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Efficacy and Physiological Responses of Payangka Fish (*Ophieleotris aporos*) to Anesthetization with Clove Oil

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Abstract: This study aims to determine the effect of clove oil concentration and time of immersion in clove oil on the anesthesia, recovery time, and physiological responses in the payangka fish (*Ophieleotris aporos*). The experimental design was completely randomized with a 6 x 3 factorial arrangement. The first factor was a concentration of clove oil consisting of six levels i.e., 0.02; 0.03; 0.04; 0.05; 0.1, and 0.2 ml/L of water. The second factor was a time of immersion consisting of three levels i.e., 1; 3; and 6.5 hours. Results showed that the concentration of clove oil significantly ($p<0.05$) affected the anesthesia time and the recovery time after immersing. The length of immersing and concentration of clove oil also significantly ($p<0.05$) affected the concentration of glucose, but not significantly ($p>0.05$) affected the concentration of cholesterol. It showed that clove oil can be used as anesthetization for payangka fish in the appropriate dose. The optimal concentration of clove oil that can be used as anesthesia for the transportation of live payangka fish is 0.02 ml/L of water. Meanwhile, for handling activities on payangka fish, a clove oil concentration of 0.03 ml/L of water is recommended.

Keywords: Anesthesia; Clove oil; *Ophieleotris aporos*

Introduction

Research on local fish needs to be done to find out the potential of these fish and support their sustainability (Setyowati & Agustin, 2022). Payangka fish (*Ophieleotris aporos*) is one of the endemic fish from Sulawesi Island, Indonesia, which lives naturally in Tondano Lake. Payangka fish is a source of animal protein. The male payangka fish also have a bright and beautiful color along their body, so many people use them as ornamental fish. Because of that this fish has a big potential to be popular fish and will be distributed to another region. Market demand for fresh fish continues to increase every year. Trading fish in live form can benefit consumers, and also the traders because the price can reach three to four times the price of dead fish. Apart from that, the nutritional content of live fish is still high and the fish meat tastes better. A common obstacle faced is that transporting fish over long distances can increase fish mortality. Therefore, the stunning method is one solution to maintain the survival rate of fish (Hidayat et al., 2018).

Fish face several potential stressors, particularly in transportation, capture, and handling procedures. Those activities may increase the organism's stress level and significantly affect fish physiology and survival (Manuel et al., 2014; Belema et al., 2017). One of the initial efforts that can be done in supporting the payangka fish aquaculture is to determine the right and good anesthetic material as the tools to reduce stress. Administering an anesthetic to fish is important as it minimizes stress during manual spawning, tagging, weighing, handling, surgery, vaccination, blood and tissue collection, and antibiotic application. For aquaculture purposes, anesthetized fish can be more

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readily transported in bulk over long distances in containers (Purbosari et al., 2019).

The use of natural materials in aquaculture activities has been widely carried out. Starting from its use as a source of feed (Halimatussakdiah et al., 2021; Azzahra et al., 2023; Efendi et al., 2023; Fatmawati., 2023) and even to post-harvest activities (Vidiastuti & Arfiati, 2023). Clove oil is one of the natural material products that have many uses (Kamble et al., 2014; Batiha et al., 2020; Nirmala et al., 2022; Kiki, 2023). Clove oil can be used as a natural anesthetic extracted from the clove plant. Several studies have shown the use of clove oil as a natural anesthetic (Barata et al., 2016). The effect of giving clove oil as a natural anesthetic agent on fish depends on several factors, such as; dose, temperature, and duration of immersion (Hur et al., 2019).

Based on the above background, this research is important to be conducted to support the availability of payangka stock (as a protein source or ornamental fish) in another region, and because of that annotation, it must be done to keep the fish still alive. So this study aimed to determine the optimal dose of clove oil as an anesthetic in handling and transporting payangka fish.

Method

Experimental design

The experimental design was completely randomized with a 6 x 3 factorial arrangement. The first factor was a concentration of clove oil consisting of three levels i.e., 0.02; 0.03; 0.04; 0.05; 0.1, and 0.2 ml/L of water. The second factor was the time of immersion consisting of three levels i.e., 1; 3; and 6.5 hours. A total of 90 fish were divided into nine groups, each group contained 5 fish (each fish was replication).

Experimental Procedure

The payangka fish (mean length: 16.0 ± 2.0 cm) used in this experiment were collected from Tondano Lake. All fishes used in this study are male. At the start of the experiment, all fish were fed commercial feed (3% per total body weight) daily. The fish were acclimated for 1 week. Before treatment, all the fish have fasted for 24 hours. The clove oil used in this research is a commercial clove oil extracted from leaves of *Syzygium aromaticum* using the steam distillation method (100% pure essential oil by Atsiri Farmer Indonesia, Gunung Putri, Bogor, Jawa Barat). Anesthesia levels and recovery times for fish were measured using a stopwatch. After anesthetizing each fish in the anesthetic tank, they were immediately moved to the corresponding recovery tank that was filled with fresh water.

The criteria for anesthesia and recovery status according to Hur et al., (2019). The criteria for suitable recovery from anesthesia were based on the movement of the mouth and operculum, and reversion to a straight position. The time spent to recover was determined when the ventilation rate after the experiment was similar to that before the experiment. The physiological response was measured after the end of the time of immersing in each treatment before moving to the recovery tank. Blood was withdrawn from the caudal vasculature of the fish using a disposable syringe (1 ml; MDI Europa GmbH, Langenhagen, Germany). The concentration of glucose and blood cholesterol were analyzed using Autochek GCU multi-monitoring System (General life Biotechnology Co.,Ltd. Taiwan). The groups that we used for physiological response i.d., 0.02 mL clove oil/L water for 1 and 3 hours of immersing; 0.03 mL clove oil/L water for 1 and 3 hours of immersing; and 0.04 mL clove oil/L water for 1 and 3 hours of immersing.

Statistical analysis

The data obtained were analyzed by using analysis of Variance (ANOVA) on Minitab version 16 program. The differences between the means of the treatment were tested by using Tukey simultaneous test. All results that significantly different were expressed with $p<0.05$.

Result and Discussion

The time of the anesthesia data is shown in Figure 1. The concentration of clove oil showed a significant difference ($p<0.05$) in the length time of anesthesia. The lowest concentration of clove oil (0.02mL/L of water) significantly ($p<0.05$) has a longer time to anesthesia the payangka fish. Otherwise, the highest concentration of clove oil (0.2mL/L of water) significantly ($p<0.05$) has a shorter time to anesthesia the payangka fish. Clove oil contains eugenol compounds which have antioxidant, antifungal, analgesic, and antiseptic activities (He et al., 2022). In general, clove oil was an efficient anesthetic at low concentrations (Fernandez et al., 2017). According to Kheawfu et al., (2022), the anesthetic mechanism of action of clove oil is based on the modulation of [^{3}H] muscimol binding to $GABA_A$ receptors located in the brain. Clove oil exerted a conspicuous depression of muscle contraction power (Fujimoto et al., 2018). A study conducted by Jannah et al., (2022) showed that the time to faint will be faster as the dose of clove oil increases. This study also showed the same result that the highst concentration gives a shorter time to faint.

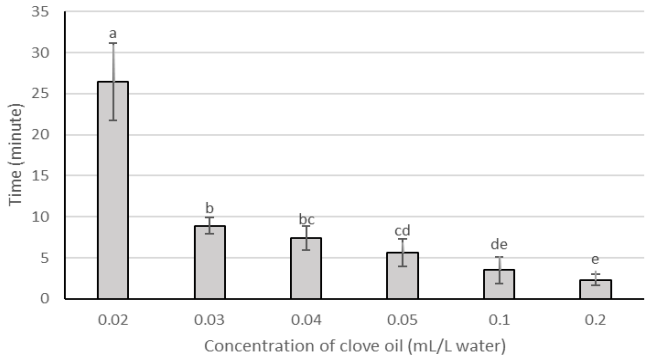

Figure 1. Time of anesthesia. Different superscripts indicate a significant difference ($p<0.05$).

The recovery time after immersing in clove oil in different concentrations showed that the concentration of clove oil has a significant effect ($p<0.05$) on the time of recovery (Figure 2). The highest concentration results in a longer length of recovery time. The fish immersed in 0.2 ml clove oil/L of water for 1 hour showed a more long time of recovery time, significantly different ($p<0.05$) from the fish that were immersed in a lower concentration of clove oil. Meanwhile, there is no significant difference ($p>0.05$) in the length of time recovery between immersing in 0.02, 0.03, 0.04, and 0.05 ml clove oil/L of water but significantly differ ($p<0.05$) with a concentration of 0.1 clove oil/L of water.

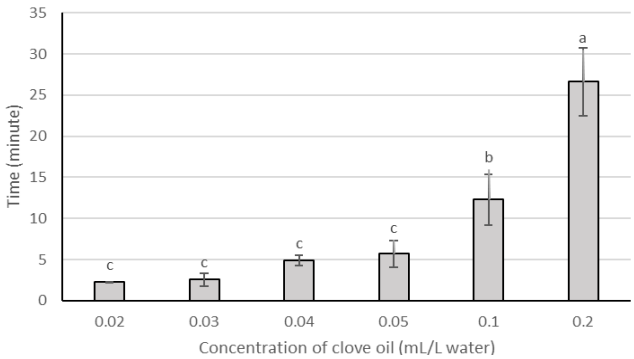

Figure 2. Time of Recovery after Immersing for 1 hour. Different superscripts indicate a significant difference ($p<0.05$).

The same pattern of recovery time in the treatment of immersing time for 1 hour also was shown on immersing time for 3 hours in a clove oil solution (Figure 3). The fish that were immersed in high concentrations still showed the highest levels of recovery time ($p<0.05$). 3 hours after immersing all the fish immersed with concentrations of 0.1 and 0.2 ml clove oil/L of water did not survive.

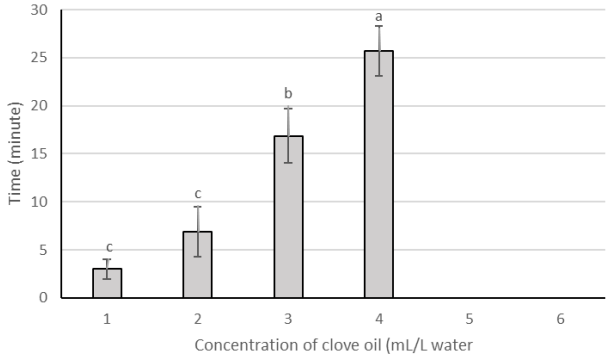

Figure 3. Time of Recovery after Immersing for 3 hour. Different superscripts indicate a significant difference ($p<0.05$).

Immersing time for 6.5 hours in clove oil solution has showed that the fish was immersed in a concentration of clove oil 0.02 ml/L of water still had a stable time for recovery (Figure 4). On the other hand, the fish in the other concentration did not survive. The concentration of glucose and cholesterol can be seen in Table 1. The length of time of immersing and the different concentrations of clove oil have a significant effect ($p<0.05$) on the concentration of glucose. On the other hand, the concentration of cholesterol did not significantly affect ($p>0.05$) by The length of time of immersing and the different concentrations of clove oil. Anaesthesia should also allow a quick and full recovery, with fishes showing normal behaviors (Nordgreen et al., 2014; Valentim et al., 2016). A study conducted by Kamble et al., (2014) showed that the smaller the concentration of clove oil, the longer the anesthetic time, and the smaller the concentration of clove oil, the faster the recovery time. The efficacy of clove oil is also influenced by species differences (Kroon, 2015). A different species showed a different response.

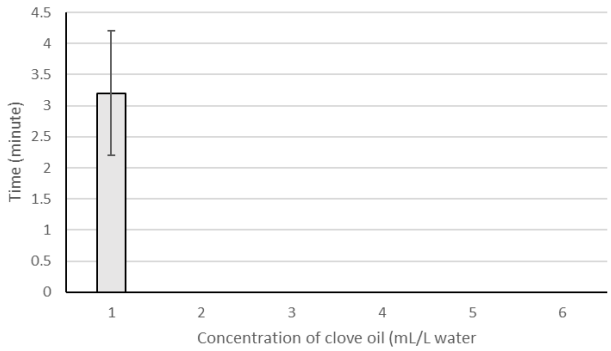

Figure 4. Time of Recovery after Immersing for 6.5 hour.

The length of recovery time is caused by the increased duration of exposure or the physical properties of clove oil**,** the increasing concentration of clove oil, and water temperature (Hur et al., 2019)**.** Generally, high doses of and long exposure to anesthetic agents lead to long recovery times and even death

because of severe hypoxia. In our research, immersing fish in different concentrations of clove oil until 1 hour of immersing showed that all the fish still survived. Information about the internal environment of the organism can be found through the biochemical profiles of blood.

Table 1. The concentration of Glucose and Cholesterol for 1 and 3 hours after immersing

Treatment	Glucose (mg/dL)	Cholesterol (mg/dL)
0.02 mL clove oil/L water for 1 hour immersing	204±26.9^{b}	200±5.7^{a}
0.03 mL clove oil/L water for 1 hour immersing	247±7.12^{ab}	156.5±62.90^{a}
0.04 mL clove oil/L water for 1 hour immersing	309±11.31^{ab}	253.5±86.97^{a}
0.02 mL clove oil/L water for 3 hour immersing	223.5±57.34^{ab}	245±25.46^{a}
0.03 mL clove oil/L water for 3 hour immersing	228.5±4.89^{ab}	171±1.41^{a}
0.04 mL clove oil/L water for 3 hour immersing	336.5±26.21^{a}	168±31.11^{a}

Note: Different superscripts in the same column indicate a significant difference

Exposure to stress can increase plasma glucose. It is because stress elevates the level of cortisol hormone. The elevated cortisol hormone results in the increasing activation of plasma glucose due to gluconeogenesis (Souza et al., 2019). The Stress response is usually triggered by physiological mechanism (Nurhaliza et al., 2023) as a compensate the imbalances produced by the stressor and recover the homeostatic status. The stress response is initiated and controlled by two hormonal systems, which lead to the production of catecholamines by the hypothalamus – sympathetic – chromaffin (HSC) axis, and corticosteroids (manly cortisol) by the hypothalamus – pituitary – Internenal (HPI) axis (Martos et al., 2014). A study conducted by Hur et al., (2019) showed that clove oil can elevate the cortisol and glucose level in olive flounder plasma at 0.5 and 1 hour after immersing. Meanwhile, research conducted by Bahi et al., (2018) showed that administration of clove oil on gilthead seabream did not affect glucose levels. According to He et al., (2022), eugenol could reduce the metabolic capacity of fish, which is shown by reducing glucose concentration. Our recent study showed that the high concentration of clove oil and the length of time of immersing can increase plasma glucose.

Our research showed that the best concentration of clove oil to be anesthetic for transporting is 0.02 ml/L of water. It was because the fish in this group showed a constant time of recovery and high survival. The appropriate concentration of clove oil for handling payangka fish is 0.03 ml/L of water. It was because at this level the fish are calm and their physiological parameter is the same as the fish in a concentration of 0.02 ml of clove oil. Meanwhile, the fish that were immersed in 0.02 ml of clove oil concentration until 3 hours of immersing still had a reaction when blood sampling. It is similar to a study conducted by Barata et al., (2016) that low concentrations of clove oil induce a similar stress response as handling without anesthesia during routine activities. According to Bradley et al., (2021), there is a two-step protocol is recommended for euthanasia: heavy anesthesia via immersion followed by an intravenous or intracardiac injection of euthanasia solution, or another secondary method of euthanasia. The study conducted by McCord et al., (2022), showed that 175 mg/L of clove oil is the ideal anesthetic and concentration for the routine laboratory use of Pacific hagfish for low risk of accidental overdose and high safety margins for both the handler and the fish. The optimum dose for using clove oil on Nilem fish is based on the speed of fainting and recovery is 0.10 mL/L (Jannah et al., 2022). This shows that each type of fish has a different tolerance to clove oil. This shows that each type of fish has a different tolerance to clove oil (Fernandez et al., 2017). According to Wojan et al., (2019) The use of anesthesia using either clove oil or MS-222 can cause discoloration of the fish's body. In fish that were given anesthesia, there was a difference in color compared to the control, there was an increase in saturation and a decrease in brightness. In our research, this was observed in male fish because they have more colorful bodies compared to females.

Conclusion

In this study, the optimal concentration of clove oil that can be used as anesthesia for the transportation of live payangka fish is 0.02 ml/L of water. Meanwhile, for handling activities on payangka fish, a clove oil concentration of 0.03 ml/L of water is recommended. Because those concentrations showed the best performance of payangka fish.

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Author Contributions

The writing of this article was carried out cooperatively. All authors have jointly contributed so that this paper can be completed. Conceptualization and methodology, L.D.R; Validation and formal analysis W.A.T; writing, N.M; review and editing, L.D.R; W.A.T. All authors have read and agreed to the published version of the manuscript

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Conflicts of Interest
The authors declare no conflict of interest

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