

## Anesthesia of silver catfish with eugenol: time of induction, cortisol response and sensory analysis of fillet

### Anestesia de jundiás com eugenol: tempo de indução, resposta ao cortisol e análise sensorial do filé

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#### ABSTRACT

The aim of this study was to identify the time of anesthetic induction and recovery of silver catfish (*Rhamdia quelen*) exposed to eugenol. It was also determined the efficacy of the anesthetic as a stress reducing agent and performed a sensory analysis of the fillets from fish exposed to this substance. The silver catfish were exposed to air for 1min to carry out biometry, and blood was collected at 0, 1 and 4 hours later. Eugenol can be used in the range of 20-50mg L<sup>-1</sup> for anesthetic induction in silver catfish, and recovery time from anesthesia was not affected by eugenol concentration. The control group showed significantly higher cortisol levels 4 hours after biometry than at time zero. Fish anesthetized with eugenol (50mg L<sup>-1</sup>) presented significantly lower plasma cortisol levels than control fish at the same time. These data indicate that eugenol inhibits the rise of cortisol in the blood. The sensory analysis test demonstrated that eugenol modifies the flavor of the fillet and therefore is contra-indicated for anesthetization of silver catfish that are intended for human consumption.

**Key words:** clove oil, *Rhamdia quelen*, sedation, stress, taste.

#### RESUMO

O objetivo deste estudo foi identificar o tempo de indução e recuperação anestésica de jundiás (*Rhamdia quelen*) expostos ao eugenol, bem como a eficácia desse anestésico na inibição do estresse e realizar análise sensorial dos filés dos peixes expostos a essa substância. Os jundiás foram expostos ao ar por um minuto para realização da biometria, e o sangue foi coletado zero, uma e quatro horas depois. O eugenol pode ser usado na faixa de 20-50mg L<sup>-1</sup> para a indução da anestesia

em jundiás, e o tempo de recuperação da anestesia não foi afetado pela concentração do eugenol. O grupo de controle mostrou níveis significativamente mais elevados do cortisol quatro horas após a biometria que no tempo zero. Os peixes anestesiados com eugenol (50mg L<sup>-1</sup>) apresentaram níveis significativamente mais baixos do cortisol plasmático do que peixes do grupo de controle do mesmo tempo. Esses dados indicam que o eugenol inibe o aumento do cortisol no sangue. O teste sensorial demonstrou que o eugenol modifica o sabor dos filés e consequentemente é contra-indicado para a anestesia do jundiá quando o filé for destinado ao consumo humano.

**Palavras-chave:** óleo de cravo, *Rhamdia quelen*, sedação, estresse, palatabilidade.

#### INTRODUCTION

Currently, procedures for managing and manipulating silver catfish (*Rhamdia quelen*) do not involve the use of anesthetics. However, due to increased interest in product quality, the use of sedatives is occasionally necessary to facilitate management and to reduce fish stress during handling. Stress induces cortisol secretion by the interrenal tissue, and this hormone causes various secondary stress responses, including increased glucose and lactate plasma levels (ROTLLANT et al., 2001; SKJERVOLD et al., 2001). Increased plasma levels of

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these parameters is an indication of glycogen mobilization and is associated with poor quality and rigidity of fish fillets (SKJERVOLD et al., 1999, 2001). High levels of plasma cortisol can also induce a decrease in the immunologic capacity of salmon (*Oncorhynchus tshawytscha*) (PICKERING & POTTINGER 1989) and channel catfish (*Ictalurus punctatus*) (DAVIS et al., 2002, 2003). Certain anesthetics, as metomidate (OLSEN et al., 1995), eugenol and isoeugenol (Aqui-S) (IVERSEN et al., 2003) can reduce or obstruct hypothalamus-hypophysis-interrenal axis activation, resulting in lower cortisol discharge (ROTLLANT et al., 2001; SKJERVOLD et al., 2001).

Clove oil is an anesthetic derived from distillation of plant parts from the *Eugenia* genus (*Eugenia caryophyllata* and *E. aromatica*) (LEE & SHIBAMOTO, 2001), and the active ingredient is eugenol (makes up to 90-95% of the clove oil), which functions as a depressor of the Central Nervous System (ANDERSON et al., 1997). Moreover, clove oil has been indicated as an alternative to traditional anesthetics as metomidate, quinaldine, and tricaine methanesulphonate because it is a natural oil and safe to use (SLADKY et al., 2001). The clove oil concentrations necessary for anesthesia induction vary by species. In channel catfish (*Ictalurus punctatus*), bluegill (*Lepomis macrochirus*) (STEHLY & GINGERICH 1999), Atlantic salmon (*Salmo salar*) (IVERSEN et al., 2003), rainbow trout (*Oncorhynchus mikiss*) (KEENE et al., 1998), black pacu (*Piaractus brachypomus*) (SLADKY et al., 2001), Chinook salmon (*Oncorhynchus tshawytscha*) (CHO & HEATH 2000) and largemouth bass (*Micropterus salmoides*) (COOKE et al., 2004) clove oil is effective at concentrations of 10-50mg L<sup>-1</sup>.

The aim of this study was to determine the optimal eugenol concentration for silver catfish anesthesia and to evaluate the time of anesthetic induction and recovery from anesthesia. Furthermore, it was examined the effect of eugenol on plasma cortisol levels of silver catfish exposed to handling stress and the sensory characteristics of the resulting fillets.

## MATERIAL AND METHODS

### Animals

Juvenile silver catfish were purchased from a fish culture and transported to the laboratory, where they were maintained for two weeks in continuously aerated 250L tanks, 21±1°C, pH 6.6-7.0, dissolved oxygen levels 5.8-7.2mg L<sup>-1</sup>. Juveniles were fasted for 24h prior to experiments.

### Anesthesia induction and recovery

Juvenile fish (2.14±0.01g and 7.0±0.1cm) were transferred to aquaria containing 1L of water and eugenol (Eugenol, Odontofarma®, Porto Alegre, Brazil) at concentrations (in mg L<sup>-1</sup>) of 5, 10, 20, 30, 40, 50, 60 or 70, first diluted in ethanol (1:20). Control experiments were performed using aquaria containing ethanol alone at the same concentration as used to the dilution of the highest eugenol concentration. To evaluate the time required for anesthesia induction, 20 juveniles were used for each concentration tested, and each juvenile was used only once, according to SCHOETTGER & JULIN (1967). The maximum observation time was 30min. After induction, juveniles were transferred to anesthetic-free aquaria to measure anesthesia recovery time.

### Measurements of plasma cortisol

Juvenile fish (194.89±12.5g and 26.0±0.6cm) were divided into two treatment groups: eugenol first diluted in ethanol (1:20) (50mg L<sup>-1</sup>) (this concentration was used because is the lower concentration to obtain anesthesia within a short period of time, around 111s, see results) or control (without anesthetic). Anesthesia was carried out in an aquarium containing 5L of water (1-2min), and biometry (weight and length measurements - time of air exposure: 1min) was performed. Following biometry, blood was collected from the caudal vein of 12 juveniles (time 0). The remaining anesthetized juveniles were placed in two 250L tanks and blood samples of 12 fish from one tank were collected 1h later and from 12 fish from another tank 4h after anesthesia. The control group was subjected to the same procedures as the test group except by the exposure to eugenol, and the fish were held while the biometric measurements were taken. Blood was collected using a Hamilton syringe, transferred to 2mL plastic tubes and later centrifuged 3000xg to separate plasma, which was kept under constant refrigeration. Plasma cortisol level was measured using a commercially available solid phase competitive chemoluminescent enzyme immunoassay kit (Immulite 2000) (Diagnostic Products Corporation, Los Angeles CA, USA). The specificity of the test was evaluated by comparing the parallelism between the standard curve and serial dilutions in PBS (pH 7.4) of the plasma samples. No differences were observed between human and fish samples. The standard curve, constructed with human samples ran parallel to that obtained using serial dilutions of silver catfish plasma. A high positive correlation (r<sup>2</sup>=0.9725) was obtained between curves. The coefficient of variation observed from fish ranged from 9 to 12%. Fish from which blood

was collected at time 0 were euthanized by severing the spinal cord immediately after blood collection. Fillets were obtained from these fish, which were later used for sensory analysis.

#### Sensory analysis

To determine whether there was a difference in the taste and odor attributes between the fillets from fish submitted to eugenol treatment (50mg L<sup>-1</sup>, 1-2min, see previous section "Measurements of plasma cortisol") and control fish (no eugenol) the difference from a standard method described by COSTELL (2002) was used. Fillets were cooked in a microwave oven (20g 1min<sup>-1</sup>) and evaluated by 26 untrained judges. The degree of taste and odor difference from control was measured on a seven-point scale, where 1=extremely better than control; 2=moderately better than control; 3=slightly better than control; 4=not different from control; 5=slightly worse than control; 6=moderately worse than control; 7=extremely worse than control. Samples were coded by random numbers and presentation of the samples included a hidden control. Sensory scores were obtained for the treated sample and for the control sample.

#### Statistical analysis

All data were submitted to a Levene test to verify the homogeneity of variance. Variance was found to be equivalent between different groups, so analysis of plasma cortisol levels were carried out using two-way ANOVA, followed by Tukey post hoc test. Data from sensory analysis were analyzed using the Mann-Whitney U test. The time of anesthetic recovery data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. Comparisons of time to anesthesia induction to stage 4 with 50mg L<sup>-1</sup> eugenol in silver catfish of two different sizes were analyzed using the Student T test. STATISTICA (version 5.1) was used for analyses and significance was set at a level of 95% (P<0.05).

## RESULTS

Fish exposed to 5 and 10mg L<sup>-1</sup> eugenol showed no evidence of anesthesia during the 30min evaluation period. In juveniles exposed to higher concentrations of eugenol, increasing eugenol concentration proportionally decreased induction time for 2 to 4 stages of anesthesia (Figure 1). No mortality was found during anesthesia induction and recovery between 20 and 50mg L<sup>-1</sup> eugenol administration. However, at 60 and 70mg L<sup>-1</sup>, some fish reached stage 5

(respiratory movements ceased) and consequently mortality increased to 20% and 65%, respectively. Ethanol alone showed no anesthetic effect.

No significant difference was found in the recovery time at the different concentrations of eugenol tested (206.4-260.2s). Likewise, there was no significant difference between the latency to anesthesia induction and recovery in juveniles of different sizes induced to stage 4 with 50mg L<sup>-1</sup> eugenol (time for anesthesia induction to stage 4-2g: 206.4±22.5s and 177g: 243.2±19.4s).

The control group showed significantly higher cortisol levels 4h after biometry than at time zero. Silver catfish anesthetized with eugenol showed significantly lower plasma cortisol levels than fish from the control group at the same time (P<0.05) (Figure 2).

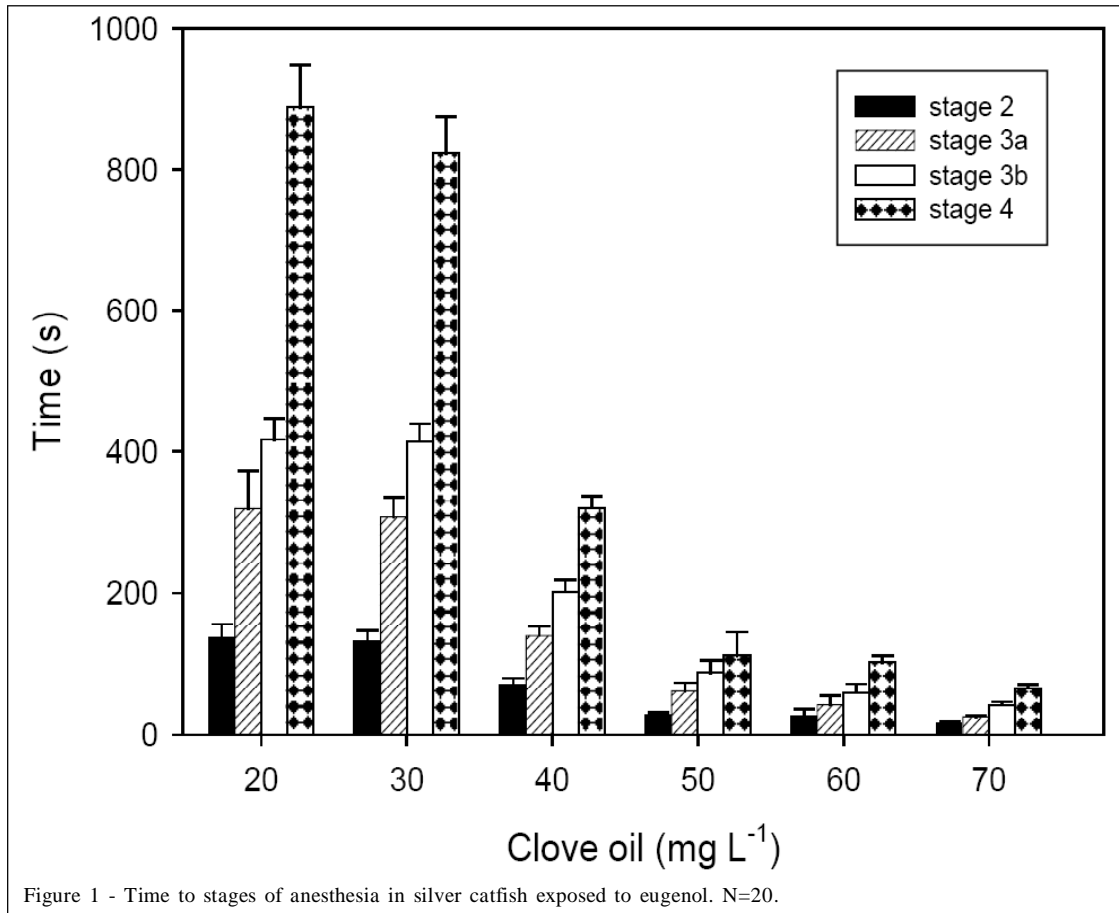
The median sensory score (interquartile range) of the odor of fillets obtained from silver catfish anesthetized with eugenol was 4 (3-5), like that from control fish 4 (3-5). However, the median taste score (interquartile range) of fillets from fish anesthetized with eugenol was significantly higher 6 (6-7) than that from control fish 4 (3-4) (P<0.05), indicating that the taste of filets from fish exposed to eugenol was considered worse than that from control fish (slightly to moderately).

## DISCUSSION

### Anesthesia induction and recovery

In this study, eugenol concentrations above 20mg L<sup>-1</sup> induced stage 4 of anesthesia in silver catfish within 15min, while lower concentrations had no anesthetic effect within 30min. A positive effect was determined by a rapid induction to stage 4 of anesthesia and a lack of harm to the fish at a concentration of 50mg L<sup>-1</sup>. At this concentration, stage 4 anesthesia was reached in 111s and death among the tested animals did not occur. Therefore, this concentration of eugenol was indicated for induction to stage 4 of anesthesia. The effect of clove oil varies according to the species, but 20-50mg L<sup>-1</sup> induced stage 4 of anesthesia within 120-360s in juvenile Chinook salmon (CHO & HEATH 2000), rainbow trout (KEENE et al., 1998), black pacu (SLADKY et al., 2001), and Atlantic salmon (IVERSEN et al., 2003).

The size of fish appears to be positively correlated with the latency to anesthesia induction (OLSEN et al., 1995), but no significant difference in the time to anesthesia induction to stage 4 with eugenol 50mg L<sup>-1</sup> was found relative to fish size for silver catfish in this study.



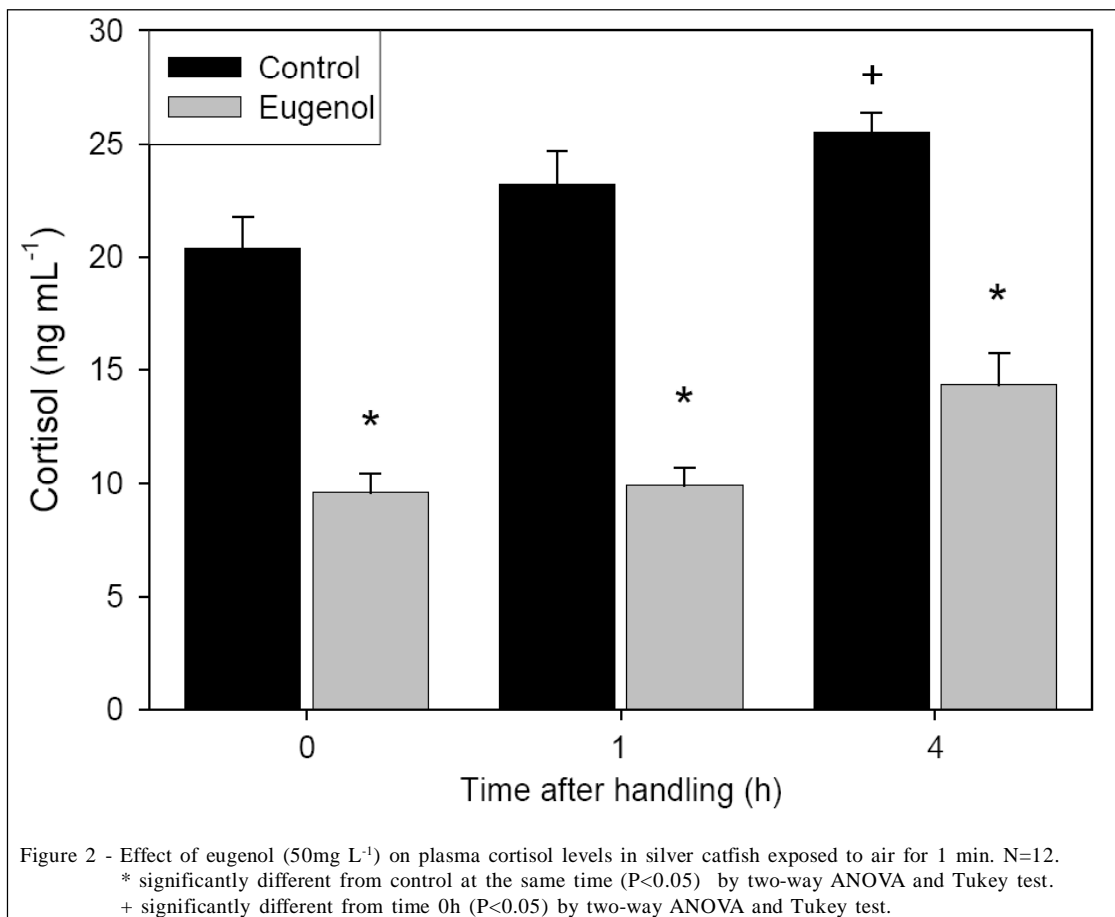
#### Evaluation of plasma cortisol

Clove oil at concentrations higher than 20mg L<sup>-1</sup> prevented plasma cortisol elevation above resting levels in Atlantic salmon, but its mechanism of action is not known. However, it is reasonable to presume that the drug disrupts transmission of sensory information to the hypothalamus (IVERSEN et al., 2003). One of the main fish responses to adverse situations is the production of catecholamines and corticosteroids, which are responsible for physiological and biochemical changes and are usually characterized as stress responses. Cortisol levels in non stressed fish vary from 5 to 51ng mL<sup>-1</sup> and after acute stress from 30 to 309ng mL<sup>-1</sup> (DAVIDSON et al., 2000). The resting cortisol level in non stressed silver catfish is 23.80±5.45ng mL<sup>-1</sup>, which is significantly lower than the level of chronically stressed fish (55.23±11.44ng mL<sup>-1</sup>) (BARCELLOS et al., 2006), and similar to the values obtained in the control fish (20-25ng mL<sup>-1</sup>) in the first two measurements. The increase of plasma cortisol values in control fish 4h after biometry was also observed by CUNHA et al. (2010) in an experiment using the same handling methodology. Cortisol

secretion is dependent on the severity and value of the stressor applied (SUMPTER et al., 1985). Rainbow trout exposed to air for 30s showed increased plasma cortisol levels 30min later (SLOMAN et al., 2001). In chronically stressed silver catfish the cortisol peak occurs 1h after exposure to a new stress factor (BARCELLOS et al., 2006). In our study at time zero the levels of plasma cortisol in control fish were already significantly higher than those anesthetized with eugenol, and a further increase (compared to time zero) occurred 4h after air exposure and handling. However, silver catfish anesthetized with eugenol presented significantly lower plasma cortisol levels than control fish at all analyzed times, which supports the hypothesis that this anesthetic prevents an increase of cortisol in the plasma at the moment of handling and air exposure. A similar result was obtained by CUNHA et al. (2010) using the essential oil of *Lippia alba* as anesthetic.

#### Sensory analysis

The sensory analysis is relevant in this study because it evaluates fish quality in the same way that the consumer would perceive fish quality.



Recent studies propose some method of anesthesia for fish slaughter practice (SAVENIJE et al., 2002; LINES et al., 2003; MATOS et al., 2010). AQUI-S™ is a fish anesthetic/sedative approved for use in several countries, and its active ingredient, isoeugenol, is approved for human consumption in the U.S. when used as a food flavoring. AQUI-S™ has been developed as an anesthetic that may be used on food-fish with no withdrawal period. Isoeugenol residues were detected in rainbow trout fillets after exposure to AQUI-S™ (MEINERTZ et al., 2006), but this compound is not known to produce adverse taste responses. Recently, it was demonstrated that exposure of *Solea senegalensis* to clove oil can assure a high quality product for the market. Moreover, the authors concluded that the results can also be applied to other species used in aquaculture as a method to assure high quality fish for human consumption (RIBAS et al., 2007). However, they did not evaluate the taste of their fish. In the present study, sensory analysis demonstrated that anesthesia with clove oil adversely affects the taste of silver catfish fillets. This change probably occurred due to the strong flavoring capacity

of clove oil, and suggests that some residues of the oil remain in the fish muscle. The Food and Drug Administration (FDA) does not approve clove oil as an anesthetic in fish for human consumption because of safety concerns (US FDA 2007). AQUI-S (or isoeugenol) is also not approved yet for use in fish (US FDA 2010). However, clove oil and eugenol have been widely used as a flavoring and antimicrobial agent in the food industry (VELLUTI et al., 2003; JECFA 2006). Although clove oil and many of its components are considered to be “generally recognized as safe” (GRAS) substances for use as food additives by the United States FDA (1978), there is some evidence of carcinogenicity for eugenol and methyleugenol (US FDA 2007). The joined results suggest that due to sensory concerns eugenol is contra-indicated for anesthesia at the moment of slaughter if the fish is intended to be used for human consumption.

Eugenol is an effective anesthetic for silver catfish and can be used at 20-50mg L<sup>-1</sup> without problems for the fish or the handlers, but care must be taken if the fish is to be used for consumption after anesthesia because eugenol leaves an unpleasant taste in the fillet.

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