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The Effects of Different Natural Anesthesia on Survival of Juvenile Sang kuriang Catfish (*Clarias gariepinus*) on Closed Wet System Transportation

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Authors' contributions

This work was carried out in collaboration between all authors. Author MRA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors KH and RG managed the analyses of the study. Authors WL managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to understand the effect of the different natural anesthesia on the survival rate of sangkuriang catfish juvenile which was transported using closed wet system fish transportation. We discussed selected natural anesthesia for effectiveness, induction times and recovery times in the application of anesthesia for transportation. The benefits of this research were as a reference to choose the best natural anesthesia for catfish juvenile transportation. This research used the experimental method on Completely Randomized Design (CRD) with 4 treatments (clove oil, nutmeg oil, lemongrass oil, and control) with 3 replications. Measured parameters were survival rate after fish transportation, survival rate after aquaculture for 7 days, induction times, recovery times,and water quality such as temperature, dissolved oxygen (DO), pH, and ammonia. The result was clove oil had high survival rate after fish transportation and cultivated for 7 days which is 98,67% and 87,33%. This treatment had quicker time for induction of catfish juvenile than other treatments which was 12.59 minutes although recovery time (10.34) minute was assumed lower than other treatments.

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Keywords: Sangkuriang catfish juvenile; induction times; recovery times; survival rate.

1. INTRODUCTION

The increasing population in Indonesia spur the need for food consumption to increase. Protein can be obtained from various sources, one of the important sources is fish. Catfish is a freshwater fish species that is easy to cultivate, can be maintained with a high stocking density and can be cultivated in marginal areas and is water efficient. Catfish is rich in nutritional content, the amount of protein reached 20% [1].

Juveniles Sangkuriang catfish (*Clarias gariepinus*) still had a high demand among cultivators. The advantage of catfish seeds was the high growth performance. Maintenance from juvenile until harvest and consumption can be achieved within 4-5 months. However, the problem that was often faced during culture was the transportation of live fish juvenile which can cause a low survival rate during and after the transportation process.

The factors that cause fish mortality during transportation were high levels of carbon dioxide (CO_2) due to the respiration process. Accumulated ammonia was formed from fish metabolism. Hyperactive fish consume more O_2 and emit high CO_2 so that O_2 may rapidly reduced which and dissolved CO_2 increases [2]. However, in principle of the transportation of live fish according to Suwetja, et al. [3], the aims of fish transportation was to reduced of fish mortality during transportation until reached the destination.

The transportation technique for live fish juvenile that was commonly used by cultivators was a wet system using a plastic container. Efforts to increase the carrying capacity have been made by reducing the amount of water used or increasing the number of fish juvenile transported. However, these efforts have not been followed to improve the survival of fish juvenile so that there are still many problems to be faced. Technical fainting used anesthesia can solve the problem.

The process of fainting under anesthesia is known as immotilization. Immotilization is an action that causes the fish's body to lose its ability to feel due to low respiratory and metabolic activities, so that the fish will undergo a physiological change from a conscious state to sedation. The anesthetic agents used for stunning fish are quite diverse. MS-222, quinaldine and benzocaine are commonly used in the stunning process of fish, but these anesthetics are chemicals and will leave a residue in the fish [4].

Natural anesthetics such as clove oil, lemongrass oil, and nutmeg oil can be applied to transported fish because these ingredients were safer to use and do not cause negative effects on fish, and do not endanger the safety of fish when consumed by humans. However, this lack of natural anesthetic ingredients, the quality of the extraction results sometimes varies from brand to brand, so a preliminary test is needed so that the dose used for anesthesia is correct and does not become toxic which can cause fish death [5].

Clove oil (*Syzygium aromaticum*) is a natural anesthetic agent that can be used in the transportation of fish [6]. Eugenol compound is the main component contained in clove oil with a content that can reach 70-96%. In the eugenol compound, several functional groups are contained, namely allyl ($-CH_2-CH = CH_2$), phenol (OH) and methoxy ($-OCH_3$) [7].

Nutmeg oil (*Myristica fragrans*) contains myristicin compounds which function as an anesthetic to reduce stress during fish transportation.This compound has the molecular formula $C_{11}H_{12}O_3$ [8]. Nutmeg oil is one type of essential oil that includes types of alcohol such as eugenol, which is also found in clove oil so that it can make fish weak and move slowly and eventually faint [9].

Lemongrass oil (*Cymbopogon citratus*) is anessential oils which contain lots of geraniol, citronelloll and citronellal compounds which can reduce the metabolic rate of fish by making fish fainted or calming fish [10].

2. MATERIALS AND METHODS

2. 1 Research Location

The research was conducted in October 2020 at CiparanjeFishery Area, Faculty ofFisheries andMarine Sciences, UniversitasPadjadjaran, Jatinangor, West Java, Indonesia.

2.2 Research Equipments

The equipment used in this research was :

- Plastic Polyethylene 24 pieces.

- Glass aquarium 20cm x 30cm x 30cm 12 pieces.
- Concrete aquarium.
- Aerator with stone and hose for aeration.
- Sieve.
- Oxygen tub.
- Stopwatch.
- Termometer.
- pH meter.
- DO test kit.
- 4 wheels vehicle.
- Micropipette.
- Spectrofotometer.
- Ruler.
- Styrofoam.
- Distillation tools.
- Book and pencil.
- Measuring cup.
- Distillation flask.

2.3 Research Materials

The materials used in this research was:

- Juvenile Sangkuriang catfish with 7-9 cm in size.
- Clove oil.
- Nutmeg oil.
- Lemongrass oil.
- Oxygen.
- PF-400 feed.
- Nessler.
- Penolpthalein
- Borat buffer
- NaOH.

2.4 Research Method

The method used in this research was anexperimental method by using Completely Randomized Design (CRD) with 4 treatments, repeated3 times:

A: Control (did not used natural anesthethic)
B: Anesthestic using clove oil
C: Anesthestic using nutmeg oil
D: Anesthestic using lemongrass oil

2.5 Research Procedure

The procedure of this research was catfish juvenile before being transported were quarantined (not fed) for 24 hours. The dose used was 0,015 ml on every natural anesthethic (taken from research that had been done) [6]. The dose was safe because it has been tested with LC_{50} test and Probit Analysis. The plastic used for transportation is filled with 1 liter of water with a density stock of 50 catfish juvenile. Anesthetic agents was inserted using a micropipette and then followed by checking the water quality such as the temperature, DO, PH, and ammonia. Before transportation was carried out, plastics were labeled and placed in stereofoam boxes. Then catfish juveniles were transported for a duration of five hours. After being transported, the water quality parameters were checked and then catfish juvenile transferred to an aerated aquarium to recover from fainting. Catfish juvenile were kept for 7 days after transportation to determine the effect of differences anesthesia on survival rate after fish transportation.

2.6 Observation Parameters

2.6.1 Survival rate

Survival rate was calculated based on the total number of catfish before transportation and after transportation. The survival rate of catfish juvenile wascalculated using the formula below [11]:

$$SR = \frac{Nt}{No}$$

Note: SR = survival rate (%), Nt = amount of fish after transportation (fish), N_0 = Initial amount of fish before transportation.

2.6.2 Induction times

Induction times were calculated from natural anesthetic put into the water in plastic until the fish juvenile were faint.

2.6.3 Recovery times

Recovery times were calculated from the fish juvenile transferred to an aerated aquarium until the fish juvenile showed active swimming and returned to normal from the effects of anesthesia.

2.6.4 Water quality

Water quality parameters checked were temperature, PH, DO, and ammonia. Measured parameters were obtained at the start of fish transportation and at the end of fish transportation.

2.7 Data Analysis

Data analysis of survival rate of sangkuriang catfish juvenile was carried out by analyzing variance(F test) with a confidence level of 95%. If theresults of the analysis of variance showedsignificantly different results, then a further testwas conducted with the Duncan test with aconfidence level of 95%. Induction times, recovery times, and water quality were analyzed descriptively.

3. RESULTS AND DISCUSSION

3.1 Survival Rate

The results showed that the highest survival rate of catfish juvenile obtained in treatment B (clove oil) was 98,67%, and for the other treatments were as following; treatment A was 20,67%, treatment C was 97,33%, and treatment D was 93,33%. The survival rates of catfish juvenile in each treatment was presented in Fig. 1.

According to Zonneveld et al. [12] changes in environmental conditions can cause stress for fish such as temperature and transportation. The fish were stressed during the anesthetic process, it is suspected that the fish cannot tolerate the anesthetic substance so that it will eventually die. In addition, fish were mainly stressed fish due to environmental changes. According to Khalil [13], many fish become stressed and fish flounder with a lot of physical activity so that the fish spend a lot of energy adapting to an anesthetic environment. The duration of anesthesia for fish juvenile also affects the stress level of the fish. The high survival rate of sangkuriang catfish juvenile in clove oil anesthetic treatment compared to other treatments was because when they were given anesthetics, catfish juvenile fainted faster than other anesthetic treatments. According to Riesma et al. [14] the condition of the fish in a fainting state can reduce stress levels.

The control treatment had an average survival rate of fish juvenile that was significantly different from the other treatments as well as the lowest value, 20.67%. There were quite a lot of fish juvenile deaths in this control treatment due to lack of dissolved oxygen because they were not given an anesthetic during transportation for five hours so that the juvenile continued to respire so that dissolved oxygen levels in plastic containers decreased and worsened the quality of water in the plastic. The dead fish juvenile cause the ammonia content in the plastic container to increase so that it affects other fish. It is suspected that the death of fish juvenile in plastic is due to stress due to the transfer of fish juvenile from the quarantine container to the plastic transportation container.

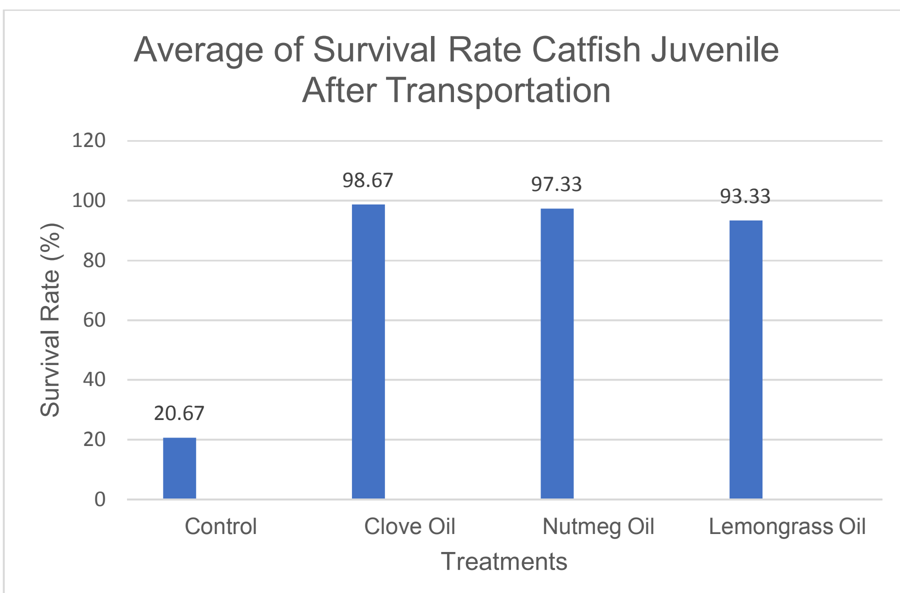

Fig. 1. Average of survival rate catfish juvenile after transportation

The results of the F test analysis using the 5% level (Table 1) showed significantly different results, meaning that there were differences between the control treatments to clove oil, nutmeg oil and lemongrass oil. The treatments of clove oil, lemongrass oil and nutmeg oil were not significantly different because each had the same notation.

The results of survival rate after aquaculture for 7 days showed that the highest survival rateof catfish juvenile was obtained in treatment B (clove oil) with 87,33%, and for the othertreatments as follows treatment A was 10%,treatment C was 81,33%, and treatment D was 85,33%. The survival rate of catfish juvenile after aquaculture for 7 days in each treatment was presented in Fig. 2.

Based on the results of sangkuriang catfish juveniles from the beginning to recovery until the aquaculture period of 7 days, the initial 3 days of aquaculture is a critical period for fish juveniles because the mortality rate is highest at the beginning of aquaculture. At the beginning of aquacultre fish juveniles, they still adapt to the new environment from plastic containers after transportation, causing the death of catfish juveniles. This is following the research of Maryani et al. [15], fish mortality in aquaculture aquariums mostly occurred at the beginning due to adaptation for the new environment and causing high fish mortality.

According to Fujaya [16] the osmoregulation process does not go well so that unnecessary substances (anesthetics) were not properly wasted. This can lead to a high fish mortality rate at the start of aquaculture. In the following days, sangkuriang catfish juveniles still died in several treatments but not many as the beginning of the aquaculture. Daily feeding, aquarium cleaned, and routine water change is one way to control water quality to remain optimal by minimizing the possibility of death of fish juveniles.

Table 1. Average of survival rate catfish juvenile after transportation

Dose(0,015 ml/L)	Average SR(%)	Notation
Control	20,67	a
Clove Oil	98,67	b
Nutmeg Oil	97,33	b
Lemongrass Oil	93,33	b

Note: Values followed by the same letter in the same column show no significant difference at the 5% test level

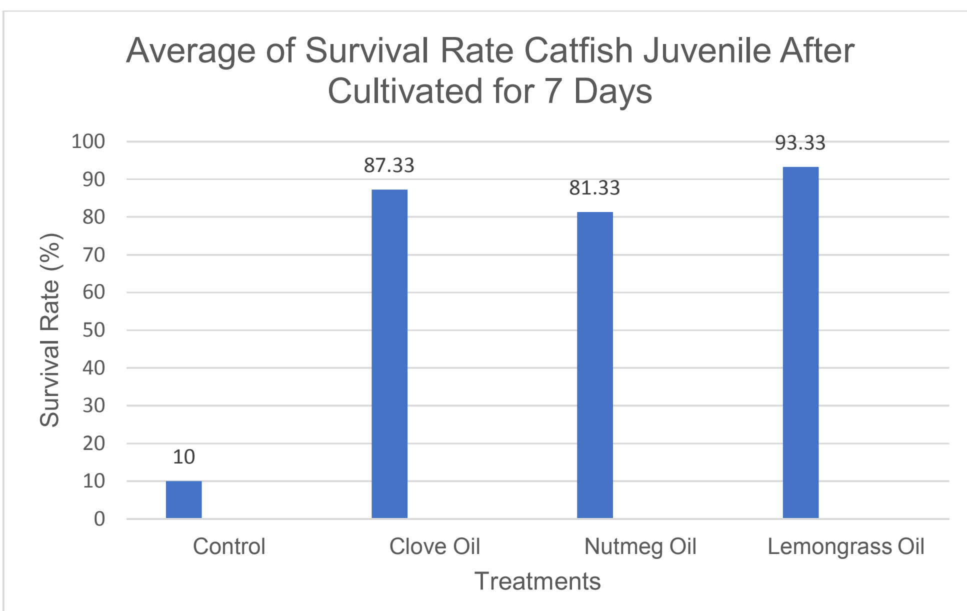

Fig. 2. Average of survival rate catfish juvenile after cultivated for 7 days

The results of the F test analysis (Table 2) using a level of 5% among the four treatments showed significantly different results, meaning that there were differences between the control treatment to clove oil, nutmeg oil and lemongrass oil. Further test results showed that the control treatment with clove oil, lemongrass oil and nutmeg oil were significantly different because they had different notations. However, the treatments for clove oil, lemongrass oil and nutmeg oil were not significantly different because they each had the same notation.

3.2 Induction Times

The induction time of clove oil resulted in the fastest induction time with an average of 12.59 minutes, while nutmeg and lemongrass oil had an average induction time of 30.05 minutes and 54.25 minutes. Clove oil anesthetic was the most effective anesthetic because according to Riesma et al. [14] induction time of less than 15 minutes is a good dose to faint fish and even better if it is less than 3 minutes. The induction times of catfish juveniles in each treatment was presented in Fig. 3.

The duration of induction of each anesthetic oil has different times for the catfish juveniles, this is because each material contains different essential oils. Eugenol from cloves has a concentration of 70% -90% [17], myristicin from nutmeg only has a concentration of 12% [18], while citronellal, citronellol, and geraniol from lemongrass add up to 50% concentration [19]. This causes clove anesthetic oil to have a shorter induction time than nutmeg and lemongrass oil.

3.3 Recovery Times

Lemongrass oil treatment has a very short recovery time of 3.22 minutes on average compared to the other two treatments, clove oil 10.34 minutes and nutmeg oil 4.40 minutes. Ongge [20] states that the entry of anesthetic substances into the blood of fish juveniles will cause the juveniles to become numb so that the process of recovering faint takes a long time and the duration of awareness was also influenced by the length of packaging. The recovery times of catfish juveniles in each treatment was presented in Fig. 4.

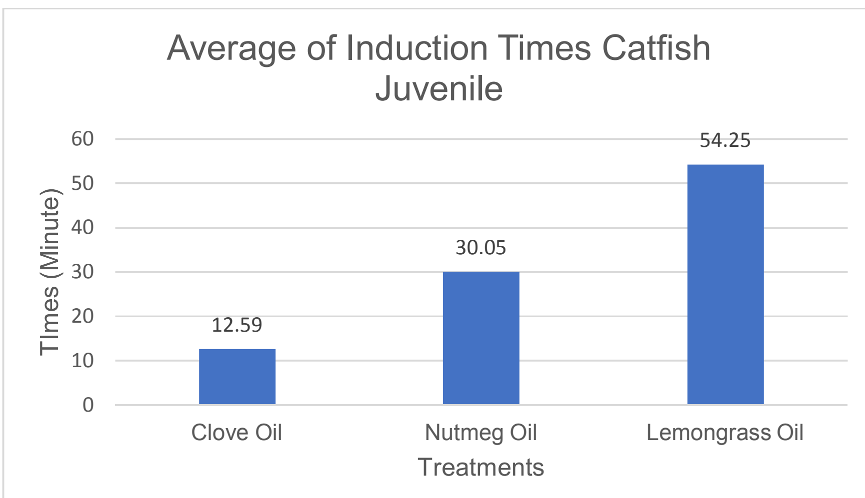

Fig. 3. Average of induction times catfish juvenile

Table 2. Average of survival rate catfish juvenile after cultivated for 7 days

Dose(0,015 ml/L)	Average SR(%)	Notation
Control	10	a
Clove Oil	87,33	b
Nutmeg Oil	81,33	b
Lemongrass Oil	85,33	b

Note: Values followed by the same letter in the same column show no significant difference at the 5% test level

Table 3. Water quality before and after transportation

Treatment	Temperature(°C)		pH		DO(mg/L)		Ammonia(mg/L)	
	a*	b**	a	b	a	b	a	b
Control	22,6	24,6	7,03	7,01	6	1	0,03	0,14
Clove Oil	22,3	25,3	7,02	6,97	6	4,6	0,02	0,03
Nutmeg Oil	22,6	24,6	7,05	6,94	6	5,3	0,03	0,06
Lemongrass Oil	22,6	25	7,03	6,95	6	5,3	0,03	0,08
Eligibility According to Literature :	22°-30°(***)		6-8,5(***)		>3mg/L(***)		<0,1(***)	

*Note : *=Before Transportation; **=After Transportation; ***=National Standardization Agency [22]*

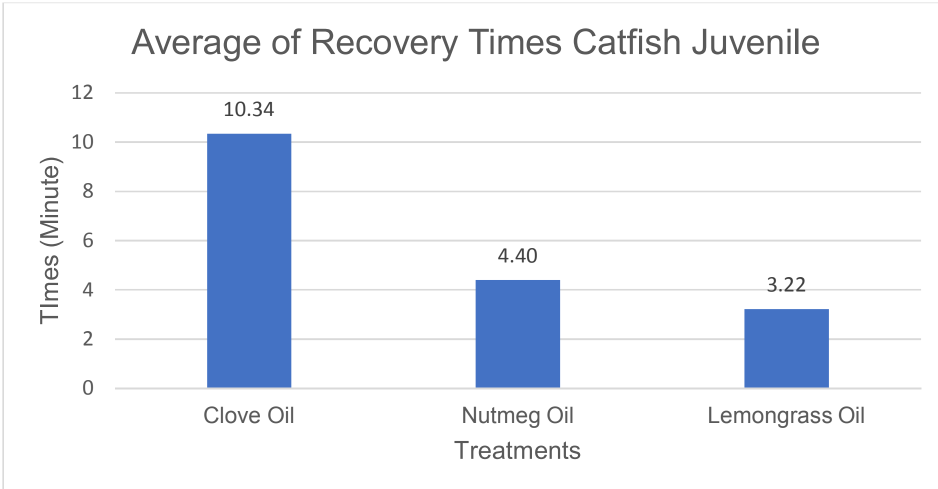

Fig. 4. Average of recovery times catfish juvenile

In the transportation process that uses the fainting technique, the gills play an important role in the fish awareness process because the anesthetic substances that are present in the fish's body are dissolved by placing them in clean water and containing very high DO. During the awareness process, water containing sufficient DO will enter through the gills and then enter the blood and will clean the remaining anesthetic substances in the body of the fish and will then discharged through fish feces [21]. Fish recovery to normal movement and swimming again took 10 minutes or less and no death was found for 15 minutes after unloading if the fish were anesthetized with the correct dose [14].

3.4 Water Quality

Water quality is the nature of water and the content of living things, energy substances or other components in water. Water quality is expressed by three parameters, namely biological parameters (presence of plankton, bacteria and etc.), physical parameters (temperature, turbidity, dissolved solids etc.), and chemical parameters (pH, DO, ammonia etc.). Water quality can indicate whether a water source was polluted or not in a certain time by comparing the water quality standards set.

The water quality of catfish juveniles in each treatment was presented in Table 3.

According to the National Standardization Agency [22], the optimal temperature for catfish farming is 25°C – 30°C. The temperature on transportation tends to be low between before transportation and after transportation because the time used for transportation of sangkuriang catfish juveniles is in the morning. Low temperatures can inhibit the metabolic rate of fish because according to Putra [23] an increase in temperature can cause an increase in the metabolic rate.

The low pH after transportation is thought to be due to the high activity of fish juveniles during the anesthetic process. According to Irianto [24], the decrease in water pH is caused by the amount of CO_2 produced from a respiration of water organisms, the reaction will tend to free H^+ so that the water pH will decrease. Before the fish faint because they are given anesthetic treatment, the fish will tend to respire quickly. With rapid respiration, a large amount of CO_2 is produced and in this reaction, H^+ is released into the water and makes the pH drop. The eligibility of the pH value for catfish farming according to the National Standardization Agency [22] is 6 - 8.5, while the data obtained in research are still at the literature eligibilty.

In the average DO value after transportation control treatment was quite small, 1 mg/ L, which resulted in very high mortality of catfish juveniles in this treatment. The control treatment did not use anesthetic so that the fish kept breathing and DO levels in the plastic packaging decreased. According to the National Standardization Agency [22] the DO value needed for catfish farming must be more than 3 mg/L.

According to the National Standardization Agency [22], the eligibility level of ammonia for the survival of catfish is <0.1 mg/L. Based on the research that had been done, after five hours of transportation the ammonia value tends to increase. This is presumably because at the beginning of the anesthetic process the metabolism of fish juveniles tends to increase due to adaptation to the new environment. The product of fish juveniles metabolism is ammonia. Ammonia value in this research is still categorized as safe because its value is not much different from the eligibility levelaccording to the National Standardization Agency [22].

4. CONCLUSION

Differences in natural anesthetic materials have no effect on the survival rate of catfish juveniles during transportation. These three natural anesthetic materials can be used to transport sangkuriang catfish juveniles. However, based on induction time, clove oil was the fastest to faint catfish juveniles which was 12.59 minutes and obtained a high survival rate both after transportation and after 7 days of aquaculture 98.67% and 87.33% although slowly times for recovery 10.34 minute.

CONSENT

As per international standard informed and written participant consent has been collected and preserved by the authors.

COMPETING INTEREST

Authors have declared that no competing interests exist.

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