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The effectiveness of immersed in extract viscera of sea cucumber *Holothuria* sp on growth, consumption level and feed efficiency in tilapia larvae *Oreochromis niloticus*

Efektivitas perendaman ekstrak jeroan teripang *Holothuria* sp terhadap pertumbuhan, tingkat konsumsi dan efisiensi pakan pada larva ikan nila *Oreochromis niloticus*

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ABSTRACT

The aim of this research was to determine the effect of sea cucumber immersion extract on growth, consumption and feed efficiency in masculinized tilapia larvae aged six to seven days. September–November 2022 was the time the research was carried out and the experimental method used in this study was a Completely Randomized Design (CRD) with five treatments and three replications. The treatment used was the soaking of sea cucumber viscera with doses of 0 ml/L (A), 1 ml/L (B), 3 ml/L for 24 hours (C), 5 ml/L for 24 hours (D), and 0.5 ml/L 17α-MT for 12 hours as positive control (E), then maintained for 60 days. Parameters observed included absolute length growth, absolute weight growth, feed consumption level, feed efficiency and water quality. The results showed that immersion sea cucumber innards for 24 hours with a dose of 1 ml/L showed the highest growth rate in absolute length and a dose of 3 ml/L showed the highest feed consumption rate.

Keywords: feed consumption rate, feed efficiency, sea cucumber viscera, tilapia fry

ABSTRAK

Tujuan dari penelitian ini adalah menentukan pengaruh ekstrak jeroan teripang terhadap pertumbuhan, konsumsi dan efisensi pakan pada larva ikan nila hasil maskulinisasi yang berumur 6 sampai 7 hari. Penelitian ini dilakukan pada bulan September–November 2022 dan metode eksperimen yang digunakan dalam penelitian ini adalah Rancangan Acak Lengkap (RAL) dengan lima perlakuan dan tiga ulangan. Perlakuan yang digunakan adalah perendaman jeroan teripang dengan dosis 0 ml/L (A), 1 ml/L (B), 3 ml/L (C), 5 ml/L selama 24 jam (D), dan 0,5 ml/L 17α-MT selama 12 jam sebagai kontrol positif (E), kemudian dipelihara selama 60 hari. Parameter yang diamati meliputi pertumbuhan panjang mutlak dan bobot mutlak ikan, tingkat konsumsi pakan, efisiensi pakan dan kualitas air. Hasil penelitian menunjukkan perendaman jeroan teripang selama 24 jam dengan dosis 1 ml/L menunjukkan laju pertumbuhan panjang mutlak yang tertinggi dan pada dosis 3 ml/L tingkat konsumsi pakan dan efsiensi pakan tertinggi.

Kata kunci: benih ikan nila, efisiensi pakan, jeroan teripang, tingkat konsumsi pakan

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INTRODUCTION

The potential of the fisheries sector in Indonesia is vast, ranging from marine fisheries resources to freshwater fisheries resources, providing significant opportunities for fisheries development, particularly in freshwater fisheries. According to Fandana *et al.* (2020), one of the most promising freshwater fish species for cultivation is Nile tilapia (*Oreochromis niloticus*), as it is a highly demanded commodity. Fujaya *et al.* (2022) stated that Nile tilapia has flesh rich in essential nutrients such as proteins, lipids, and minerals. The cultivation process of Nile tilapia is relatively easy, and the fish exhibit rapid growth. According to Iskandar *et al.* (2021), male Nile tilapia grow faster than females, making them more economically advantageous, whereas the slower growth rate of female Nile tilapia can hinder production efficiency.

To address this issue, sex reversal techniques have been applied to convert female Nile tilapia into males, a process known as masculinization. Masculinization is a sex differentiation technique aimed at altering the phenotypic sex from female to male using steroid hormones (androgens). The most commonly used synthetic androgen is 17α-methyltestosterone. Beyond its role in sex reversal, this hormone also promotes growth through three mechanisms: stimulating appetite, enhancing protein synthesis, and suppressing gonadal development (Andi *et al.*, 2020). However, the use of 17α-methyltestosterone poses environmental and public health risks, leading to its prohibition by the government through regulatory policies (Permen, 2019). Consequently, there is a need for natural alternatives for Nile tilapia masculinization. One potential yet underutilized aquaculture byproduct that contains high levels of steroid hormones is the visceral organs of the sand sea cucumber (*Holothuria scabra*).

Sea cucumbers (*Holothuridae* sp.) are marine organisms with high nutritional value, as reported by Huwae *et al.* (2021). One of the underutilized parts of sea cucumbers in aquaculture is their viscera, which consists of internal organs. According to Amanullah (2021), the steroid content in sea cucumber viscera includes testosterone, which can be utilized for masculinization, as demonstrated in giant freshwater prawns (*Macrobrachium rosenbergii*) (Susanto *et al.*, 2017), guppy fish (*Poecilia reticulata*) (Emilda, 2012; Saputra *et al.*, 2018), and freshwater lobsters (*Cherax quadricarinatus*) (Susanto *et al.*, 2018). Additionally, it has been used to enhance the reproductive performance of male black tiger shrimp (*Penaeus monodon*) (Hidayani *et al.*, 2024).

According to Susanto *et al.* (2024), immersion in a sea cucumber viscera extract combined with honey can increase the percentage of male Nile tilapia up to 83.33%. The highest concentration of testosterone in sea cucumbers is found in reproductive organs (testes, ovaries, cortex, and placenta) as well as the intestines, which form the largest part of the viscera (Emilda, 2012). This aligns with the findings of Susanto *et al.* (2023), which stated that the extraction of 1 kg of fresh sea cucumber viscera yields 21.28 g (2.128%) of steroid compounds. GC-MS and NMR analyses confirmed that the molecular weight of the extracted steroid is 288.42, identifying it as testosterone (Hidayani *et al.*, 2024).

Beyond its role in masculinization, steroid hormones function as receptors that facilitate protein transport into cells, thereby enhancing protein metabolism and improving the efficiency of dietary protein utilization. The presence of additional steroid hormones in the body increases the protein metabolism rate through the stimulation of mRNA synthesis (Heriyati *et al.*, 2015). Proper protein metabolism requires an adequate supply of dietary protein, with feed being a primary source. A high protein metabolism rate can stimulate appetite, which directly influences feed consumption. The consumed feed is then efficiently utilized for growth (Aslamyah & Fujaya, 2010).

Based on the aforementioned discussion, it is crucial to evaluate the effects of sea cucumber viscera extract on the growth rate, feed consumption, and feed efficiency of masculinized Nile tilapia larvae. Furthermore, no previous studies have specifically investigated the impact of sea cucumber viscera extract on these parameters in Nile tilapia larvae. Therefore, this research aims to determine the optimal dosage of sea cucumber viscera powder for enhancing growth, feed consumption, and feed efficiency in masculinized Nile tilapia (*Oreochromis niloticus*) larvae.

MATERIALS AND METHODS

Preparation of sea cucumber viscera powder

The extraction process begins by removing the intestines of the sea cucumber and placing them in a container, followed by freezing in a freezer for 24 hours. The container with the viscera is

then placed in a freeze dryer for 24 hours at a temperature of -75°C and a pressure of -0.1 MPa. Once dried, the sea cucumber viscera are blended and sieved using a fine mesh (0.42 mm). The extracted sea cucumber viscera powder is then ready for use in the immersion process of Nile tilapia larvae.

Preparation of sea cucumber viscera powder solution

The immersion solution is prepared by dissolving the sea cucumber viscera powder in a methanol solution at a ratio of 1 mL per container, each containing 1 liter of water. Aeration is applied to ensure the complete dissolution of the extract in water.

Immersion and experimental containers

The immersion process utilizes four sterile aquariums, each measuring 11×11×17 cm³, pre-filled with 2 liters of clean water that has been left to settle for 24 hours. For the experimental setup, fiber tanks are partitioned using fine mesh nets to create 15 separate enclosures, each measuring 11×11×17 cm³, and pre-filled with two liters of clean water, also left to settle for 24 hours. Each immersion and rearing container are equipped with aeration, labeled according to the treatment codes and replications.

Fish rearing

Once the test substance is dissolved, it is added to the immersion containers, each containing 2 liters of water according to the treatment labels. Aeration is applied, and the solution is left for one hour to ensure complete dissolution. Next, Nile tilapia larvae are introduced into the immersion containers, with a stocking density of 90 larvae per container for each treatment. The larvae are immersed for 12 hours in the methyltestosterone treatment, while those in the sea cucumber viscera powder treatment are immersed for 24 hours.

After the immersion process, six- to seven-day-old Nile tilapia larvae are transferred to rearing containers with a stocking density of 30 larvae per container. During the rearing period, larvae are fed three times daily with finely ground artificial feed. Feeding is administered at a dose of 5 g/day during the first month and 25 g/day during the second month. The feed used in the first month is finely ground pellet feed (powdered form), while in the second month, whole pellets measuring 0.5–0.7 mm are provided. To maintain water quality, uneaten feed and feces are removed through siphoning. Water quality is monitored regularly throughout the rearing period, ensuring a clean and stable environment.

Experimental design

This study used a Completely Randomized Design (CRD). Five treatments are tested, with each treatment consisting of three replications: negative control with no immersion in sea cucumber viscera extract, treatment 1 used 1 mL/L for 24 hours, treatment 2 used 3 mL/L for 24 hours, treatment 3 used 5 mL/L for 24 hours, and positive control with 0.5 mg/L 17α-methyltestosterone (17α-MT) for 12 hours (Robbani, 2017).

Research parameters

Absolute weight growth

To calculate absolute weight growth, sampling is conducted at the beginning (after the larval immersion process) and at the end of the study. Sampling is performed by weighing five test fish larvae per container. For the final sampling, each test organism is weighed in its entirety. The obtained data is then calculated using the following formula (Sutrisno *et al*., 2020):

$$W = Wt - W0$$

Note:
W = Absolute weight growth (g)
Wt = Average weight of test fish at the end of the study (g)
W0 = Average weight of test fish at the beginning of the study (g)

Absolute length growth

To calculate absolute length growth, sampling is conducted at the beginning and end of the study. The obtained data is then calculated using the following formula (Suparlan *et al*., 2020):

$$L = Lt - Lo$$

Note:
L = Absolute length growth (mm)
Lt = Average total length of test fish at the end of the study (mm)
Lo = Average total length of test fish at the beginning of the study (mm)

Feed consumption

During the rearing period, feed administration follows the satiation method. Based on this method, feed consumption is determined by weighing the

feed daily, calculated as the difference between the total feed provided and the remaining feed per number of fish in the container throughout the maintenance period. Feed weighing is conducted in the morning before feeding, while the remaining feed is weighed in the afternoon after feeding. The range of remaining feed weight is 3.4–297.7 g/week. Additionally, the weight of dead fish larvae is also recorded throughout the rearing period. Feed consumption is calculated using the following formula (Nugraha *et al*., 2018):

$$TKP = F1 - F2$$

Note:
TKP = Feed consumption (g)
F1 = Initial feed amount (g)
F2 = Final feed amount (g)

Feed efficiency

The consumed feed is utilized primarily for growth. Feed efficiency (EP) can be determined using the following formula (Rizki & Mukti, 20 22):

$$EP = \frac{(Wt + Wd) - Wo}{F} \times 100$$

Note:
EP = Feed efficiency
Wt = Total weight of test fish at the end of the study (g)
Wd = Total weight of test fish that died during the study (g)
Wo = Total weight of test fish at the beginning of the study (g)
F = Total weight of feed consumed during the study (g)

Data analysis

The data were analyzed using analysis of variance (ANOVA), followed by Tukey's test as a post hoc analysis if a significant effect was observed, to determine the relationship between dosage in each treatment and the research parameters. Water quality data were analyzed descriptively.

RESULTS AND DISCUSSION

Results

Absolute length growth

Statistical analysis showed that immersion treatment with a 1 mL dose of sea cucumber viscera solution had a significant effect on the absolute length growth of Nile tilapia larvae (Figure 1).

Absolute weight growth

Unlike absolute length growth, statistical analysis showed no significant differences in absolute weight growth among treatments. However, descriptively, the treatment with methyl resulted in the highest absolute weight growth compared to other treatments, while the lowest absolute weight growth was observed in the 3 mL/L dose treatment (Figure 2).

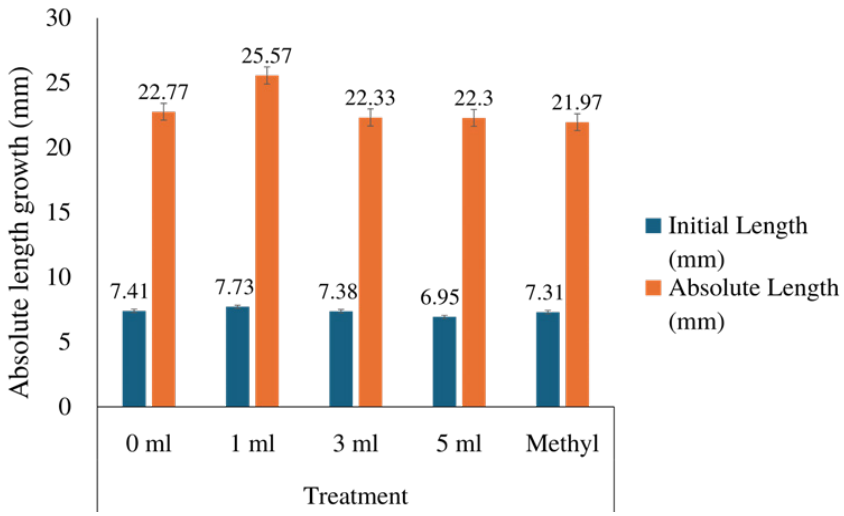

Figure 1. Absolute body length growth of Nile tilapia larvae immersed in sea cucumber viscera solution at different doses.
Note: A (0 mL/L); B (1 mL/L); C (3 mL/L); D (5 mL/L); E (0.5 mg/L 17α-MT). Different letters indicate significant differences between treatments at a 5% level (P<0.05).

Feed consumption and feed efficiency

Feed consumption and feed efficiency levels of Nile tilapia (*Oreochromis niloticus*) larvae subjected to immersion in sea cucumber viscera solution and reared for 60 days are presented in Table 1. The average feed consumption of Nile tilapia larvae ranged from 14.21 ± 0.44 g to 16.88 ± 0.44 g. Based on the analysis of variance (ANOVA), treatment C showed a significant difference compared to treatment A, but no significant differences were observed between treatments B, C, and E. The average feed efficiency of Nile tilapia larvae subjected to sea cucumber viscera immersion over 60 days is presented in Table 1. The feed efficiency values ranged from 66.97 ± 8.51% to 84.47 ± 24.74%. ANOVA results indicated that the mean feed efficiency values were not significantly affected (P>0.05) by the dose of sea cucumber viscera applied through feed immersion.

Water quality

The measurement of water quality parameters is presented in Table 2, which includes temperature, ammonia, dissolved oxygen (DO), and pH.

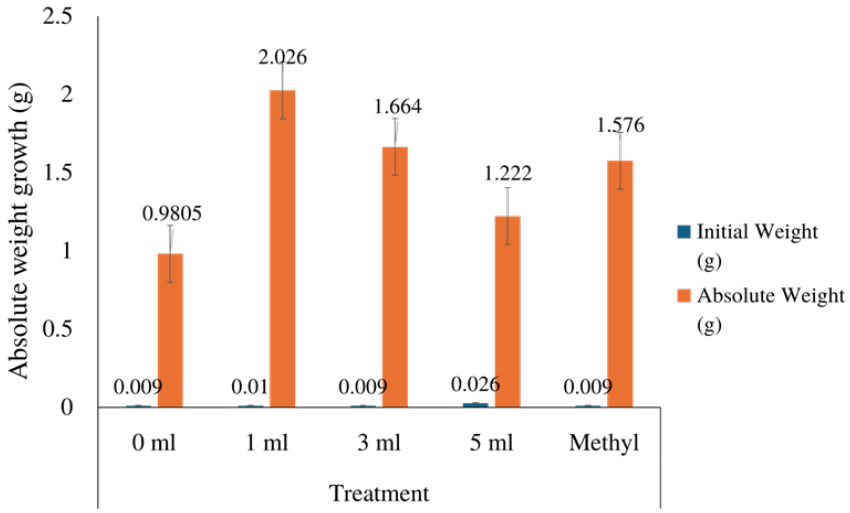

Figure 2. Absolute weight growth of Nile tilapia larvae immersed in sea cucumber viscera solution at different doses. Note: A (0 mL/L); B (1 mL/L); C (3 mL/L); D (5 mL/L); E (0.5 mg/L 17α-MT).

Table 1. Average feed consumption and feed efficiency of Nile tilapia (*O. niloticus*) larvae per tank during the 60-day rearing period.

Treatments	Feed consumption (g) ± sd	Feed efficiency (%) ± sd
0 ml/L (control)	14.10 ± 0.24[a]	78.23 ± 35.44[a]
1 ml/L	14.69 ± 0.79[a]	66.97 ± 8.51[a]
3 ml/L	16.54 ± 0.85[b]	84.47 ± 24.74[a]
5 ml/L	14.21 ± 0.29[a]	81.33 ± 35.38[a]
Methyltestosterone (17α-MT) (+ Control)	15.00 ± 0.38[ab]	82.08 ± 8.71[a]

Table 2. Water quality parameters of Nile tilapia (*Oreochromis niloticus*) during the 60-day rearing period.

No.	Parameters	Units	Measurement results	
			Average results	References (Siegers *et al.*, 2019)
1	Dissolved oxygen	mg/L	6.8	>5
2	pH	No unit	7	6-8
3	Water temperature	Celcius (°C)	29	28-32
4	Ammonia	ppm	0.017	<1

Discussion

Immersion of Nile tilapia larvae in sea cucumber viscera solution resulted in a significant difference in absolute length growth, particularly at doses of 1 mL/L and 3 mL/L compared to the 0 mL/L treatment. However, overall, all treatments exhibited normal absolute length growth in Nile tilapia, ranging from 1.90 to 2.09 cm (Scabra *et al*., 2022). Conversely, in terms of absolute weight growth, although no significant differences were observed between treatments, the application of sea cucumber viscera led to higher weight gain, ranging from 1.6 to 2 g in treatments with 1 mL/L, 3 mL/L, and 5 mL/L doses. This suggests that immersion with sea cucumber viscera has the potential to enhance fish growth.

According to Emilda (2012), sea cucumbers are marine organisms with high protein content, low-fat levels, and are traditionally believed to have aphrodisiac properties due to their high steroid content, especially in their viscera. Amanullah (2021) stated that the steroid compound present in sea cucumber viscera is testosterone, which has two physiological activities: androgenic and anabolic effects (Asad *et al*., 2023). Testosterone is an anabolic-androgenic steroid that facilitates growth hormone release from the pituitary gland in fish. Anabolic steroids have potential applications in aquaculture as they can enhance bone density, red blood cell mass, body weight, and muscle growth in masculinized fish (Dias-Neto *et al*., 2017; Fauzan *et al*., 2017; Abaho *et al*., 2022).

In general, male Nile tilapia grow faster, with a growth rate of 1.53–2.69 g/day, compared to female tilapia, which grow at only 0.83–1.05 g/day until they reach market size (Hutagalung, 2020). However, in this study, weight gain did not show significant differences among treatments, likely because the larval stage is characterized by length growth rather than weight gain. According to Prasetiyo *et al.* (2020), length growth differs from weight growth length growth is always positive (an organism continuously grows in length), whereas weight growth can be both positive and negative. Additionally, the relatively short rearing period (only two months) may have influenced the results. Typically, fish body weight increases more rapidly after 10 weeks and becomes more evident after 150 days of rearing (Ayuningtyas *et al*., 2015).

Heriyati *et al.* (2015) also noted that tilapia exhibit noticeable growth when they reach sexual maturity, which occurs at five to six months of age (150–200 g) in aquaculture ponds. Therefore, this study may not fully capture differences in weight growth, but the increased length growth suggests a potential for higher overall growth if the fish are reared to maturity. The faster growth of male tilapia is likely attributed to higher digestive efficiency, better nutrient metabolism, and hormonal regulation (Pradeep *et al*., 2012). According to Susanto *et al.* (2018), steroid hormones increase androgen levels, making fish more aggressive and influencing metabolic rates. This leads to enhanced appetite and faster growth rates. Testosterone-containing hormone immersion may also stimulate thyroid function, internal physiological processes, and insulin secretion from pancreatic β-cells in fish (Fauzan *et al*., 2017).

According to Zhou *et al.* (2018), fish growth is significantly influenced by feed consumption levels. The feed consumption test in this study revealed differences among fish subjected to different doses of sea cucumber viscera immersion, indicating that specific doses may influence feed intake in Nile tilapia. As known, fish feed consumption depends on various factors, such as feeding habits, water quality, feed composition, palatability, and physiological conditions (Andriani & Rostika, 2021). Therefore, the effect of immersion in sea cucumber viscera powder on fish feed consumption varies among individuals and species, such as Nile tilapia. The immersion of Nile tilapia larvae in sea cucumber viscera powder significantly influenced feed consumption.

According to Hanief *et al.* (2014), growth rate increases with higher feed consumption. However, each fish species has an optimal feed intake level, beyond which additional feed consumption does not further enhance growth. Excess feed that surpasses the fish's optimal intake level is excreted as feces. The high testosterone content in sea cucumber viscera stimulates protein synthesis by improving the utilization efficiency of amino acids from feed (Howard *et al*., 2020). Protein is a critical nutrient necessary for growth, tissue repair, and metabolism. Amino acids play an essential role in regulating metabolism, stimulating growth hormone secretion, and determining feed quality (Rahael *et al*., 2019).

Furthermore, Howard *et al.* (2020) stated that testosterone supplementation enhances satellite cell proliferation and myogenin expression, indicating that testosterone promotes cell cycle entry and muscle development. Therefore,

immersion in sea cucumber viscera at an appropriate dose can improve feed consumption in Nile tilapia. Unlike feed consumption, feed efficiency analysis did not show significant differences among treatments. This suggests that the tested immersion doses of sea cucumber viscera powder did not have the expected impact. Several key factors influence growth and feed utilization efficiency, including feed composition, species-specific dietary needs, fish size, and water quality.

Generally, sea cucumbers contain two dominant amino acids: glycine and glutamic acid (Sroyraya *et al*., 2017). Glycine functions as a natural feed attractant, enhancing feed intake, growth, and overall consumption in fish (Khasani, 2013). Glutamic acid plays a role in physiological responses by facilitating ammonia detoxification. It binds to ammonia within the fish's body, converting it into glutamine, a process regulated by glutamine synthetase (GSase).

However, the amino acids in sea cucumber viscera alone were insufficient to significantly enhance growth and feed utilization efficiency in Nile tilapia (Kurniasih *et al*., 2020), as they were only administered through immersion treatment. This may explain the low feed efficiency values observed in the study. The low feed efficiency values may also be attributed to the low lysine content in sea cucumber viscera, which is only 0.13% in *Holothuria scabra* (Wen *et al*., 2010). Lysine deficiency can lead to poor absorption in larvae, slower growth rates, and reduced appetite, ultimately limiting metabolism and nutrient assimilation. Additionally, the immersion method itself has inherent limitations.

The effects of immersion may primarily influence long-term genetic or physiological conditions in fish, rather than providing immediate and continuous benefits, such as improved feed efficiency. Rohma *et al.* (2012) explained that feed efficiency is determined by the type and amount of essential nutrients in the diet. The higher the feed efficiency, the better the fish's response to the diet, leading to faster growth. In this study, the similar feed efficiency values across treatments indicate that immersion treatment did not significantly impact short-term feed utilization.

According to the results showed in Table 2, the measured water quality parameters in the rearing media of Nile tilapia larvae included dissolved oxygen (DO), pH, water temperature, and ammonia levels. The average DO level recorded during the study was 6.8 mg/L. According to Siegers *et al.* (2019), the optimal DO level for Nile tilapia is >5 mg/L. Dissolved oxygen is crucial for fish survival, as insufficient oxygen supply to the brain can lead to stress and mortality due to the inability of body tissues to bind oxygen in the blood. The average water temperature recorded was 29°C.

According to Siegers *et al.* (2019), the optimal temperature range for Nile tilapia growth is 28–32°C. Temperature significantly influences fish activity and appetite—temperatures below 25°C reduce fish activity and suppress appetite, while temperatures below 12°C can lead to hypothermia and death. Conversely, temperatures above 35°C cause respiratory distress due to increased oxygen consumption. The average pH recorded was 7.0, which aligns with the optimal pH range for Nile tilapia growth as reported by Siegers *et al.* (2019). A stable pH is crucial for fish health, as fluctuations outside the optimal range can lead to stress, increased disease susceptibility, and potential mortality.

The average ammonia level recorded was 0.017 ppm. According to Siegers *et al.* (2019), ammonia levels below 1 ppm are considered safe for Nile tilapia survival. High ammonia concentrations can impair oxygen transport in the blood, reduce appetite, and increase disease susceptibility. The water quality conditions observed during the study were within the optimal range for Nile tilapia larvae, ensuring a favorable environment for growth and adaptation. These conditions allowed the larvae to thrive and adjust to their surroundings, minimizing stress and mortality risks.

CONCLUSION

Based on this study, it can be concluded that a dose of 1 ml/L of sea cucumber viscera extract had the most significant effect on absolute length growth of Nile tilapia larvae (Oreochromis niloticus) but did not affect body weight gain. Furthermore, a dose of 3 ml/L of sea cucumber viscera extract increased feed consumption but did not improve feed efficiency in Nile tilapia larvae.

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