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Swimming Bladder Imaging Morphometry and Potential for Reducing Heart Rate in the Use of Clove Oil as an Anesthetic for Jatimbulan Tilapia (*Oreochromis niloticus*)

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Abstract: Jatimbulan tilapia has the potential to be developed in Indonesia because it is easy to cultivate. The use of anesthetics in the dry transportation process and medical examination of fish often uses clove oil because it is cheap and easy to obtain. This research was conducted to determine the effect of using clove oil as an anesthetic on the swim bladder and heart rate. 35 adult Jatimbulan tilapia fish, 4 months old, male, were divided into 7 treatment groups, namely control (P1), clove oil anesthesia induction dose of 20 ppm (P2), 30 ppm (P3), 40 ppm (P4), 50 ppm (P5), 60 ppm (P6) and 70 ppm (P7). The swimming pool measurement method uses lateral projection radiography imaging and echocardiography to measure heart rate. The results of the Tukey test showed that there was no difference in the average length of the swim bladder for each group, while the doses of P5 (50 ppm) and P6 (60 ppm) caused an increase in the width of the swim bladder compared to P1 (control) and P2 (20 ppm). Group P7 (70 ppm) had the lowest average heart rate compared to all groups, namely 49 ± 3.69 times per minute. The conclusion of this research is that the use of clove oil at a dose of 20-70 ppm for anesthesia of Jatimbulan tilapia does not affect the dimensions and structure of the swim bladder, but it is necessary to anticipate a decrease in heart rate that occurs with increasing doses.

Keywords: Aesthetic; Clove oil; Echocardiography; Jatimbulan tilapia; Radiography; Swim bladder

Introduction

Freshwater fish cultivation in tropical climates such as Indonesia is in great demand because it helps economic development (Sarwana et al, 2019; Sutiani et al., 2020; Syafei, 2017). Tilapia (*Oreochromis niloticus*) is one of the freshwater fish commodities that is widely cultivated in Indonesia as a food fish and is in great demand by the world market because it has the ability to reproduce well, and has a high survival rate and economic value (Amri & Khairuman, 2007; Rakhman et al., 2017; Jayadi et al., 2020; Jayadi et al., 2021). One type of typical Indonesian tilapia, namely Jatimbulan tilapia, has the advantage of being able to grow quickly and be large in size with a high survival rate of up to 85% (Arfiati et al., 2021). In the process of transporting fish using the dry method and medical handling of sick fish, anesthesia is required. Dry technical transportation is carried out using anesthesia and lowering the temperature to keep the fish alive for a longer period of time (Jailani, 2000; Utomo, 2001; Nitibaskara et al., 2006; Hasan, 2018).

The use of anesthesia is an important tool that can be widely applied in fisheries management programs. Anesthesia is used to reduce stress associated with handling and/or transporting fish. Anesthesia is widely used both in cultivated populations and in other field situations (Taylor et al., 2017; Føre et al., 2021; Svendsen

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et al., 2021). The stress response in fish is described by a primary response which includes the release of stress hormones such as catecholamine and cortisol into the circulatory system, followed by secondary responses such as changes in glucose levels, electrolyte balance, heart rate, and a tertiary response, namely changes in activity. If the fish cannot adapt to the stressor at this stage, it will have impacts such as changes in behavior and growth and can sometimes even result in the fish dying (Iwama et al., 2008).

Clove oil is one of the agents often used for fish anesthesia because it is easy to obtain and relatively cheap. Eugenol as the active substance in clove oil can provide an anesthetic effect, capable of reducing the work of the nervous system, thereby providing a calming effect on fish, reducing stress and is effective at low concentrations at an affordable price (Palimbu & Mandiangan, 2019). Telemetry studies on salmon after recovery from surgery using benzocaine anesthetic showed heart rate activity ranging from 20-40 bpm. Currently there is no information regarding the effects of clove oil on the swim bladder and heart of Jatimbulan tilapia. The swim bladder in fish contains gas to maintain hydrostatic balance due to changes in the external environment. Changes in the swim bladder have the potential to cause the fish to lose its buoyancy. The aim of this research is to test various doses of clove oil to determine the anesthetic effect on the swim bladder and heart rate of Jatimbulan tilapia fish as a reference for dose selection using non-invasive methods of radiographic and echocardiographic images.

Method

Test Animals

Jatimbulan tilapia, male, adult, 4 months old, 35 fish, body weight 100 – 200 g from UPT PBAT Umbulan, Pasuruan, East Java. The fish pellet feed given is 3% of body weight per day. Monitoring water conditions is seen through the parameters of temperature, pH, brightness and oxygen levels. The optimal temperature for the development of tilapia is 25-30˚C, pH 7 – 8.5 with a brightness level of 30-40 cm and oxygen levels > 5 ppm (Lukman et al., 2014). The density when rearing tilapia fish ranges from 20–25 fish/m^2. Draining the maintenance tank is carried out when the water starts to become cloudy.

Anesthesia Preparation

In this study, commercial clove oil (PT Usfi®) was used. The experimental animals were divided into 7 groups, namely control and 6 administration groups. Group P1 was the control, P2 had a dose of 20 ppm clove oil, P3 had a clove oil dose of 30 ppm, P4 had a clove oil dose of 40 ppm, P5 had a clove oil dose of 500 ppm, P6 had a clove oil dose of 600 ppm and P7 had a clove oil dose of 70 ppm, dose modification based on research another was carried out on zebrafish (Rahim, 2017).

Research Flow

Before treatment, the fish were fasted for about 12 hours to reduce the interference of ingesta and chyme in the digestive tract organs. 1 L of water is put in a container then clove oil is given according to the dosage. Anesthesia induction is carried out by placing the fish in a tub filled with water mixed with clove oil. The duration of fish anesthesia is 60 minutes. Monitoring fish anesthesia is through behavioral observation. Signs of anesthetized fish include euquilibrium, decreased activity and slowed operculum movements. Evaluation of observation parameters, namely swim bladder morphometry and heart rate. Radiography uses a 50 kvp x-ray machine with 20 mAs, flat panel detector Exprimer DR Tech® with measurements using Econsole® software. Heart rate measurements with M-mode were taken during the systolic and diastolic phases using a Mindray M5Vet ultrasound machine, 3.5 - 4 MHz phased array transducer, by placing the transducer in the opercular slit in the sagittal plane. Jatimbulan tilapia heart detection using color Doppler mode at 50 minutes. Taking lateral projection radiographs at 60 minutes after induction of anesthesia.

Ethical Feasibility

All research procedures have received appropriateness from the Brawijaya University research ethics commission (No. 151-KEP UB-2022).

Data Analysis

Data analysis used Statistical Package for the Social Sciences (SPSS) software with the OneWay ANOVA test, with a confidence level of 95% (α = 0.05) and continued with the Tukey test.

Result and Discussion

Jatimbulan tilapia that have been anesthetized have clinical signs in the form of decreased activity, slowing of operculum movement, loss of balance and slowly reaching the bottom of the container. The anesthetic agent of clove oil is induced through water which will be absorbed by the blood capillaries of the gills to the spinal cord and brain to be distributed throughout the body through blood vessels and tissues. Furthermore, eugenol inhibits the cyclooxygenase enzyme, thereby reducing pain mediators which results in a decrease in sensory responses, muscle relaxation and sedation (Anggitasari, 2018; Dewi et al., 2018; Anggitasari, 2022).

Evaluation of the Nile Tilapia swim bladder using radiography can be seen in Figure 1 showing the intra ceolomic structure with the swim bladder appearing to be normal in the form of a tubule with one gas opacity chamber, no fluid in it, and no change in location. Skeletal bones appear normal along the dorsal swim bladder. This normal structure was seen at all clove oil doses of 20 ppm, 30 ppm, 40 ppm, 500 ppm, 60 ppm and 70 ppm. Other internal organs are difficult to visualize because they have the same opacity.

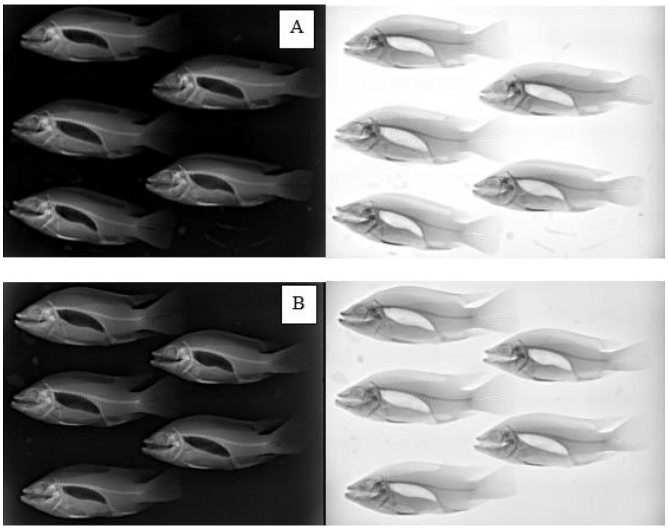

Figure 1. Lateral radiograph of Jatimbulan tilapia fish looks normal. The swim bladder appears to consist of a single chamber dominating the center of the abdomen with radiolucent gas opacity. The negative contrast shows the dominance of the white swim bladder; (A) Administration of clove oil at a dose of 50 ppm; (B) Clove oil dose 40 ppm

This is in accordance with previous studies that appropriate radiographic techniques allow identifying several internal abnormalities and knowing the prognosis. Cases that can be identified include fish with abnormal body shapes, swimming and buoyancy disorders, and suspected foreign objects. Radiography has the advantage of being a non-lethal, non-invasive procedure that provides permanent images. In some types of fish, the swim bladder structure has one to three connected chambers. Organs such as the heart, liver, gonads, and kidneys are difficult to visualize due to the lack of radiographic contrast. Abnormalities of the swim bladder can be seen using radiography, namely displacement of location, excessive inflation, rupture and fluid content (Wildgoose, 2003).

The results of measuring the dimensions of the swim bladder, taken by measuring the length and width axes, are shown in Table 1. Based on the One Way ANOVA test, there was a significant effect ($p < 0.05$) of administering clove oil in a dose range of 20-70 ppm on the swim bladder, but from the results of the Tukey test, the average swim bladder length between treatments showed the same dimensions, namely 5.88 ± 0.12 cm (P1); 5.49 ± 0.16 cm (P2); 5.96 ± 0.17 cm (P3); 6.07 ± 0.14 (P4); 5.99 ± 0.33 cm; 6.38 ± 0.17 cm and 5.73 ± 0.27 cm. Changes were found in the width of the swim bladder at the P5 (50 ppm) dose, namely 1.84 ± 0.37 cm and P6 (60 ppm) 1.86 ± 0.36 cm, experiencing increased dilation compared to P1 (control) 1.40b ± 0.09 cm and P2 (20 ppm) 1.36 ± 0.12 cm.

Table 1. Swimming Bladder Dimensions of Jatimbulan Tilapia Fish in Groups Given Various Doses of Clove Oil

Treatment	Mean length ± SD (cm)	Mean width ± SD (cm)
P1 (control)	5.88ab ± 0.12	1.40b ± 0.09
P2 (20 ppm)	5.49ab ± 0.16	1.36b ± 0.12
P3 (30 ppm)	5.96ab ± 0.17	1.50ab ± 0.45
P4 (40 ppm)	6.07ab ± 0.14	1.74ab ± 0.12
P5 (50 ppm)	5.99ab ± 0.33	1.84a ± 0.37
P6 (60 ppm)	6.38a ± 0.17	1.86a ± 0.36
P7 (70 ppm)	5.73ab ± 0.27	1.64ab ± 0.56

Note: different superscript notations indicate significant differences ($p < 0.05$) between treatments

As a hydrostatic organ, the swim bladder in fish tends to contain the right amount of gas needed to make the specific gravity of the fish the same as the specific gravity of the water in which the fish swims, so that it can stop in the middle of the water and tends not to rise or fall, this normal condition is called neutral buoyancy. In an anesthetized condition, Jatimbulan tilapia sinks to the bottom of the container tank. Physiologically, gas has the property of being compressible while water cannot, so an increase in external or atmospheric pressure through the body wall will reduce the volume of gas in the swim bladder, causing the fish to sink in the water (negative buoyancy condition). The use of clove oil is thought to be able to reduce blood vessel activity so that fish experience impaired buoyancy control function. According to Alexander (1966) under normal conditions, gas retention in the swim bladder can occur by the mechanism of increased blood flow due to capillary dilation and increased oxygen due to local dissociation of oxygen from oxy-hemoglobin which can trigger oxygen secretion in the swim bladder. Gas exchange occurs by simple diffusion between the vascular rete and the swim bladder lumen where the partial pressure of a particular gas exceeds the partial pressure of that gas in the bloodstream.

A decrease in blood capillary activity can be triggered by a decrease in heart rate. Based on Table 2, the higher dose of clove oil given causes a significant decrease in the average heart rate ($p < 0.05$) where the P7 group (70 ppm) has an average heart rate the lowest heart rate compared to all treatment groups reached 49 ± 3.69 bpm minutes. In groups P1 (control) to P4 (40 ppm), the heart rate was in the range of 61-65 bpm, higher than groups P5, P6 and P7. This high heart rate can be related to the high activity of the adrenaline hormone in fish. At low doses of anesthesia, it is indicated that some fish show that complete anesthetic relaxation has not occurred. Based on preliminary research by Snelderwaard (2006) which used transmitter telemetry on Goldfish (*Carassius auratus*), administering an intermediate dose of adrenaline increased heart rate by 62 bpm and at high doses caused an increase of up to 71 bpm.

Heart rate monitoring can use ultrasonography with color Doppler detection. The heart is small in size and its location close to the operculum, making it difficult to identify due to operculum movement. The blue color (Figure 2A) shows blue cardiac blood flow moving away from the transducer. Furthermore, measuring the heart rate using M-mode echocardiography by measuring the rate of one period during systole and diastole will automatically display the rate per minute.

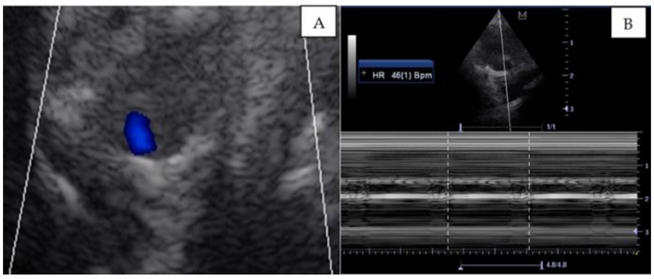

Figure 2. Heart sonogram during treatment with 50 ppm clove oil. (A) Detecting the location of the heart with color Doppler shows blue blood flow; (B) M-mode echocardiography of Jatimbulan tilapia heart rate

M-mode is an adaptation of B-Mode used to evaluate moving structures such as the heart. A mode that utilizes Doppler technology records the movement of blood towards or away from the transducer (probe) thereby creating a color image based on the flow pattern (Novelo & Tiersch, 2012).

Ultrasound in fish, has been used to study blood flow and venous contraction in the cuttlefish Sepia officinalis. The heart was well visualized in all fish. Image collection time can generally be completed within 3 minutes after induction of anesthesia. This procedure was well tolerated and there were no deaths associated with this procedure. Image quality in female fish, especially fish containing many eggs, is often lower than in male fish, thereby affecting the accuracy of ventricular measurements and strain analysis (King et al., 2005). Heart rate is a more sensitive indicator than fish swimming activity after surgery.

In Figure 2B, the results of measuring the heart rate of one of the tilapia fish during anesthesia induction of 50 ppm, namely 45 bpm (beats per minute).

Table 2. Measurement of the Heart rate of Tilapia after Induction of Anesthesia with Clove Oil

Treatment	Average heart rate ± SD (beats per minute)
P1 (control)	$61^{bcd} \pm 5.96$
P2 (20 ppm)	$63^{bcd} \pm 3.00$
P3 (30 ppm)	$71^{d} \pm 4.27$
P4 (40 ppm)	$65^{cd} \pm 6.94$
P5 (50 ppm)	$54^{bc} \pm 2.21$
P6 (60 ppm)	$55^{bc} \pm 5.50$
P7 (70 ppm)	$49^{a} \pm 3.69$

Note: different superscript notations indicate significant differences ($p < 0.05$) between treatments

The condition of bradycardia occurs because respiratory and heart rates generally decrease as the dose and duration of anesthesia increases (Harms, 2003). Echocardiography studies in zebrafish show that when using tricaine, the average heart rate reaches 63-72 bpm. At the 9th minute of anesthesia, bradycardia occurred because the anesthetic agent has the potential to cause negative chronotopic effects and a decrease in ventricular contraction (Wang et al., 2017). In some species, for example tuna, opercular movements are minimal or even non-existent due to the ventilation mode (ram ventilation). This condition is to maintain adequate ventilation through the flow of oxygenated water through the gills. Even though the fish appears to be well ventilated, bradycardia and increased resistance to gill capillary flow have the potential to cause accumulation of erythrocytes at the capillary bed and become edematous and cause hypoxemia (Stemper & Neiffer, 2009). Most anesthetic agents have negative chronotropic effects so optimizing drug selection and dosage is very important to avoid the effects of drug-induced bradycardia on measurements of cardiac chamber size and function (Wang et al., 2017).

Conclusion

Giving clove oil at a dose of 20-70 ppm for anesthesia for Jatimbulan tilapia does not affect the dimensions and structure of the swim bladder, but it is necessary to anticipate that a decrease in heart rate will occur as the dose increases.

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Author Contributions

Contribution of author involvement in scientific work, namely conceptual, methodology, investigation and writing: Dian Vidiastuti; supervision, writing, review and editing: Diana Arfiati.

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Conflicts of Interest

All authors declare no conflict of interest.

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