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# Hematological biomarkers in tilapia: Effect of residual concentrations of clove oil and florfenicol

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## ABSTRACT
The use of clove oil as an anesthetic in fish management and florfenicol in the treatment of bacteriosis can leave residues in water causing damage to fish and environmental health. Hematological biomarkers are changes in blood parameters detected in organisms exposed to some agent or pollutant in water. Residues of clove oil and florfenicol on water may interfere with hematological biomarkers, impairing their interpretation during water quality monitoring. The objective of this study was to evaluate the effect of residual concentrations of clove oil and florfenicol on water on the response of hematological biomarkers in tilapia. The blood variables were evaluated in juvenile tilapias maintained for three and seven days in an aquarium system with a useful volume of 200 L without water recirculation. Three treatments with three replications were used, each aquarium containing nine tilapias as a sampling unit. Experiment 1 was composed of the treatments: control (without diluent and without clove oil), clove oil at a concentration of 0.5mg/L with alcohol diluent at a concentration of 10μL/L and only alcohol diluent at a concentration of 10μL/L. Experiment 2 was composed of the treatments: 0.0 (control); 0.5 mg/L and 5 mg/L florfenicol. The blood variables collected during the two experiments showed no significant difference, as did the water monitoring parameters, such as pH, dissolved oxygen, temperature, conductivity, and total ammonia. Residual concentrations of clove oil and florfenicol in water do not interfere in the response of hematological biomarkers in tilapia.

**Keywords:** *Oreochromis niloticus*, Eugenol, Antibiotic, Pollutant, Residues.

## 1 INTRODUCTION

Tilapia farming began in Brazil in the 1970s. Although not a native species, Nile tilapia (*Oreochromis niloticus*), the main species produced in Brazil, was introduced in 1971 (SCHULTER, FILHO, 2017). Native to Africa, Israel and Jordan, tilapia have easy reproduction, excellent quality white meat, great market value, low production costs and adapt to the most extensive to the most intensive farming systems, being able to be cultivated in waters with high salinities and low temperatures. (OLIVE TREE *et al.*, 2007). The expansion of aquaculture in Brazil has been showing a growing and significant increase, with an increase of 4.7% recorded in 2021 with a production above 840 thousand tons (PEIXEBR, 2022). Therefore, it is essential to adopt efficient productive management based on the development of innovative environmental systems and monitoring in order to optimize the environmental management and management of aquaculture (ISHIKAWA et al., 2020).

The development and validation of research methodologies and protocols for water analysis and environmental quality, especially monitoring using biomarkers and bioindicators, are essential to ensure the expansion and consolidation of aquaculture in Brazil (SIDONIO *et al*., 2012; ISHIKAWA *et al*., 2020). The biomarkers show the expressions of changes in animal homeostasis with biological response, whether at the biochemical, molecular and/or cellular level, in relation to the toxicological effects of chemical substances, while the biomarkers are plant and animal species that express the initial symptoms of environmental stress through the effect of contaminants. (ADAMS, 2002; VAN DER OOST; SINGER, VERMEULEN, 2003).

It should be noted that the causes of stress in fish are practically inevitable when it comes to the routine management of fish farming, and even environmental variations and natural factors also influence (URBINATI, CARNEIRO, 2004). Some strategies have been pointed out in order to alleviate the effects of stress in fish, both to stimulate the immune system (FUJIMOTO *et al*., 2005), and the use of anesthetics in some management practices (INOUE; SANTOS NETO, MORAES, 2004).

Anesthetics play an extremely important role in fish farming, as several substances are often used to anesthetize fish in order to relieve the stress reactions suffered in their management. MS-222 (tricaine methane sulfonate), quinaldine, benzocaine and phenoxyethanol have been widely used in Brazil, but some side effects are observed such as mucus loss, irritation in the gills and eyes. Thus, natural substances such as clove essential oil (eugenol) are proposed as an alternative anesthetic, as it is a natural product of affordable cost and with no apparent risk of poisoning (INOUE; SANTOS NETO, MORAES, 2003).

Clove essential oil is the result of the distillation of the leaves and flowers (including stalks) of clove trees (Eugenia aromatica) and the *active substance is eugenol, with a concentration ranging from 70% to 90% of the total composition of clove essential oil, considering it a safe anesthetic, of great efficacy, presenting a wide safety margin for the fish and absence of toxicity for the operator in the doses used in fish* (ISAACS, 1983).

Also as a result of stress, the consequences of damage to fish include increased susceptibility to pathogenic and infectious diseases (GIMBO *et al*., 2008). It is then necessary to use medications and antibiotics during the routine management of a production, such as florfenicol.

Florfenicol is considered an important antibiotic used in aquaculture and, currently, in Brazil, it is one of the few antibiotics for veterinary use that is registered and regulated for use (Ministry of Agriculture, Livestock and Supply, 2016).

Taking into account the frequent use of clove oil and florfenicol in aquaculture with the probability of residues remaining in the cultivation water, the objective of this study was to evaluate the effect of residual concentrations of clove oil and florfenicol in water on the responses of hematological biomarkers in tilapia.

## 2 MATERIAL AND METHODS

### 2.1 PLACE OF THE EXPERIMENTS

The experiments were conducted at the Aquaculture and Ecotoxicology Laboratory of Embrapa Environment, located in Jaguariúna, São Paulo, Brazil, and were carried out concurrently, but independently and without any interference between the two experiments. Both belong to the same research project and were approved by the Ethics Committee on the Use of Animals CEUA (Embrapa Environment) Protocol No. 007/2019 and Protocol No. 008/2019.

### 2.2 EXPERIMENTS AND TREATMENTS

In this study, fish from two experiments were carried out in a system of 18 aquariums with a useful capacity of 200 L, without water recirculation (Figure 1). Nine tilapia were used per aquarium, provided with supplementary aeration by means of a radial air compressor. The tilapia were purchased from a producer registered with Embrapa Environment's CEUA. The two experiments were completely randomized, with three treatments: the control (without the addition of clove oil and without the addition of diluent - common alcohol) in the water, the treatment with the addition of clove oil at a concentration of 0.5mg/L (100 times lower than the concentration used to anesthetize tilapia), with the addition of alcohol diluent at a concentration of 10µL/L and the treatment with the addition of only the oil diluent, i.e. from alcohol to 10µL/L of water. In the experiment with florfenicol, the following concentrations were used for the treatments: control (without the addition of florfenicol); 0.5mg of florfenicol/L of water (100 times lower than the concentration used in the treatment of bacteriosis) and 5mg of florfenicol/L of water (10 times lower than the concentration used in the treatment of bacteriosis). Three replicates were used for each treatment, with each aquarium containing nine tilapia as a sampling unit.

Figure 1 - Aquarium system of the experiments without water recirculation. Photo: Márcia Mayumi Ishikawa

## 2.3 PHYSICOCHEMICAL PARAMETERS OF WATER

During the experimental period, water quality parameters were monitored in the morning. With the aid of a multiparameter probe (U-50, Horiba, Minami-ku, Kyoto, Japan), pH, temperature (°C), dissolved oxygen (mg L-1) and electrical conductivity (µScm-1) were measured. Ammonia was also measured daily using the Labcon test kit.

## 2.4 HAEMATOLOGICAL ANALYSIS

After the period of three and seven days, three fish from each repetition were used for biometry and blood tests, totaling 27 fish. Biometry (weight in g and length in cm) and blood collection were performed after anesthetic induction with benzocaine 100 mg $^{L-1}$ (immersion bath). Blood collection was performed by caudal puncture with the aid of syringes containing EDTA (3%). Hematocrit (Htc%) was determined using the microhematocrit method according to GOLDENFARB; BOWYER, BROSIOUS (1971), hemoglobin (Hb; g dL-1) by the cyanomethaemoglobin (HCN) method, total plasma protein (TPP) with the aid of a refractometer, the blood glucose test was performed with the aid of a glucometer. The total number of erythrocytes was determined by the hemocytometer method, in a Neubauer chamber. After these procedures, the fish were euthanized by anesthetic deepening with benzocaine at 100 mg $^{L-1}$.

During the experiments, the occurrence of mortality and clinical symptoms such as injuries, hemorrhages or behavioral changes were evaluated.

# 3 RESULTS AND DISCUSSION

The parameters measured in the water quality monitoring of experiment 1 are shown in Table 1.

During the seven days of the experiment, there were no significant differences (p>0.05) for the physicochemical parameters measured. Although there were no significant differences, it was possible to observe that the total ammonia of the control group in three days (Table 1) maintained mean values of 1.74 ppm, but with a high standard deviation. This was due to the fact that the system does not have water recirculation, which favored the increase in its concentration. The temperature was around 25.7°C.

Table 1. Physicochemical parameters of tilapia water quality in a controlled environment with sublethal concentration of clove oil.

| **Physicochemical Parameters at 3 days** | | | | | |
|---|---|---|---|---|---|
| **Treatments** | **Temp. (°C)** | **ph** | **OD (mg $^{L-1}$)** | **Cond. (µScm$^{-1}$)** | **Ammonia (ppm)** |
| **Control** | 25.73± 0.47A | 6.88± 0.27A | 6.39± 0.70A | 0.08± 0.0A | 1.74± 1.16a |
| **Alcohol** | 25.85± 0.65A | 6.88± 0.25A | 5.76± 0.38a | 0.08± 0.0A | 1.55± 0.88a |
| **Clove Oil + Alcohol** | 25.79± 0.75a | 6.90± 0.21a | 6.37± 1.23a | 0.08± 0.0A | 1.34± 0.59a |
| **Physicochemical Parameters at 7 days** | | | | | |
| **Control** | 25.76± 0.46a | 6.60± 0.13a | 5.95± 0.76a | 0.09± 0.01a | 2.09± 0.34a |
| **Alcohol** | 25.71± 0.50A | 6.68± 0.14a | 5.41± 0.51a | 0.09± 0.01a | 1.90± 0.33a |
| **Clove Oil + Alcohol** | 25.70± 0.44a | 6.66± 0.15A | 5.51± 0.23a | 0.09± 0.01a | 1.76± 0.37a |

Temp. = Temperature; DO = Dissolved Oxygen; Cond. = Conductivity
Means followed by different letters in the same column differ according to Tukey's test ($P < 0.05$).

The parameters measured in the water quality monitoring of experiment 2 are shown in Table 2. During the seven days of experiment of experiment 2, there were no significant differences (p>0.05) in the physicochemical parameters measured.

No clinical signs or fish mortality were observed during the experiments, reinforcing that the residual concentrations of clove oil and florfenicol did not cause harm to the health of the tilapia.

Table 2. Physicochemical parameters of tilapia water quality in a controlled environment with sublethal florfenicol concentration.

| Physicochemical Parameters at 3 days | | | | | |
|---|---|---|---|---|---|
| **Concentration (mg $^{L-1}$)** | **Temp. (°C)** | **pH** | **ID (mg $^{L-1}$)** | **Cond. (μScm$^{-1}$)** | **Ammonia (ppm)** |
| **Control** | 25.98± 0.44a | 6.87± 0.28A | 6.20± 0.89a | 0.09± 0.01a | 1.61± 0.89a |
| **0.5** | 26.07± 0.48a | 6.98± 0.15a | 5.86± 0.51a | 0.08± 0.0A | 1.44± 0.46a |
| **5** | 26.10± 0.56a | 6.97± 0.17A | 5.86± 0.50A | 0.08± 0.0A | 1.39± 0.89a |
| **Physicochemical Parameters at 7 days** | | | | | |
| **Control** | 26.36± 0.58a | 6.67± 0.09A | 6.27± 0.75A | 0.09± 0.01a | 1.50± 0.25A |
| **0.5** | 26.57± 0.75a | 6.73± 0.21a | 6.22± 0.77a | 0.09± 0.01a | 1.72± 0.26a |
| **5** | 26.61± 0.75a | 6.71± 0.17a | 5.60± 0.28A | 0.09± 0.01a | 1.72± 0.36a |

Temp. = Temperature; DO = Dissolved Oxygen; Cond. = Conductivity
Means followed by different letters in the same column differ according to Tukey's test ($P < 0.05$).

The mean weights (g) and lengths (cm) of the fish and their respective coefficients of variation for experiment 1 are shown in Table 3. The observed parameters showed differences within the expected range to ensure the homogeneity of the treatments.

Table 3. Biometric parameters of tilapia in a controlled environment with sublethal concentration of clove oil.

| Biometric parameters at 3 days | | | | | | |
|---|---|---|---|---|---|---|
| **Treatments** | **Weight (g)** | **CV (%)** | **C. Standard(cm)** | **CV (%)** | **C. Total (cm)** | **CV (%)** |
| **Control** | 52.19±8.85 | 16,97 | 11.96±0.83 | 6,95 | 14.50±0.90 | 9,37 |
| **Alcohol** | 47.17±10.72 | 22,72 | 11.51±1.21 | 10,55 | 14.08±1.32 | 6,18 |
| **Clove Oil + Alcohol** | 48.95±12.77 | 26,09 | 11.79±0.76 | 6,41 | 14.56±1.03 | 7,09 |
| **Biometric Parameters at 7 days** | | | | | | |
| **Control** | 56.66±11.35 | 20,03 | 12.18±0.76 | 6,47 | 14.86±0.95 | 6,41 |
| **Alcohol** | 52.51±9.03 | 17,20 | 11.74±0.76 | 6,44 | 14.47±0.98 | 6,76 |
| **Clove Oil + Alcohol** | 53.27±9.17 | 17,22 | 11.90±0.77 | 6,22 | 14.69±0.93 | 6,35 |

C. Standard = Standard Length; C. total = Total length; CV = Coefficient of variation

The mean weights (g), lengths (cm) of the fish and their respective coefficients of variation for experiment 2 are shown in Table 4. Although the coefficient of variation was higher in some treatments, the averages were kept within the expected range to ensure the homogeneity of the treatments.

Table 4. Biometric parameters of tilapia in a controlled environment with sublethal florfenicol concentration.

| Biometric parameters at 3 days | | | | | | |
|---|---|---|---|---|---|---|
| **Concentration (mg L-1)** | **Weight (g)** | **CV (%)** | **C. Standard(cm)** | **CV (%)** | **C. Total (cm)** | **CV (%)** |
| **Control** | 74.81±13.02 | 17,41 | 13.42±0.62 | 4,59 | 16.40±0.80 | 4,89 |
| **0.5** | 61.54±30.11 | 48,94 | 13.18±1.08 | 8,19 | 16.31±1.44 | 8,81 |
| **5** | 70.82±13.38 | 18,89 | 13.09±1.06 | 8,13 | 16.02±1.02 | 6,36 |
| **Biometric Parameters at 7 days** | | | | | | |
| **Control** | 74.64±7.66 | 10,27 | 13.53±1.07 | 7,90 | 16.62±1.12 | 6,76 |
| **0.5** | 74.80±13.02 | 18,75 | 13.31±1.23 | 9,20 | 16.39±1.32 | 8,07 |
| **5** | 73.22±15.92 | 21,75 | 13.19±0.78 | 5,92 | 16.24±0.93 | 5,72 |

C. Standard = Standard Length; C. total = Total length; CV = Coefficient of variation

Hematocrit, total plasma protein, hemoglobin, erythrocytes, and blood glucose levels did not show significant differences (p>0.05) in response to the treatments used (Tables 5 and 6). These data reinforce that the residual concentrations of clove oil and florfenicol did not cause harm to the health of tilapia.

Table 5. Blood variables of tilapia in a controlled environment with sublethal concentration of clove oil.

| Blood Variables at 3 days | | | | | |
|---|---|---|---|---|---|
| **Treatments** | **Htc (%)** | **PPT (g $L^{-1}$)** | **Hb (g dL)** | **Erit. (x106 L)** | **Blood Glucose(mg dL)** |
| **Control** | 33.61± 2.92a | 4.17± 0.35a | 10.58± 1.14a | 1.64± 0.57a | 46.56± 9.37a |
| **Alcohol** | 31.44± 4.19a | 4.0± 0.5A | 10.90± 2.18a | 2.08± 0.62a | 46,11± 10,87a |
| **Cavo Oil + Alcohol** | 32.61± 4.08a | 3.79± 0.67a | 10.87± 0.88a | 1.57± 0.58a | 41.67± 8.51a |
| **Blood Variables at 7 days** | | | | | |
| **Control** | 28.22± 3.23a | 4.02± 0.34a | 12.34± 2.18a | 1.94± 0.62a | 43.11± 8.37a |
| **Alcohol** | 27.44± 4.59a | 4.19± 0.25a | 11.57± 1.68a | 1.66± 0.36a | 54,33± 14,76a |
| **Clove Oil + Alcohol** | 28.0± 4.12a | 4.30± 0.66a | 12.96± 1.60A | 1.84± 0.26a | 42.78± 8.77a |

Htc = Hematocrit; PPT = Total Plasma Protein; Hb = Hemoglobin; Erit. = Erythrocytes
Means followed by different letters in the same column differ according to Tukey's test (P < 0.05).

Table 6. Blood variables of tilapia in a controlled environment with sublethal florfenicol concentration.

| Blood Variables at 3 days | | | | | |
|---|---|---|---|---|---|
| **Concentration (mg L-1)** | **Htc (%)** | **PPT (g $L^{-1}$)** | **Hb (g dL)** | **Erit. (x106 L)** | **Blood Glucose(mg dL)** |
| **Control** | 31.5± 4.45A | 4.22± 0.44a | 9.92± 1.08A | 1.87± 0.49a | 44.33± 5.94a |
| **0.5** | 34.67± 8.67a | 4.33± 0.71a | 10.85± 1.13a | 1.85± 0.50A | 45.11± 5.46a |
| **5** | 35.22± 4.77a | 4.11± 0.33a | 11.25± 0.71a | 1.72± 0.35a | 43,11± 11,15a |

| Blood Variables at 7 days | | | | | |
|---|---|---|---|---|---|
| **Control** | 29.89± 3.26a | 4.33± 0.71a | 10.01± 0.62a | 2.02± 0.24a | 45.89± 9.17a |
| **0.5** | 31.11± 2.32a | 4.22± 0.44a | 10.50± 1.59a | 2.01± 0.30A | 43.0± 8.79a |
| **5** | 30.33± 1.94a | 4.11± 0.60A | 10.59± 2.25A | 2.08± 1.05a | 40.78±5.17A |

Means followed by different letters in the same column differ according to Tukey's test ($P < 0.05$).

According to McDONALD; MILLIGAN (1997), stress causes hemoconcentration in many teleost fishes, altering hematocrit values and other hematological parameters. On the other hand, PETERSON (1990) clarifies that the increase in hematocrit is observed as a result of erythrocyte swelling, decrease in plasma volume, increase in the number of red blood cells or a combination of these factors. In relation to fish submitted to sublethal concentrations of clove oil, there was no significant difference, corroborating the results of WAGNER; SINGER, McKINLEY (2003), who did not observe changes in the hematocrit levels of fish anesthetized with clove oil. In this study, it was observed that there were no significant changes in blood glucose in the two experiments, reinforcing the absence of stress in the fish.

With the increasing use of clove oil in fish management, several studies have been conducted to test its potential as an anesthetic. Although these studies present data for anesthetic induction, it is possible to use the results for comparison and confirmation that at residual concentrations the substance does not have the potential to alter hematological parameters in tilapia, allowing the use of these biomarkers in the monitoring of other agents or pollutants without the interference of this clove oil residue.

According to SIMÕES *et al.* (2012), the concentration of 250 mg $^{L-1}$ of clove oil was selected as the most appropriate to achieve the total arrest of the opercular movements of the test organisms, presenting significant differences in hematocrit and hemoglobin values. On the other hand, in the present study, the concentrations tested were 2,500 times lower, showing that in residual concentrations, the hematological response was different from those obtained with anesthetic concentrations, favoring the interpretation of the results of hematological biomarkers during water quality monitoring.

The use of the antibiotic florfenicol has been studied to evaluate its potential in the treatment of bacteriosis in fish (CARRASCHI *et al*., 2011; GAIKOWSKI *et al*., 2013; OLIVEIRA *et al*., 2018). The frequent use of this antibiotic in therapeutic doses favors the occurrence of residual concentrations in the water, and monitoring is important to ensure water quality. In this work, it was observed that these residual concentrations do not cause changes in hematological parameters in tilapia, allowing the use of these biomarkers in the monitoring of other agents or pollutants without the interference of florfenicol residue. For the standardization of biomarkers in fish in water quality monitoring, it is important to evaluate other biomarkers, such as biochemical, enzymatic, and pathological biomarkers (ISHIKAWA *et al*., 2020).

## 4 CONCLUSION

Residual concentrations of clove oil and florfenicol in the water do not interfere with the response of hematological biomarkers in tilapia.


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## REFERENCES


ADAMS, M. Biological indicators of aquatic ecosystem stress. American Fisheries Society, EUA. 2002, p. 656.

CARRASCHI, S. P.et al. Eficácia do florfenicol e da oxitetraciclina no controle de *Aeromonas hydrophila* em pacu (*Piaractus mesopotamicus*). Arquivo Brasileiro de Medicina Veterinária e Zootecnia, v. 63, n. 3, p. 579-583, 2011.

FUJIMOTO, R. Y.et al. Efeito da suplementação alimentar com cromo trivalente em pacu, *Piaractus mesopotamicus* (Holmeberg, 1887) mantido em diferentes densidades de estocagem. Parâmetros Fisiológicos. Boletim Instituto da Pesca, v. 31, n. 2, p. 155-162, 2005.

GAIKOWSKI, M. P.et al. Safety of florfenicol administered in feed to tilapia (*Oreochromis sp.*). Toxicologic Pathology, v. 41, p. 639-652, 2013.

GIMBO, R. Y.et al. Diferentes concentrações de benzocaína na indução anestésica do lambari-doraboamarelo (*Astyanax altiparanae*). Revista Brasileira de Saúde e Produção Animal, v. 9, n. 2, p. 350- 357, 2008.

GOLDENFARB, P. B.; BOWYER, F. P.; BROSIOUS, E. Reproducibility in the hematology laboratory: the microhematocrit determination. American Journal of Clinical Pathology, v. 56, p. 35-39, 1971.

INOUE, L. A. K. A.; SANTOS NETO, C. dos; MORAES, G. Clove oil anaesthetic for juveniles of matrinxã Brycon cephalus (Gunther, 1869). Ciência Rural, v. 33, n. 5, p. 943-947, 2003.

INOUE, L. A. K.; SANTOS NETO, C. dos; MORAES, G. Standardization of 2-phenoxyethanol as anesthetic for juvenile brycon cephalus (Gunther, 1869): the use in field procedures. Ciência Rural, v. 4, n. 2, p. 563- 565, 2004.

ISAACS, G. Permanent local anaesthesia and anhidrosis after clove oil spillage. Lancet, v. 321, p. 882, 1983

ISHIKAWA, M. M.; QUEIROZ, J. F. de; NASCIMENTO, J. L. do; PÁDUA, S. B. de; MARTINS, M. L. Uso de biomarcadores em peixe e boas práticas de manejo sanitário para a piscicultura. Jaguariúna: Embrapa Meio Ambiente, 2020. 28 p. (Embrapa Meio Ambiente. Documentos, 126).

Ministério da Agricultura, Pecuária e Abastecimento. Sobre produtos veterinários, dez. 2016. Disponível em: <https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-pecuarios/produtos-veterinarios/sobre-produtos-veterinarios>. Acesso em: 24 jul. 2020.

McDONALD, G.; MILLIGAN, L. Ionic, osmotic and acid-base regulation in stress. In: IWANA, G. W.et al. (Ed.). Fish stress and health in aquaculture. Cambridge: University Press, 1997. p. 119-144.

OLIVEIRA, E. G. de et al. Produção de tilápia: mercado, espécie, biologia e recria. Teresina: Embrapa Meio-Norte, 2007. 12 p. (Embrapa Meio-Norte. Circular Técnica, 45).

OLIVEIRA, T. F.et al. Recurrent *Streptoccoccus agalactiae* infection in Nile tilapia (*Oreochromis niloticus*) treated with florfenicol. Aquaculture, v. 493, p. 51-60, 2018.

PEIXE BR 2022. Anuário peixe BR da piscicultura. Disponível em: <https://www.peixebr.com.br/anuario2022/>. Acesso em: 14 dez. 2023.

PETERSON, M. S. Hypoxia-induced physiological changes in two mangrove swamp fishes: sheepshead minnow, *Cyprinodon variegatus* lacepede and sailfin molly, *Poecilia latipinna* (Lesueur). Comparative Biochemmstry and Physiology. Part A, v. 97, p. 17-21, 1990.

SCHULTER, E. P.; VIEIRA FILHO, J. E. R. Evolução da piscicultura no Brasil: diagnóstico e desenvolvimento da cadeia produtiva de tilápia. Rio de Janeiro: *Instituto de Pesquisa Econômica Aplicada*, 2017. p. 13-14.

SIDONIO, L.et al. Panoramada aquicultura no Brasil: desafios e oportunidades. Agroindústria BNDES Setorial, v. 35, p. 421-463, 2012.

SIMÕES, L. N.et al. The use of clove oil as an anesthetic for advanced juvenile tilapia (*Oreochromis niloticus*). Acta Scientiarum. Animal Sciences, v. 34, n. 2, p.175-181, 2012.

URBINATI, E. C.; CARNEIRO, P. C. F. Práticas de manejo e estresse dos peixes em piscicultura Intensiva. Disponível em: <https://www.researchgate.net/publication/286776959_Praticas_de_manejo_e_estresse_dos_peixes_em_piscicultura>. Acesso em: 25 jul. 2020.

VAN DER OOST, R.; BEYER, J.; VERMEULEN, N. P. E. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, v. 13, p. 57-149, 2003.

WAGNER, G. N.; SINGER, T. D.; McKINLEY, R. S. The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquaculture Research, v. 34, p. 1139-1146, 2003.