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THE USE OF NATURAL CLOVE OIL ANESTHETICS IN THE TRANSPORTATION OF NIRVANA TILAPIA JUVENILE (*OREOCHROMIS NILOTICUS*)

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KeyWords
Closed system transportation, clove oil concentration, induction and conscious recovery time, survival rate.

ABSTRACT

This research aims to analyze the time of induction and recovery, determine the effective concentration of clove oil for anesthesia and analyze the survival rate of nirvana tilapia using clove oil that is transported. This research uses the experimental method Factorial Randomized Group Design (FRGD) which consists of two factors namely four levels of concentration factors (13, 15, 17 x 10^{-3} ml/L and controls) and three-level duration factors (3, 6 and 9 hours) The parameters observed during the research took place were the time of induction and recovery nirvana tilapia juvenile, post-transportation survival and water quality in the form of DO, pH, ammonia and temperature. The results showed that the higher the concentration given, the faster the time of induction and the longer the time to recovery, the effective concentration for transportation of tilapia nirvana juvenile was 13 x 10^{-3} ml/L with the duration of transportation for 9 hours due to post survival transportation are 99.17% and the effect of clove oil concentration and duration of transportation has a significant effect on post-transportation survival.

INTRODUCTION

The aquaculture sector plays an important role in supporting economic growth, increasing the income and welfare of the community and opening employment opportunities. Aquaculture has increased since 2009, with growth of 43.76%. According to the Directorate General of Aquaculture (2016), since 2013 West Java is the largest producer of tilapia from several provinces that produce tilapia, amounting to 205,951 tons. The level of fish consumption in West Java is 20-31.4 kg/capita (Central Bureau of Statistics 2017).

One of the superior varieties of tilapia is tilapia nirvana. Nirvana or wanayasa tilapia can be known by the community as a result of the selection of GIFT (Genetic Improvement of Farmed Tilapia) and GET (Genetically Enhanched of Tilapia) tilapia from the Philippines by the Wanayasa Juvenile Development Center (WJDC) Wanayasa, Purwakarta Regency. The advantage of Tilapia Nirvana lies in its growth speed. Maintenance since the larvae weigh up to 650 grams can be achieved within a period of 6 months, besides that the superiority of Nirvana tilapia has a thick meat structure compared to other types of tilapia (Ghufran 2011).

Market demand for Nirvana tilapia has now penetrated the export market. Marketing of fish is usually done in a living state (Suwetja et al 2015). In marketing live fish using the transportation method is a solution to distribute juvenile or brood to the intended area. According to Nani et al (2015), live fish transportation is divided into two, namely transportation of dry systems without using water and wet system transportation using water. There are several factors that cause the death of fish during transportation, namely high levels of carbon dioxide (CO_2) due to the process of respiration, accumulation of ammonia formed from the metabolism of fish, and hyper active fish so that it consumes a lot of O_2 and releases CO_2 and causes the dissolve oxygen (DO) in the water. decreases and CO_2 gas rapidly increases (Junianto 2003).

One attempt to reduce mortality in the process of transporting fish juvenile by anesthetizing or anesthesia in fish during transportation. According to Fauziah (2006), anesthetic ingredients can be synthetic chemicals or natural ingredients. Chemicals used for anesthesia such as MS-222, benzocaine, metomidate, phenoxy ethanol, quinaldine, chinaldine (Coyle et al 2004). The use of chemicals according to Supriyono et al (2010) as an anesthetic material can leave residues that are harmful to fish, humans, and the environment. One of the natural anesthetics is clove oil according to Fauziah et al (2011).

Clove oil has a large component of eugenol (70-80%) which has properties as a stimulant, local anesthetic, carminative, antiemetic, antiseptic, and antispasmodic (Somaatmadja 2001). Clove oil is rich in eugenol content of 70 - 79% (Purbani 2006) so that it can be used as a natural anesthetic.

This research aims to analyze the induction and recovery time of nirvana tilapia juvenile, determine the effective concentration of clove oil for tilapia nirvana juvenile anesthesia and analyze the survival rate of nirvana tilapia juvenile using clove oil after being transported.

MATERIAL AND METHODS

The materials used in this research include 1440 Tilapia Nirvana (Oreochromis niloticus) juvenile originating from Ciparay and Cibiru Juvenile Development Center with a size of 4-6 cm, Bratachem brand clove oil, pure oxygen, water samples, $MnSO_4$, O_2 Reagents, H_2SO_4, Na-thiosulfate, signette and nessler solution. This research uses an experimental method with a factorial randomized group design pattern (FRGD) consisting of four levels of concentration factors, three-level duration factors and repeated three times.

The transportation of nirvana tilapia juvenile in this research was carried out in a closed system with a density of 20 fish/liter of water using clove oil which was diluted by 1000 times with different concentrations (controls, 13, 15 and 17 x 10-3 ml / L). Tilapia nirvana seeds are transported in three levels of time, 3, 6 and 9 hours at 3:00 a.m. WIB. After being transported, tilapia nirvana seeds are kept for 7 days to determine the post-transportation effect on fish survival.

The parameters observed in this research were induction and recovery time, post-transportation survival rate and water quality during transportation which included DO, ammonia, temperature and pH.

Survival Rate

The survival of juvenile is observed when dismantling transportation packaging. To find out the survival rate of fish can be calculated from the comparison of the number of fish that live at the end of the period with those that live at the beginning of the period (Effendie 1978). To find out the survival of the fish, use the following formula:

$$SR\ (\%) = \frac{Nt}{No} \times 100\%$$

Description:
SR = Survival of fish during the experiment.
Nt = Number of fish at the end of the experiment.
No = Number of fish at the beginning of the experiment

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Water Quality Parameters

Observation of water quality is carried out by measuring water quality at before and after transportation. Measurements of water quality in situ include pH, and temperature while ex situ includes DO and ammonia in the Laboratory of Aquatic Resource Management Faculty of Fisheries and Marine Sciences Padjadjaran University.

Ammonia measurements using the spectrophotometric method using the following formula (Clesceri et al 1998):

$$Ammonia\ Value = \frac{1000}{25}\ x\ \frac{Sample\ Absorbances}{Standard\ Absorbances}\ x\ 5\ microgram$$

Description:
Sample Absorbances = Calculated absorbance from the sample.
Standard Absorbances = Calculated absorbance from the standard.

DO measurements use the winkler titration method using the following formula (Leonore et al 1988):

$$DO\ Value = \frac{8000\ x\ ml\ Na - tiosulfat\ x\ N\ Na - tiosulfat}{50\ x\ \frac{(V_0 - 2)}{V_0}}$$

Description:
Vo = Volume of winkler bottle

Data Analysis

The survival rate data was tested by ANOVA (F test) at the 95% confidence level. If there are significant differences between treatments in the F test, then it is continued with Duncan's multiple distance at the confidence level of 95% (Gasperz 1991). Data on induction time, recovering conscious of fish and water quality data were analyzed descriptively.

RESULTS AND DISCUSSION

Induction Time

The time of induction is when the fish is conscious until the fish faints because the anesthetic is given. The induction time starts when the fish is put into a plastic that has been given anesthetic treatment according to the treatment until the fish faints. The following is a diagram of the time of induction of tilapia nirvana seeds which can be seen in Figure 1.

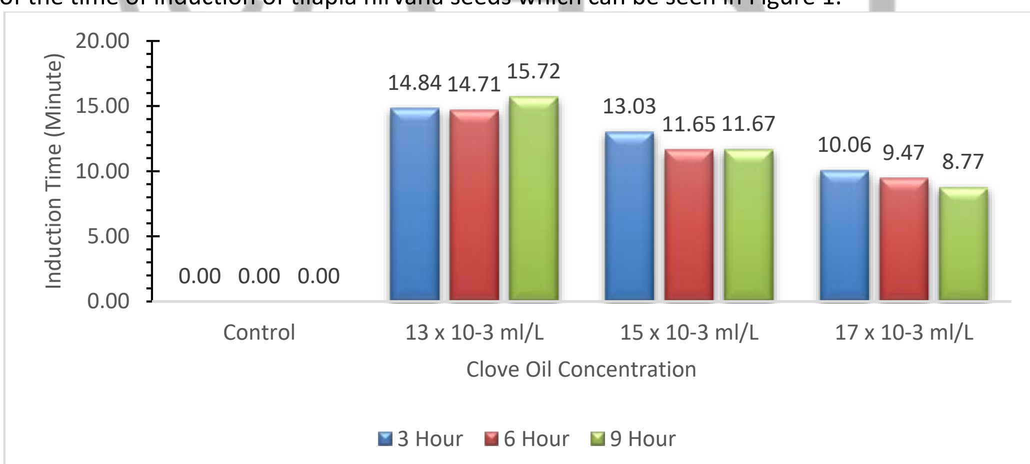

Figure 1. Average Diagram of Nirvana Tilapia Juvenile Induction Time

The average length of induction was 14.84, 14.71 and 15.72 minutes at a concentration of 13 x 10^{-3} ml/L for transportation for 3, 6 and 9 hours, respectively. Fish have been seen swimming randomly not in the same direction of entering minute 6 - 8, meaning the fish has begun to the beginning of the anesthesia stage. By the 9th minute some fish have entered the stage of losing some and total balance. When entering minutes 13-15 minutes the fish have entered the stage of losing reflex, which means it has lost total consciousness, operculum slows down but regularly (Bowser 2001).

The time of induction for transportation for 3, 6 and 9 hours at a concentration of 15 x 10^{-3} ml/L respectively was 13.03, 11.65 and 11.67 minutes. Minutes 5 - 7 fish have been seen swimming randomly not in the same direction which means that the fish has entered the beginning of the anesthesia stage. Minutes 8 - 10 fish have entered the stage of losing partial and total balance seen

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from the movement of fish affected by clove oil. Fish that have entered the stage of losing balance partly have the characteristics of muscles starting relaxation, the movement of the operculum increases, and reacts only to strong stimuli from the outside, if losing total balance has the characteristics of losing balance, but the movement of the operculum slowly decreases and stabilizes (Bowser 2001). Minutes 11-13 fish have entered the total induction stage which is indicated that the fish is in a dorsal recumbency position.

The time of induction for transportation for 3, 6 and 9 hours at 17 x 10^{-3} ml/L respectively was 10.06, 9.47 and 8.77 minutes. Minutes 3 - 4 fish have entered the initial stage of anesthesia with marked fish starting to swim randomly not in the same direction. Minutes 5 - 6 fish have begun to calm down when swimming, which is a fish that has entered the stage of losing some balance, the characteristics of the muscles have begun to relax and react when there is a strong stimulation from outside the container (Bowser 2001). Minutes 8 - 10 fish have entered the total induction stage where the fish has been completely sedated by the anesthetic given. The characteristics of a total fainted fish are that the fish is already in the dorsal recumbency position, the operculum movement is slowing down but regularly.

Concentrations of 13 and 15 x 10^{-3} ml/L had an induction time that tended to fluctuate at each treatment hour and at a concentration of 17 x 10^{-3} ml/L had an induction time which tended to decrease at each treatment hour. This is due to differences in the size of the fish used at each treatment hour. According to Gunn (2001), fish that have a larger gill space can be fast and efficient in absorbing anesthetics. This is in accordance with this research where the treatment of 3 and 6 hours using fish with a range of sizes 4 - 4.5 cm while the 9 hour treatment uses fish with a size range of 5-6 cm. This can occur because of the limitations of existing materials.

The higher the concentration of clove oil given, the faster the time of induction of the fish tested. The fastest induction (fainting) at a concentration of 17 x 10^{-3} ml/L with a time of 8.77 minutes and the longest at a concentration of 13 x 10^{-3} ml/L with a time of 15.72 minutes. Daud et al (1997) in Riesma et al (2016) stated that the induction time of less than 15 minutes is a good concentration to stun fish and is better than 3 minutes. In this research, the induction time is less than 15 minutes, which means that the concentration of 13, 15, 17 x 10^{-3} ml / L is considered good in stunning fish.

Recovery Time

Recovery time is the process of realizing the affected fish the anesthetic is unconscious until it reaches consciousness. The time to recovery starts when the affected fish anesthetic is put into the awareness container and finished when the fish has regained consciousness or the fish has been swimming actively again. The following is a diagram of the recovery time of conscious tilapia nirvana seeds can be seen in Figure 2.

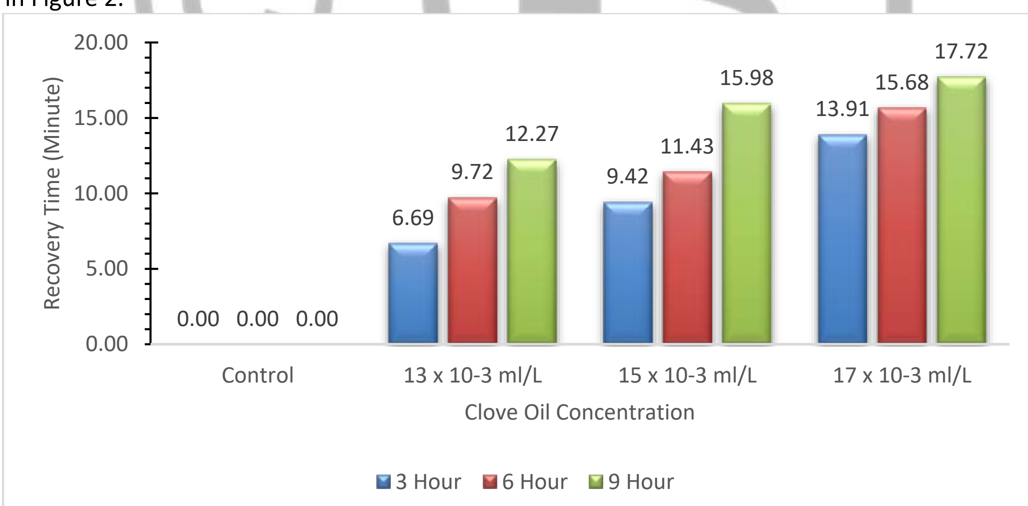

Figure 2. Average Diagram of Time to Recovery Nirvana Tilapia Juvenile

Recovery time for the duration of transportation 3, 6 and 9 hours, each at a concentration of 13 x 10^{-3} ml/L was 6.69, 9.72 and 12.27 minutes. At a concentration of 15 x 10^{-3} ml/L, recovery time for transportation duration 3, 6 and 9 hours was 9.42, 11.43 and 15.98 minutes, respectively. At a concentration of 17 x 10^{-3} ml/L, recovery time for the duration of transportation 3, 6 and 9 hours was 13.91, 15.68 and 17.72 minutes, respectively.

Figure 2 shows, the higher the concentration and the longer the packing period for fish that have been given anesthetic, the longer the time for recovery is needed. According to Robertson et al (1987) in Riesma et al (2016) the use of anesthetic materials with different doses will affect the level of consciousness. Ongge (2001) states that the entry of anesthetics into the blood will cause

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numbness so that when the process of awareness will require a long time and the duration of awareness is also influenced by the length of packaging.

Based on the research that has been carried out, the process of awareness of nirvana tilapia juvenile after being transported with various concentrations and the duration of transportation is found to have an average recovery time of 6.69 to 17.72 minutes. Recovery of fish to normal movements and swimming takes 10 minutes or less and there is no death for 15 minutes after demolition when anesthetized fish is properly concentrated (Gilherdus and Marking 1987 in Riesma et al 2016).

According to Ravael (1996) in Gunawan (2015), gills have an important role in the process of recovery because the dissolution of anesthetics that exist in the body of a fish in a state of fainting is placed in clean water. When the awakening process, water containing enough dissolved oxygen will enter through the gills and then enter the blood will clean up the remnants of the anesthetic that is in the body of the fish will then be discharged through the drain.

Post Transportation Survival Rate

Based on the research that has been done, the survival rate of fish with different concentrations and duration of transportation can be seen in Figure 3.

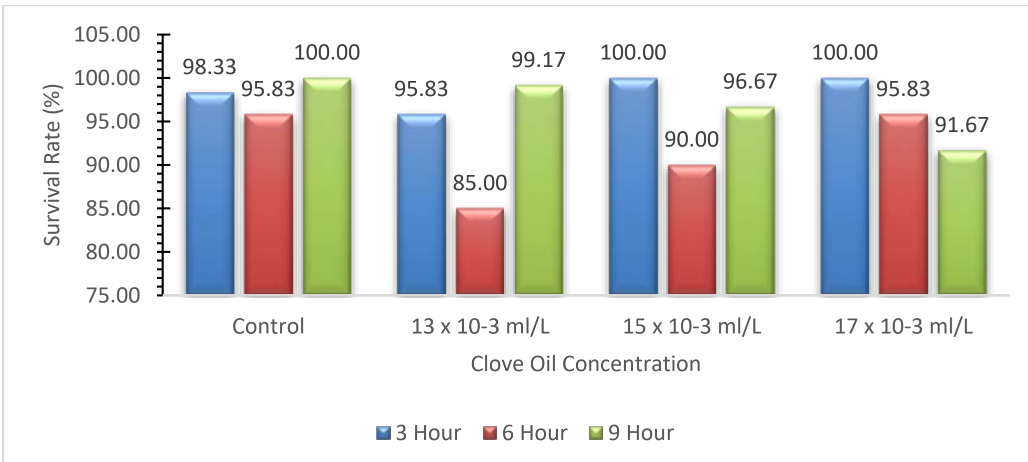

Figure 3. Diagram of the Survival Rate of Nirvana Tilapia Juvenile

The average survival rate of nirvana tilapia juvenile ranges from 85 - 100%. In the 3 hours duration of transportation with a concentration of 13, 15, 17 x 10^{-3} ml/L and control, the highest average at concentrations of 15 and 17 x 10^{-3} ml/L are 100% and the lowest at concentrations of 13 x 10^{-3} ml/L which is 95.83%. The occurrence of death during transportation is thought to be the cause of the fish experiencing stress during the anesthesia process. Allegedly the stress comes from transferring fish from the sinking tub to the plastic. Because according to Zonneveld et al (1991) in Hariyanto (2008) changes in the environment can cause fish stress such as temperature and transportation. Fish stress when the anesthesia process is suspected fish can not tolerate anesthetizing substances so that many experience death.

In addition to stressed fish because of environmental changes, according to Khalil (2013) many fish become stressed and flounder with lots of physical activity so that fish consume a lot of energy to adapt to an environment that is given an anesthetic. The duration of fish anesthesia also affects the stress level in fish. The high survival rate in treatment 15 and 17 x 10^{-3} ml/L compared with other treatments because when the fish were treated with this concentration the fish had fainted faster than other concentrations. According to Riesma et al (2014) the condition of fish in a state of fainting can reduce stress levels.

The duration of transportation for 6 hours showed that the survival rate was significantly different from the highest survival rate in the control treatment and 17 x 10^{-3} ml/L at 95.83% and the lowest at concentrations of 13 x 10^{-3} ml/L at 85% . The presence of a large number of fish deaths at a concentration of 13 x 10^{-3} ml/L was suspected of having fish that died shortly after being treated with clove oil, which worsened the quality of water in the plastic. Dead fish causes an increase in the content of ammonia in the plastic so that it affects other fish that are in a state of fainting. It is also suspected that the death of fish in plastic is due to fish in a stressful state due to the transfer of fish from the sinking tub to the plastic so that the fish cannot tolerate the content of eugenol compounds in clove oil. Dayat and Sitanggang (2004) state that the use of anesthetic materials must be careful because basically they are toxic.

On transportation with a 9 hours duration, the survival rate is significantly different. The lowest survival rate was at a concentration of 17 x 10^{-3} ml/L are 91.67% and the highest in the control treatment are 100%. The cause of mortality in this treatment is presumably because the fish was exposed to the Tilapia Lake Virus Desease (TiLV) after being transported for 9 hours. The trigger for the

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emergence of TiLV after transportation is thought to be due to high stress levels because the duration of transportation is too long. Fish exposed to TiLV itself have bleeding features around fish skin and peeled fish skin (FQASC 2017). Not only at a concentration of 17 x 10^{-3} ml/L, the cause of death at concentrations of 13 and 15 x 10^{-3} ml/L was suspected because of TiLV Desease because some fish at that concentration also had the same characteristics, namely the dorsal fin, tail and the body is bleeding. According to Fish Quarantine Agency, Fisheries Product Quality and Safety Control (FQASC) 2017, Triggers the emergence of TiLV in fish due to high density, low temperature and high stress levels in fish.

Water Quality Parameters

Oxygen solubility when transported is from 4.8 to 9.4 mg/L. The control treatment has a DO value that tends to be lower than the treatment given anesthetic treatment. This is due to oxygen consumption in the control treatment is higher than the anesthesia treatment. According to Tahe (2008), the purpose of anesthesia is to calm fish so that the rate of oxygen consumption, carbon dioxide production and the rate of fish metabolism decreases. From the DO values obtained, according to Kep.23/MEN/2012 DO values for the survival of nirvana tilapia are more than 2 mg/L.

The average value of pH during transportation is 5.25 - 6.83. The low pH after transportation is thought to be due to high fish activity during the anesthesia process. According to Irianto (2005), a decrease in water pH is caused by the amount of CO_2 produced from a respiration of aquatic organisms, the reaction will tend to free H^+ so that the pH of the water will drop. Feasibility of pH values for the survival of nirvana tilapia seeds according to Kep.23/MEN/2012 5 - 8,5 while the data obtained in research is not much different from the feasibility of literature for survival rates.

The average value of ammonia during transportation is 0.013 - 0.043. According to NSA (2009) the level of ammonia feasibility for tilapia survival <0.02 mg/L. Based on the results of the research obtained, after transportation many treatments of ammonia values> 0.02 mg/L were suspected because at the beginning of the fish anesthetic process, fish metabolism tended to increase due to adaptation to the new environment. Ammonia values in this research are still categorized as safe because the value does not differ much from the level of feasibility of ammonia according to NSA (National Standardization Agency) in 2009.

The temperature of transportation ranges from 20 - 24.9°C. According to Kep.23/MEN/2012, the optimal temperature in tilapia maintenance is 22 - 32 ° C. The temperature in transportation tends to be low after transportation because the time used for transportation is early morning. Low temperatures can inhibit fish metabolism because according to Putra (2015) temperature increases can cause an increase in metabolic rate.

Conclusion

Based on the research that has been carried out, it can be concluded that:

1. The higher the concentration of clove oil given to nirvana tilapia juvenile with the aim of anesthesia in closed system transportation, the faster the time of induction and the longer the time to recovery.
2. Effective treatment of clove oil for the purpose of anesthesia for transportation is a 13 x 10^{-3} ml/L with a duration of transportation for 9 hours because the survival rate is 99.17% in post-transportation. The effect of clove oil concentration and duration of transportation has a significant effect on post-transportation survival rates.
3. The parameters of water quality during transportation are still considered quite good because the results and level of feasibility according to the literature are not very different.

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