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EFFECTIVENESS OF CLOVE OIL (*Syzygium aromaticum* L.) AS A NATURAL ANAESTHETIC IN SEURUKAN FISH JUVENILE (*Osteochilus vittatus*)

EFEKTIVITAS MINYAK CENGKEH (*Syzygium aromaticum* L.) SEBAGAI BAHAN ANESTESI ALAMI PADA BENIH IKAN SEURUKAN (*Osteochilus vittatus*)

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Abstract

Seurukan fish (*Osteochilus vittatus*) is a freshwater fish of economic value whose supply still relies heavily on wild catches. In aquaculture, particularly during grading, transportation and research, anaesthetics are needed to reduce stress and to improve survival. Common synthetic anaesthetics may leave residues and harm the environment, so that safe natural alternatives are required. Clove oil (*Syzygium aromaticum* L.), which contains eugenol, is known to have anaesthetic and sedative properties in fish. This study aimed to determine the effect and the optimal dose of clove oil as a natural anaesthetic for seurukan juveniles. It was conducted in October 2025 at the Hatchery of the Faculty of Fisheries and Marine Science, Teuku Umar University, using a completely randomised design with four treatments and three replications: a control without clove oil (P0) and doses of 0.02 ml/L (P1), 0.04 ml/L (P2) and 0.06 ml/L (P3). The parameters observed were induction and sedative time, blood glucose, operculum opening frequency, survival and water quality. Clove oil had no significant effect on survival or blood glucose ($P>0.05$), but it lowered the operculum opening frequency and accelerated induction. A dose of 0.04 ml/L was the most effective, giving an optimal anaesthetic response, a good recovery time and the highest survival (96.67%).

Keywords: blood glucose, clove oil, natural anaesthetic, *Osteochilus vittatus*, survival rate

Abstrak

Ikan seurukan (*Osteochilus vittatus*) merupakan ikan air tawar bernilai ekonomis yang pasokannya masih bergantung pada tangkapan alam. Anestesi diperlukan saat sortasi, transportasi, dan penelitian untuk mengurangi stres ikan. Anestesi sintetis berpotensi meninggalkan residu dan mencemari lingkungan sehingga diperlukan alternatif alami yang aman. Minyak cengkeh (*Syzygium aromaticum* L.) yang mengandung eugenol diketahui bersifat anestetik dan sedatif pada ikan. Penelitian ini bertujuan menentukan pengaruh dan dosis optimal minyak cengkeh sebagai anestesi alami pada benih ikan seurukan. Penelitian dilaksanakan pada Oktober 2025 di Hatchery Fakultas Perikanan dan Ilmu Kelautan Universitas Teuku Umar menggunakan rancangan acak lengkap empat perlakuan tiga ulangan, yaitu kontrol (P0), 0,02 ml/L (P1), 0,04 ml/L (P2), dan 0,06 ml/L (P3). Parameter yang diamati meliputi masa induksi dan sedatif, glukosa darah, bukaan operkulum, kelangsungan hidup, dan kualitas air. Minyak cengkeh tidak berpengaruh nyata terhadap kelangsungan hidup dan glukosa darah ($P>0,05$), tetapi menurunkan bukaan operkulum dan mempercepat induksi. Dosis 0,04 ml/L paling efektif karena menghasilkan respons anestesi optimal, waktu pemulihan baik, dan kelangsungan hidup tertinggi (96,67%).

Kata kunci: anestesi alami, glukosa darah, kelangsungan hidup, minyak cengkeh, *Osteochilus vittatus*

1. Introduction

Seurukan fish (*Osteochilus vittatus*) is a freshwater species originating from Aceh that has a high economic value because of its good protein content and its tender flesh. Market demand for this fish is relatively high, with a price ranging from IDR 30,000 to IDR 35,000 per kilogram (Ibrahim et al., 2019). However, the supply of seurukan fish still depends on catches from the wild, a condition that may threaten the sustainability of the resource. Efforts to develop the culture of this species face several obstacles, including slow growth and the limited availability of good-quality seed (Samsuli et al., 2023). One of the seurukan production centres is in Nagan Raya, from which the seed is distributed to fish farmers, and during seed transport one of the handling measures applied is the use of anaesthetic agents.

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Anaesthesia plays an important role in aquaculture activities, especially in spawning, grading, transportation and physiological research, because it suppresses metabolic activity, reduces stress and increases the survival rate of fish (Arlanda et al., 2018). Synthetic anaesthetics such as tricaine methanesulfonate (MS-222), ether and propoxate have long been used for this purpose. Their application is nevertheless limited because they may leave harmful residues, affect fish health and are prohibited in several countries (Chilmawati and Amalia, 2022). The search for natural anaesthetic agents that are effective, safe and environmentally friendly is therefore greatly needed.

Clove oil (*Syzygium aromaticum* L.) has long been used as a natural anaesthetic for various cultured fish. Its main component, eugenol (70-95%), has sedative, antiseptic and local anaesthetic properties (Wimadani and Fitri, 2024). Several studies have demonstrated the effectiveness of clove oil as an anaesthetic in tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*) and giant gourami (*Osphronemus gouramy*) (Kurniawan et al., 2021; Irhami et al., 2023). Clove oil is known to reduce physiological activity, accelerate the induction process and provide a better recovery than several other natural anaesthetic agents (Darmawati et al., 2021).

Most of the available studies, however, are still limited to popular cultured species for which physiological data are already widely available. To date, no comprehensive study has examined the effectiveness of clove oil as an anaesthetic for seurukan fish (*O. vittatus*), even though this species has a significant ecological and economic value in Indonesia (Mahendra and Supriadi, 2020; Martisa et al., 2024). This knowledge gap raises the question of the optimal dose of clove oil that can reduce the physiological stress response of the fish, namely blood glucose, operculum opening frequency, induction time and recovery time, without reducing the survival of the juveniles. This study therefore aims to determine the effect of different doses of clove oil on the physiological response of seurukan juveniles and to determine the optimal dose that supports a high survival rate and a normal recovery.

2. Materials and Methods

2.1. Preparation

This study was conducted from 15 to 28 October 2025 at the Hatchery of the Faculty of Fisheries and Marine Science, Teuku Umar University, Meulaboh. The containers used were twelve jars with a capacity of 16 L and twelve container boxes with a capacity of 25 L. All containers were washed thoroughly, dried, filled with 10 L of fresh water per container and labelled according to the treatment and the replication applied. Before the fish were stocked, the water was allowed to settle, and aeration was installed in the recovery containers.

The test animals used in this study were 120 seurukan juveniles measuring 10-12 cm. The juveniles were first held in a cleaned tarpaulin pond and acclimatised for three days, during which they were fed commercial pellets twice a day, so that the fish were able to adjust to the water conditions used in the study and errors caused by environmental factors could be minimised. Each experimental unit was stocked with 10 fish. The clove oil used was a commercial product that is widely available on the market (Cap Gajah brand).

2.2. Experimental Design

This study used a completely randomised design (CRD) with four treatments and three replications. The clove oil doses applied in this study refer to Kurniawan et al. (2021), and the design tested was as follows:

P0 : Control (without clove oil)
P1 : Clove oil 0.02 ml/L
P2 : Clove oil 0.04 ml/L
P3 : Clove oil 0.06 ml/L

2.3. Fish Rearing

After the anaesthetic treatment and the recovery process had been completed, the juveniles were reared for 14 days in order to determine whether the clove oil solution had a side effect on the fish, particularly on the survival rate, and to help the fish return to a normal condition after body functions such as respiration and equilibrium had been disturbed by the anaesthesia. Feeding started 24 hours after recovery to avoid additional stress on the digestive system of the fish and to prevent pollution of the rearing water by uneaten feed. Feed was given twice a day using the ad satiation method. Siphoning was carried out every two days, with a water replacement of 50-70% depending on the degree of water fouling.

2.4. Test Parameters

2.4.1. Blood glucose

Blood glucose in the fish body is the main source of energy and an essential substrate for metabolism, particularly in the brain cells. The blood volume of fish ranges from 1.5 to 3% of the body weight, and a normal fish blood glucose level contains 40-90 mg/dL (Nasichah et al., 2016). Glucose is important in meeting the high energy demand under stress, because stress diverts energy from normal metabolic processes towards the activation of physiological systems (Mulyani, 2023). The blood glucose level was measured using a glucometer as the measuring device and strips as the sample holder. The measurement was carried out after the fish had regained consciousness; the blood sample was taken by puncturing the caudal peduncle, and the blood that emerged was placed onto the strip for the glucose reading.

2.4.2. Induction and sedative time

The induction time was measured from the moment the anaesthetic agent was applied until the fish became immotile, a condition indicated by a slowing of the operculum movement and by the fish remaining motionless at the bottom of the container. A shorter induction time is preferable because it reduces the duration of the stress experienced by the fish during the induction process; the induction time differs between fish species and depends on the weight of the fish and on the type of anaesthetic agent used (Irhami et al., 2023). The sedative time is the time required by the fish to regain consciousness after fainting. To accelerate the recovery, the fish were transferred into water fitted with aeration and were then observed until they became active again. Both periods were recorded using a stopwatch. The behavioural responses of the fish at the various levels of anaesthesia are presented in Table 1.

Table 1.
Behavioural response of fish at different levels of anaesthesia

Condition of the fish	Behavioural response of the fish
Normal	Reactive to external stimuli; operculum movement and muscle contraction are normal.
Light sedation	Reactivity to external stimuli slightly reduced, operculum movement slows and equilibrium remains normal.
Sedation	No reactivity to external stimuli except under strong pressure; operculum movement is slow and equilibrium is maintained.
Partial loss of equilibrium	Muscle contraction is weak, swimming is irregular, the fish reacts only to vibration and strong touch, and operculum movement is rapid.
Total loss of equilibrium	Muscle contraction ceases, operculum movement weakens but remains regular, and the spinal reflex disappears.

No reflex movement	No reactivity, operculum movement is slow and irregular, the heartbeat is slow and reflexes are absent.
Medullary collapse	Operculum movement and respiration cease, and the heartbeat stops a few minutes later.

Source: Midani (2021)

2.4.3. Operculum opening frequency

The operculum protects the head region and regulates the mechanism of water flow during respiration, and it also serves as the route through which water enters and leaves the body. The frequency of operculum opening increases when the fish is in clean water, whereas in polluted water the operculum tends to remain closed. This occurs because the effect of toxic substances on the fish alters the morphology of the gills and causes mortality over a long period. In addition, toxic substances may damage the respiratory function of the gills, so that the metabolic processes in the body are disturbed (Nasution et al., 2023). Observation of the operculum was carried out after the addition of the clove oil solution, by counting the number of opening and closing movements of the operculum from the beginning of the application of the anaesthetic agent until the fish lost consciousness.

2.4.4. Survival rate

The survival rate indicates the percentage of fish that remained alive until the end of the study. The survival rate of the fish was calculated using the formula of Effendie (1997):

$$SR\ (\%) = Nt\ /\ No\ x\ 100$$

Notes:
SR = Survival rate (%)
Nt = Number of fish at the end of rearing (individuals)
No = Number of fish at the beginning of the study (individuals)

The survival rate was calculated at two stages of observation, namely the initial and final survival rate. The initial survival rate was calculated after the anaesthetic procedure had been completed and the fish had reached recovery condition, by recording the number of live fish after the anaesthetic treatment. This stage describes the survival of the fish as a direct effect of the administration of the anaesthetic. The final survival rate was calculated after the fish had been reared for 14 days under the predetermined rearing conditions; the number of live fish at the end of the rearing period was recorded and used to calculate the final survival rate, which reflects the ability of the fish to survive after anaesthesia during the rearing period.

2.4.5. Water quality

Water quality in this study was measured three times, namely on day 0, day 7 and day 14. The water quality parameters measured were temperature, pH, dissolved oxygen (DO) and ammonia.

2.5. Data analysis

The data obtained were grouped and tabulated in Microsoft Excel 2013. An analysis of variance (ANOVA) was subsequently performed using SPSS 20.0 software to examine the effect of the treatments. Where a significant difference was found, Duncan's multiple range test at a confidence level of 95% was applied to determine the best treatment.

3. Results and Discussion

3.1. Results

The effect of the clove oil solution used as an anaesthetic agent on the initial survival rate, the blood glucose level, the operculum opening frequency and the final survival rate of the seurukan juveniles is presented in Table 2. The analysis of variance showed a significant effect (P<0.05) on the operculum opening frequency, whereas the initial survival rate, the blood glucose level and the final survival rate did not differ significantly between the treatments (P>0.05).

Table 2.
Effect of the clove oil solution on the survival rate, blood glucose and operculum opening frequency of seurukan juveniles

Parameter	P0	P1	P2	P3
Initial SR (%)	100±0[a]	100±0[a]	100±0[a]	100±0[a]
Blood glucose (mg/dL)	72.67±8.06[a]	84.67±2.49[a]	79.00±8.83[a]	74.00±15.75[a]
Operculum opening (times)	770±29.44[b]	303±71.34[a]	324±54.81[a]	397±107.71[a]
Final SR (%)	90±0.8[a]	90±0.8[a]	96.67±0.5[a]	86.67±0.9[a]

Note: SR, survival rate. Different letters within the same row indicate significant differences between treatments (P<0.05). The values presented in the table represent the mean and standard deviation.

The induction time and the sedative time of the seurukan juveniles were observed to determine the time required by the fish to lose consciousness and to regain consciousness during the anaesthetic process. The results are presented in Table 3. The control treatment (P0) is not presented because, in the absence of clove oil in the medium, the fish did not reach the immotile stage.

Table 3.
Induction time and sedative time of the seurukan juveniles

Treatment	Induction time (minutes)	Sedative time (minutes)
P1	03:46 - 10:24	04:02 - 08:44
P2	04:19 - 10:19	04:31 - 08:50
P3	04:28 - 07:49	04:45 - 07:09

The frequency of operculum opening of the seurukan juveniles was observed to determine the level of consciousness of the fish based on the number of operculum openings. The results of the observation are presented in Table 4.

Table 4.
Frequency of operculum opening of the seurukan juveniles

Treatment	Opening from the first minute until the sedative stage	Optimum 120 times/minute (Prariska et al., 2017)
P0	770 (05:00)	600
P1	303 (03:46)	452
P2	324 (04:19)	518
P3	397 (04:28)	536

The results of the measurement of the water quality parameters carried out during the anaesthetic process and the rearing period, namely temperature, pH, dissolved oxygen and ammonia, are presented in Table 5.

Table 5.
Water quality measurements during the study

No	Parameter	Sampling 1	Sampling 2	Sampling 3	Optimum
1	Temperature (°C)	26.7 - 28.3	28.4 - 28.6	28.9 - 29.4	26 - 30[a]
2	pH	7.89 - 8.27	7.95 - 8.22	7.90 - 8.17	6.5 - 9[b]
3	DO (mg/L)	5.3 - 6.5	5.4 - 6.4	4.4 - 5.3	>4[c]
4	Ammonia (mg/L)	0	0 - 0.1	0 - 0.05	<0.2[d]

Note: DO, dissolved oxygen. Optimum values follow [a]Azhari et al. (2017); [b]Mayana et al. (2016); [c]Diansyah et al. (2024); [d]Majid (2024).

3.2. Discussion

3.2.1. Initial survival rate

Based on the observations, the initial survival rate in all treatments (P0, P1, P2 and P3) reached 100%, which indicates that all of the fish were in a healthy condition and did not experience prolonged stress during the handling process, and that the water quality after the application of the anaesthetic was still within the optimum category. The analysis of variance showed that the survival rate of the fish under the influence of clove oil as an anaesthetic agent did not differ significantly (P>0.05), and Duncan's test showed that P0, P1, P2 and P3 had no significant effect on the survival rate of the seurukan juveniles.

Survival is one of the important indicators for assessing whether a clove oil solution is safe to use as an anaesthetic agent for seurukan juveniles. Clove oil contains anaesthetic compounds that are well tolerated by the fish body and are therefore able to induce unconsciousness rapidly. Eugenol, the compound contained in clove oil, is absorbed through the respiratory tissue, subsequently diffuses into the blood and spreads throughout the body, and may cause the nervous system to cease functioning (Iyeda et al., 2024). The use of eugenols as an anaesthetic agent causes the fish to lose its equilibrium and lowers its metabolic activity, which in turn reduces metabolic production (Saragih et al., 2025).

The physiological response of the seurukan fish can be observed from changes in the frequency of operculum opening, an increase in mucus production and, under extreme stress, mortality caused using clove oil. Stressed fish can also be recognised from an increase in the rate of operculum movement, from gulping air at the water surface and from a decrease in the level of activity and movement of the fish (Firdaus et al., 2018). A substance is considered toxic if it can cause mortality in an organism within less than 14 days after its application (Novitasari and Prasetio, 2017). These results therefore show that the use of a clove oil solution as a natural anaesthetic agent is effective and safe only at a low or optimal dose, whereas the use of a high dose may increase the risk of a decline in the survival rate of the seurukan fish.

3.2.2. Blood glucose

Blood glucose is one of the important variables in assessing the stress level of fish. In this study, the use of a clove oil solution as a natural anaesthetic agent had no significant effect on the blood glucose level of the seurukan fish. The measurement of blood glucose is important because it can identify the stress level of an organism (Pratama et al., 2023). The ideal blood glucose level for fish ranges from 40 to 90 mg/dL (Shabrina et al., 2018). Based on the measurements, P0 (72.67±8.06 mg/dL), P1 (84.67±2.49 mg/dL), P2 (79.00±8.83 mg/dL) and P3 (74.00±15.75 mg/dL) were all still within the optimal blood glucose range. The analysis of variance showed that the administration of the clove oil solution as a natural anaesthetic agent had no significant effect (P>0.05) on the blood glucose content of the seurukan fish. This is attributable to the physiological adaptation of the fish, since the doses applied were still within the tolerance range of the seurukan fish.

The physiological response of fish in facing stress is divided into three stages, namely the primary, the secondary and the tertiary response. The primary response is characterised by an increase in the catecholamine and corticosteroid hormones; the secondary response is indicated by an increase in the blood glucose level and by osmoregulatory disturbance; and the tertiary response can be observed at the whole-organism level, such as growth, immune response, disease resistance and behaviour (Djauhari et al., 2020). The main indicator that may cause stress in fish is the cortisol level in plasma, and the second indicator is an increase in blood glucose. When cortisol increases, the activity of insulin declines, and a decrease in insulin raises the blood glucose level and may cause the fish to become stressed (Haq et al., 2022). Excessive blood glucose level also has a negative impact on the fish body, such as a reduction in blood volume and in the number of leucocytes, a decrease in the glycogen reserves of the liver, shrinkage of the stomach diameter and thinning of the mucosal layer (Yunus and Yushra, 2023).

3.2.3. Induction time and sedative time

One of the natural anaesthetic agents that has long been known by the community is clove oil, which contains the compound eugenol. This compound can be used as a natural material to anaesthetise fish, in that eugenol is absorbed through the gills, spreads into the bloodstream and then enters the nerve cells, so that the fish loses consciousness and faints. The mixing of eugenol or essential oil into the medium can influence the decrease in fish metabolism; this condition is caused by a central nervous system that can no longer be controlled, so that the response of the fish to external stimuli progressively declines (Becker et al., 2016).

The anaesthesia of fish using natural materials takes place through the circulatory system and through the process of diffusion into the body tissues. This mechanism allows the anaesthetic compound to enter the body through the anaesthetic medium, so that the supply of oxygen to the brain is inhibited and physiological changes occur that shift the fish from a conscious to an unconscious condition (Putri et al., 2021). The induction time and sedative time parameters were used to determine the effect of the various doses of clove oil solution on the length of time required by the fish to lose consciousness and on the length of time required by the fish to regain consciousness.

Based on the results of the study, the fastest average induction time occurred in treatment P3, at minute 04:28-07:49, and the slowest in treatment P1, at minute 03:46-10:24, whereas in P0, the control treatment without the application of clove oil solution, the fish did not show a significant response or behavioural change and did not reach the fainting stage because no anaesthetic agent was added to the medium. According to Rachimi and Khoiron (2016), the optimal anaesthetic time is achieved when the induction period is relatively short, so that the risk of prolonged stress in the fish can be reduced; a good anaesthetic agent has an induction time of less than 15 minutes and preferably less than 3 minutes. The differences in time indicate that an increase in the concentration of the clove oil solution accelerates the induction time. This is caused by the higher concentration of the eugenol compound and by the size of the fish, since a higher eugenol content causes small fish to show a faster response to the active compound, because the absorption of the compound proceeds more optimally.

Eugenol acts on the consciousness of the fish through a rapid process that begins when the fish takes up the compound from the water through its highly vascularised gills. Once in the bloodstream, eugenol rapidly reaches the central nervous system, including the brain. There, eugenol acts by binding to and modulating certain nerve receptors, particularly the GABA receptors, which inhibit the transmission of normal nerve signals, the metabolic rate and the respiratory system, and which ultimately induces a state of unconsciousness, immobilisation and a total anaesthesia that is temporary in nature. The higher the dose administered, the greater the amount of eugenol that enters the body, so that the induction process can proceed more rapidly. Conversely, a low dose produces a slower effect on the loss of consciousness, so that the lower the dose administered, the longer the time required by the fish to faint (Rifa'i, 2016).

Fish that have lost consciousness can be recognised from the cessation of swimming and from the fish remaining motionless at the bottom, from the absence of a response to the stimuli given, and from an operculum opening that begins to slow down (Nurkholifah et al., 2022). The stages of fish behaviour up to the loss of consciousness comprise the normal condition, active movement, loss of equilibrium, remaining motionless at the bottom of the water, a slowing of the operculum movement, fainting and finally death (Saputra et al., 2017).

Based on the results of the study, the sedative period of the seurukan juveniles showed the shortest duration in treatment P3 (0.06 ml/L), with a recovery time of 04:45-07:09, and the longest duration in treatment P2 (0.04 ml/L), with a recovery time of 04:31-08:50. It can therefore be concluded that the administration of a higher dose (0.06 ml/L) provided a more stable anaesthetic effectiveness. The duration of the recovery of consciousness was measured from the moment the fish were placed in a container filled with clean water until the fish showed signs of regaining consciousness from the fainting condition, such as swimming normally again, moving actively, responding to external stimuli and no longer displaying a weakened physical condition (Hardiyani et al., 2020). This may be influenced by the physiological responses of the fish, such as the metabolic process and the detoxification process within the body of the fish.

The recovery time is strongly influenced by the treatment dose and by the oxygen content of the rearing or recovery container. Water with a high dissolved oxygen content plays an important role in supporting the physiological functions of the fish, particularly in the process of eliminating the anaesthetic residues that remain in the body of the fish. The oxygen that enters the water through the gills is carried into the blood and therefore helps to transport the residues present in the body of the fish towards the excretory organs, from which they are subsequently released (Mariana et al., 2015). Fish that have regained consciousness are generally recognised first from the movement of the gills, followed by swimming movements that are initially weak and that eventually return to normal.

3.2.4. Frequency of operculum opening

Based on the observations obtained, the frequency of operculum opening of the fish under the influence of the clove oil solution as an anaesthetic agent differed between treatments. In P0 the operculum opening was approximately 770 times, counted from the first minute to the fifth minute; the operculum opening frequency in this treatment was comparatively high because no clove oil solution was applied, so that it still falls within the normal or optimal range of operculum opening. In P1 the frequency of operculum opening was approximately 303 times, counted from the first minute until the fish fainted; in P2 the frequency was approximately 324 times, counted from the first minute until the fish fainted; and in P3 the frequency was approximately 397 times, counted from the first minute until the fish fainted. The analysis of variance showed that the frequency of operculum opening differed significantly between treatments ($P<0.05$).

The movement of the operculum when the fish is close to fainting can be observed from an operculum opening that is initially rapid and then slows as time proceeds, which causes the condition of the fish to become calm and finally to result in a loss of equilibrium (Erlangga et al., 2024). As the immersion in the clove oil solution proceeds, the frequency of operculum opening of the fish declines and slows down, which indicates that the metabolic activity of the fish decreases as a result of the effect of the compound used during the anaesthesia.

The frequency of the operculum opening movement is one of the signs that determines whether the fish has entered the anaesthetic phase, a phase in which the condition of the fish has begun to decline and in which the response to external stimuli has weakened. Such a condition may indicate that fish experiencing severe stress can eventually die (Rachmawati et al., 2010). Dead fish can generally be recognised from the symptom in which the mouth and the gills no longer move and the body floats to the water surface (Kurniaji et al., 2022). When the operculum opening declines, the volume of water passing over the gills is reduced; when the function of the gills is disturbed, the uptake of oxygen from the aquatic environment declines, which may result in the death of the fish as a consequence of hypoxia or a lack of oxygen (Wijaya et al., 2023).

3.2.5. Water quality

The mean temperature during the study was within the optimal range, namely 26.7-29.4 °C. The optimal temperature for the survival and growth of seurukan fish is 26-30 °C (Azhari et al., 2017). The mean pH of the water during the study was also within the optimal range, namely 7.89-8.27. A pH value in waters ranging from 6.5 to 9.0 is still within the range that can be accepted for the survival and rearing of seurukan fish (Mayana et al., 2016). The mean dissolved oxygen content during the study ranged from 4.4 to 6.5 mg/L. The optimal dissolved oxygen content of the water for rearing seurukan fish is more than 4 mg/L (Diansyah et al., 2024).

The mean ammonia content in the first sampling was 0 mg/L, whereas in the second sampling the ammonia content was 0-0.1 mg/L and in the third sampling 0-0.05 mg/L. The optimum concentration of dissolved ammonia in water for rearing nilem fish is below 0.2 mg/L (Majid, 2024). High ammonia levels in the rearing medium originate from the metabolism of the fish, such as faeces and urine, and may also originate from feed residues that are not fully consumed by the fish. In order to prevent a high ammonia concentration in the rearing medium, siphoning and water exchange can be carried out with the aim of lowering the ammonia content.

3.2.6. Rearing after anaesthesia

Based on the results obtained, the highest survival rate during the rearing period occurred in treatment P2 (0.04 ml/L), with a survival rate of 96.67% and an average of 9-10 seurukan fish remaining per replicate. The lowest survival rate was found in treatment P3 (0.06 ml/L), with a survival rate of 86.67% and an average of 8-10 seurukan fish remaining per replicate. The analysis of variance showed that the survival rate of the fish under the influence of the use of the clove oil solution did not differ significantly ($P>0.05$), and Duncan's test likewise showed that P0, P1, P2 and P3 had no significant effect.

The low survival rate in P3 was caused by the use of the clove oil solution at a dose that was too high, namely 0.06 ml/L, which was not tolerated by the body of the fish. This occurs because the physiological system of the fish is unable to tolerate the effect of an excessively high concentration of the chemical compounds contained in the clove oil solution. An excessive stress response causes a disturbance of the metabolism and of the respiratory system, which ultimately leads to mortality (Lestari and Syukriah, 2020).

The rearing of the fish was carried out in order to evaluate whether the clove oil solution had a long-term impact on the condition of the fish and whether the fish were able to return to a normal condition. After immersion, the eugenol residue stored in the body of the fish declines more rapidly than that of other compounds, although the rate and the time of elimination depend strongly on the species, the dose, the duration of exposure and the water temperature; this compound declines sharply within a period of 48 hours (Silvianti et al., 2016). During the rearing period, feeding was started 24 hours after recovery in order to prevent additional stress on the digestive system of the fish and to prevent pollution of the aquatic environment by feed that is not eaten by the fish, since residual feed is converted into ammonia, a compound that is toxic to fish. During the first week of rearing, siphoning was carried out every day with the aim of removing the waste that remained at the bottom of the rearing container, and water exchange was carried out routinely, because the appearance of excessive mucus may cause the fish to become stressed.

The success of rearing fish after anaesthesia is measured on the basis of the survival rate together with physiological indicators such as the body colour and the response of the fish to feed. The high survival rate in P2 was caused by the administration of the clove oil solution at a dose that was still within the safe limit, namely 0.04 ml/L, a dose that can still be tolerated by the fish and that does not cause severe physiological disturbance.

4. Conclusion

A dose of 0.06 ml/L (P3) produced the fastest induction time, but a dose of 0.04 ml/L (P2) was the best for anaesthesia because it resulted in the highest survival rate (96.67%) and remained safe for the physiology of the fish. Clove oil had a significant effect on the frequency of operculum opening, but it did not disturb the blood glucose level or the water quality, and it is therefore proven to be an effective and safe natural anaesthetic alternative for seurukan fish (*Osteochilus vittatus*).

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