia and also have decreased needs for oxygen.

The dissolved oxygen concentration of the water at the beginning and at the end of the experiment did not vary significantly between the different treatments (Table 3). According to Vidal et al. (2007), the increase in activity is the beginning of the behavior observed in an animal submitted to general anesthesia.

There was no change in the pH of the water at the beginning of the present study (pHI) and at the end (pHF) during transport simulation (pH 5.50), which was slightly below the ideal range (pH 6.0 to 8.5) for tilapia, according to Kubitza (1998).

Table 3. Temperature averages, dissolved oxygen and initial and final of the treatments tested.

Treatment	TI (ºC)	TF (ºC)	ODI (mg/L)	ODF (mg/L)
Benzocaine 20ml/L	21,76± 0,17	21,33± 0,57	6,56± 0,11	6,03± 0,0
Benzocaíne 40ml/L	21,73±0,92	21,33± 0,57	6,56± 0,11	6,00± 0,0
Eugenol 3mg/L	21,83±0,87	21,03± 0,57	6,56± 0,0	6,00± 0,0
Eugenol 6mg/L	22,03± 0,57	21,36± 1,05	6,56± 0,0	6,00± 0,0
Eugenol 3mg/L +Salt 5g/L	22,06±0,60	21,36± 0,63	6,36± 0,0a	5,93± 0,0a
Eugenol 6mg/L +Salt 10g/L	21,50±0,86	21,33± 0,63	7,00± 0,11	6,00± 0,0
Salt 5g/L.	21,83±0,60	21,33± 0,57	6,90± 0,0	6,00± 0,0
Salt 10g/L.	21,20±0,30	21,33± 0,57	6,63± 0,0	6,00± 0,0
Control	21,83±0,57	21,33± 0,56	6,63± 0,0	6,00± 0,0
C.V.	1,21	0,48	2,92	0,44

TI- initial temperature; TF - Final temperature; ODI - Initial dissolved oxygen; ODF - Final Dissolved Oxygen. Within each column, mean values (± standard errors) followed by dissimilar superscript lowercase letters are significantly different (Tukey test; *P* < 5%)

In the present study it was observed that the animals submitted to the anesthetic eugenol (clove oil) obtained darker coloration in comparison to the fish of other treatments. This result is in agreement with Vidal et al. (2008), who described the irritation reaction evidenced by hyperactivity at the first contact of the fish with the use of clove oil in piavuçu juveniles, *Leporinus macrocephalus* (1.77 ± 0.69 g).

The highest anesthetic induction time of 120 minutes was for eugenol 3ml / L solution that presented significant difference with the other treatments, followed by benzocaine solution 20 mg / L with 86.66 minutes, and the solutions with the shortest induction time were for benzocaine solutions 40 mg / L, eugenol 6 ml / L and eugenol + salt (6 ml / L + 10 g), which do not present significant difference between them. This higher efficiency of anesthetic inducers at higher concentrations indicates a direct and positive relationship between increased anesthetic concentration and decreased induction time, which favors the non-potentiation of stress in manipulated fish and, thus, an improvement in animal welfare (KUBITZA, 1998; ROSS; ROSS, 2008). Similar results were found in Ross and Ross (2008); Inoue et al. (2003); Vidal et al. (2008); Okamoto et al. (2009).

The anesthetic return time with benzocaine 40 mg/L, eugenol 6 ml/L and eugenol + salt (6ml/L + 10g) was higher in relation to the treatments with lower concentrations (Table 4). These results corroborate with some investigators that the increase in anesthetic exposure time or a greater amount of these elements to be metabolized leads to a reduction in induction time followed by an increase in recovery time (MASSONI, 1999; VIDAL et al., 2008).

Tables 4. Mean values of induction time (TI), return time (TR) and survival rate of Nile tilapia (*Oreochromis niloticus*) fingerlings.

Treatment	TI (seconds)	TR (minutes)
Benzocaine 20 mg./L	86,66±5,77b	10,00± 0,0f
Benzocaene 40 mg./L	30,00±0,0d	15,00± 0,0 e
Eugenol 3ml./L	120,00±0,0a	20,00± 0,0 d
Eugenol 6ml./L	30,00±2,88d	40,00± 0,0 b
Eugenol + Salt (3ml./L + 5g.)	60,00±0,0c	30,00± 0,0 c
Eugenol + Salt (6ml./L + 10g.)	33,33±2,88d	48,33± 2,8a
Salt 5g/L	0,00± 0,00 e	0,00± 0,0 f
Salt 10 g/L	0,00± 0,00 e	0,00± 0,0 f
Control	0,00± 0,00 e	0,00± 0,0 f
C.V.	104,00	99,70

Within each column, mean values (± standard errors) followed by dissimilar superscript lowercase letters are significantly different (Tukey test; $P < 5\%$).

The immediate survival rate was 100% of fingerlings for all treatments tested. The survival rate at 24 hours and at 48 hours after the experiment was also 100%, with no significant differences between treatments. The data showed that all treatments with anesthetics and sodium chloride are effective and safe in the transport of tilapia, complying with the purpose of keeping the maximum number of live and viable animals along the trajectory, being able to prevent the deleterious effects of stress can compromise the lives of animals up to 48 hours after release in the new environment. The data are in agreement with the results obtained with the use of the same substances done by Ross and Ross, (2008); Okamoto (2008); Oliveira (2009); Pramod et al. (2010) found that the use of 20mg / L benzocaine was sufficient to induce sedation for 48 hours in ornamental fish of P. filamentosus species, alleviating transport stress, as well as improving the post-transport survival rate. Massoni (1999) reported in his research that the ideal drug is one that allows a rapid recovery of the animal, thus minimizing the adverse effects and possible undesirable physiological interactions, allowing reflex returns, normal animal capacities and reducing the chance of predation, disease susceptibility, parasitism or even its own death.

The different concentrations of sodium chloride did not result in sedative effects for the treated fish, but, like the other treatments, maintained high survival rates for these animals. The addition of sodium chloride to water has been used for the prevention of stress and increased survival rate in the transport of matrinxã (*Brycon cephalus*) (URBINATI; CARNEIRO, 2001). Sodium chloride, although it does not cause sedative effects, contributed to a high survival rate and could, therefore, be considered a more economical and easily acquired alternative within the production system of Nile Tilapia.

CONCLUSION

Benzocaine 40mg/L was considered the most effective anesthetic for tilapia because it presented shorter induction and recovery times.

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