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Enhanced Growth Potential of Tilapia (*Oreochromis niloticus*) Through Maggot-Based Feeding in Multi-Trophic Systems

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Abstract

Highly nutritious insects, such as black soldier fly (BSF) larvae, also referred to as maggot, have been evaluated as feed in monoculture systems, but their use in multi-trophic systems has not been previously investigated. This study used maggot-based feed in a multi-trophic system on a laboratory scale to evaluate the survival and growth performance of tilapia *(Oreochromis niloticus)*. Tilapia were cocultured with freshwater lobster, freshwater clams, and paddy. Four experimental diets were used including a commercial pellet as a control (CP), live maggots (LM), dried maggots (DM), and supplemented maggots (SM). Tilapia (initial weight, 4.1 ± 0.2 g) and other organisms were reared in plastic tanks (water volume 50 L) and randomly distributed into 12 tanks, each containing 20 individual tilapia. The experimental diets were given four times a day at a feeding rate of 10% tilapia biomass. After 28 days of feeding, the survival and growth of tilapia on the CP diet were 90.0% and 2.8% day^{-1}, LM 93.3% and 2.7% day^{-1}, DM 93.3% and 3.3% day^{-1}, and SM 90.0% and 3.1% day^{-1}, respectively. There was no significant effect ($P > 0.05$) on the survival performance among the experimental diets. However, the growth confirmed by Specific Growth Rate (SGR) showed a significant effect ($P < 0.05$). SGR values were found to be significantly higher in the DM and SM diets than in the CP diet. An important finding of this research is the potential of maggot-based feed to increase the growth of tilapia in multi-trophic systems without impairing their survival.

INTRODUCTION

Waste products from feed represent a challenge for sustainable aquaculture. Artificial feed, while contributing to increased production, is a source of waste, particularly in monoculture systems (Boyd *et al*., 2020). For example, Nederlof *et al*. (2021) calculated that 39-63% of nitrogen (N) and 18-30% of phosphate (P) in the feed are released as solid and dissolved waste. The assimilation of feed nutrients by tilapia is only

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38% N and 31% P, with the rest wasted as waste (Sri-uam *et al*., 2016). The protein nutrition supplied in tilapia feed is between 35-40%, but 65% is wasted in aquaculture (Craig *et al*., 2017). Kurniawan *et al*. (2023) reported that the use of feed in tilapia aquaculture produces feed waste and feces of 15% and 20% respectively.

There is no consensus on the best system for sustainable aquaculture, but current documentation provides some solutions (Thomas *et al*., 2021). One such solution is the integrated multi-trophic aquaculture (IMTA) system, which is a form of good aquaculture practice. The concept of this system is a co-culture of species with different trophic levels, including fed species, organic extractive species, and inorganic extractive species (Chopin *et al*., 2012). This system reduces waste by utilizing byproducts from one organism as input for organisms at another trophic level (Nederlof *et al*., 2021). IMTA also offers economic benefits from multiple harvested species (Knowler *et al*., 2020).

Artificial feed also has a direct relationship with the production costs of commercial aquaculture. The main raw material for this feed is fish meal, which is expensive. Fish meal is a high-quality protein source of protein with an excellent amino acid profile, and its composition is 50–70% of the total raw materials used in fish feed (Macusi *et al*., 2023). However, the current trend is to increase the price of fish meal on a global scale (Sharifinia *et al*., 2023). Meanwhile, the feed industry in Indonesia relies heavily on fish meal from abroad (68% imported), which causes feed prices to become increasingly expensive (Luhur *et al*., 2021). In the aquaculture industry, feed costs account for 60–70% of the total production costs, impacting profitability (Macusi *et al*., 2023).

Efforts to reduce the amount of fish meal in the feed are an important step to reduction production costs. Therefore, there is a need to find alternative protein sources to replace fish meal (Macusi *et al*., 2023). Due to their nutritional composition, animal protein sources are considered ideal replacement sources ideal (Luthada-Raswiswi *et al*., 2021). Studies have demonstrated that black soldier fly (BSF) larvae *(Hermetia illucens)* or maggots are the most suitable candidates to replace fish meal, as they have high protein content, a similar amino acid profile to fish meal, and are rich in carbohydrates, fatty acids, vitamins, and minerals (Henry *et al*., 2015). Furthermore, BSF is easy to cultivate and is suitable for growing and breeding in Indonesia, which has a tropical climate (Wardhana, 2017).

Tilapia species *(Oreochromis niloticus)* are commercially important, ranking third in world production of freshwater fish groups (FAO, 2022). Indonesia is the world's second-largest producer in Asia (Mehar *et al*., 2023). This species has several favorable biological and economic attributes, including high consumer preferences and its ability to utilize a variety of feed ingredients (Zhao *et al*., 2020). The beneficial effects of maggots as feed for this species have been widely reported but are limited through monoculture systems (Agbohessou *et al*., 2021; Devic *et al*., 2018; Tippayadara *et al*., 2021; Wuertz *et al*., 2022). Processing methods have also been reported to influence the nutritional value of maggots (Zulkifli *et al*., 2022). Additionally, adverse effects have been observed, namely reduced consumption of maggots due to their high fat and energy content (Ahmad *et al*., 2022), leaving them as waste.

This study, laboratory experiments to assess the protein content of different processed maggot forms and their impact on the survival and growth performance of tilapia co-cultured with freshwater lobster *(Cherax quadricarinatus)*, freshwater clams *(Pilsbryoconcha exilis)*, and paddy *(Oryza sativa)*. Paddy cultivation was carried out using the floating method. As far as our knowledge, this is the first study to develop such a research model. Combining good aquaculture practices through IMTA systems and finding the best feed forms is a promising strategy to address the complexity of the ecological and economic impacts of using artificial feed, which is crucial for the development of sustainable aquaculture.

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METHODOLOGY

Ethical Approval

The experimental protocols complied with guidelines and regulations in Indonesia for the care and use of animals and plants. The health and environmental conditions in the experimental tanks were monitored daily through visual observation of animal and plant behavior and by checking water quality parameters. After the experiment, the aquatic animals and plants were handed over to the Aquaculture Institute of Moncongloe for further rearing.

Place and Time

The research was conducted from June to August 2023 at the experimental facility of the Aquaculture Institute Laboratory of Moncongloe, Maros, South Sulawesi, Indonesia.

Research Materials

The main research materials include BSF fly larvae (maggots), artificial feed, aquatic species (tilapia, freshwater lobster, freshwater clams), plant species (paddy), Rockwool, and freshwater, all of which were locally and commercially sourced. Live and dried BSF fly larvae were purchased from a maggot producer, UA Makassar, Indonesia. A special tilapia feed was used as the artificial feed (MS Pro 891; MS, Indonesia). Rockwool (Cultilene, Indonesia) was used as the paddy planting medium. Fresh water was supplied from natural sources in the laboratory.

The tilapia fingerlings (with an initial body weight of 4.1 ± 0.2 g) were obtained from the Fish Seed Center, Gowa Regency. Freshwater lobster (individual initial body weight 1.5 ± 0.2 g) were sourced from the LAT Manggala commercial farm, in Makassar City. Freshwater clams (with an initial body weight of 13.2 ± 0.2 g) were collected from farmers' catch at Garanta irrigation, in Bulukumba Regency. Meanwhile, paddy seedlings (initial height 10.3 ± 0.2 g) were obtained from farmers in the Bone Regency (Figure 1).

Figure 1. Cultivated organism fingerlings: (a) tilapia, (b) freshwater lobster, (c) freshwater clams, (d) paddy.

The main research equipment was commercially provided, including a plastic tank measuring 50×50×55 cm (length×width×height) (Gator, Indonesia), plastic netpot D10 cm (Agnis, Indonesia), an air pump (Resun LP60, China), digital scales with a precision of 0.1 g (WH-B28, China), and water quality measuring instruments (5 in 1 AZ 86031, China). Other main equipment, such as trays and shelters, were produced at the experimental location. Following the guidebook, a tray measuring 25×25 cm was constructed from PVC pipe (Heriansah *et al.*, 2023). The shelter was designed from a 2.5-inch PVC pipe (length 5 cm) filled with 35 plastic straws.

Research Design

Four experimental diet groups were randomly designed in triplicate, including live maggot (LM), dry maggot (DM), supplemented maggot (SM), and commercial pellet (CP) diets as controls (Figure 2).

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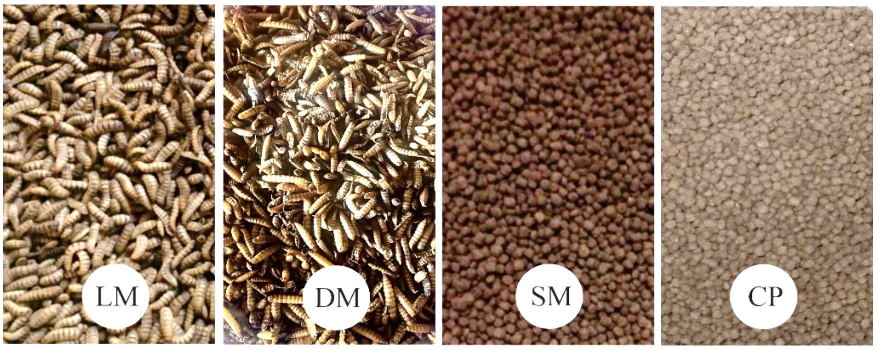

Figure 2. Experimental diets: live maggot (LM), dried maggot (DM), supplemented maggot (SM), commercial pellets (CP).

The approximate protein composition of each experimental diet was determined before use. Additionally, two key parameters for assessing aquaculture production performance (survival and growth) were evaluated for each treatment diet. The total tilapia in each tank was counted and weighed to determine these parameters. Fish survival expressed as a percentage of live fish at the end of rearing, was calculated using the equations Agbohessou *et al*. (2021):

$$\text{Survival}(\%) = 100\text{x}\frac{\text{Number of fish in the end of experiment}}{\text{Number of fish in the initial of experiment}}$$

Growth is a mathematical value used to express the increase in body weight over time (g). The experiment lasted for a short duration (28 days), and the tilapia were still in the juvenile stage. Therefore, the weight gain is still in the exponential phase (Lugert *et al*., 2016). For this reason, the growth indicator measured in this experiment was the Specific Growth Rate (SGR), which was determined using the equation of Agbohessou *et al*. (2021) :

$$\text{SGR}(\%\text{day}^{-1}) = 100\text{x}\frac{[\text{Ln}(\text{final weight, g}) - \text{Ln}(\text{initial weight, g})]}{\text{rearing time}}$$

Work Procedure

Preparation

The experimental preparation was divided into two stages: preparation of the cultivating organisms and preparation of the experimental diets. Aquatic animal fingerlings (tilapia, freshwater lobsters, and freshwater mussels) were carefully transported in plastic bags filled with oxygenated freshwater. Paddy seedlings were transported in polybags. Each aquatic animal was collected and placed in a plastic tank for 5 days before the experiment. During the adaptation phase, an experimental diet was prepared according to the treatment.

The LM and DM diets in the pre-pupal stage as well as CP measuring 0.4 cm from the producer were stored in a cool place in plastic boxes and zip-lock bags until use. The SM diet was prepared independently at the experimental site, following the method described Heriansah *et al*. (2021). The dried maggots were blended and then sifted (60 mesh) until they formed flours. Then, 1 kg of commercial feed was dissolved in 100 mL of aquadest and mixed with 40 g of maggot flour. Next, it was glued using 2 g of egg white as a binding agent. The SM diet was then dried in the sun and stored in a cool, ventilated place using a zip-lock bag until further use. The crude protein content in the treatment diet was determined using the Kjeldahl method (AOAC, 2005).

Rearing

Rearing for 4 weeks indoors was carried out in plastic tanks containing approximately 50 L of fresh water. Shelters were installed in each tank to accommodate the lobsters. Next, 10 individuals each of tilapia, lobster, and clams were distributed randomly into 12 tanks. Paddy is stocked seven days after the introduction of aquatic animals to allow for the absorption of dissolved nutrients. Four clumps of paddy were placed in a tray using a net pot and rock wool and then floated on the surface of the water tank.

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An illustration of the experimental tank design is shown in Figure 3.

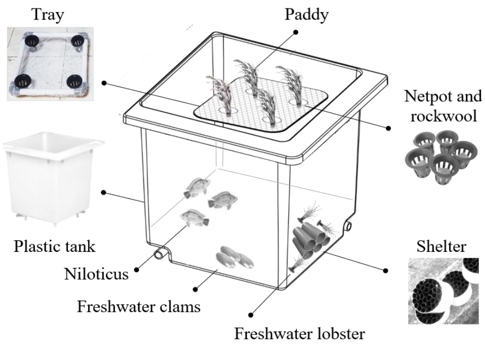

Figure 3. Illustration of the experimental tank design.

Tilapia were fed the treated diet four times a day at four-hour intervals (at 07:00, 11:00, 15:00, and 19:00 local time) at a rate of 10% of their body weight. Lobsters were also fed four times a day with carrot slices at a level of 5% of their body weight, 30 minutes after the tilapia feeding. The leftover feed and feces were not removed from the tank for use by other organisms in the system. The aeration was supplied continuously to the tank through an air stone diffuser from the air pump. Water quality parameters (temperature, dissolved oxygen, pH, and ammonia) were monitored daily to maintain values suitable for each organism.

Data Collection

The mortality of tilapia was recorded daily to evaluate survival performance during the experiment and total mortality was calculated at the end of the experiment. To evaluate growth performance, each tilapia fish was weighed on days 0, 7, 14, 21, and 28 of the experimental period. Weighing was also intended to adjust the amount of feed given based on the average increase in weight and biomass.

Data Analysis

Normal distribution and homogeneity of data variance were verified with Shapiro-Wilk-test and Levene-test and the results met the assumptions of parametric statistics. One-way ANOVA followed by Tukey's post hoc test at a significance level of 95% ($P<0.05$) was applied to compare different treatment diets. Statistical Package for the Social Sciences (SPSS) 25.0 software (SPSS Inc., Chicago, IL, USA) was used to conduct statistical analysis in this study. Water quality data was analyzed descriptively by comparing the tolerance range of every cultivated organism based on valid references.

RESULTS AND DISCUSSIONS

Protein Content of Experimental Diets

Maggots are a protein-rich feed ingredient, but their concentrations can be affected by processing methods. These methods can also impact the nutritional composition of maggots (Devic *et al*., 2018; Zulkifli *et al*., 2022). In this study, maggots were processed in various forms, and it was found that they had different protein contents. The proximate analysis revealed that live maggots (LM) had the highest crude protein contents, followed by dried maggots (DM), supplemented maggots (SM), and commercial pellets (CP). The protein content of the four treatment diets differed significantly ($P < 0.05$) (Figure 4).

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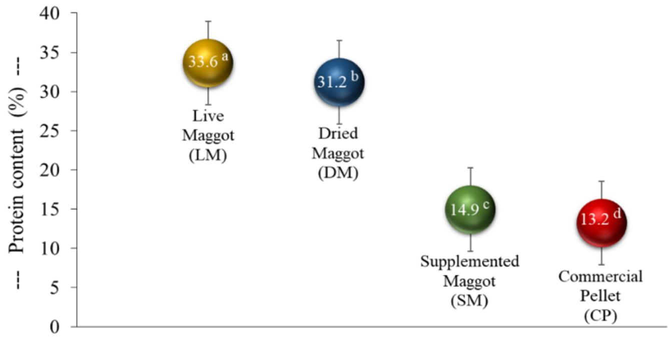


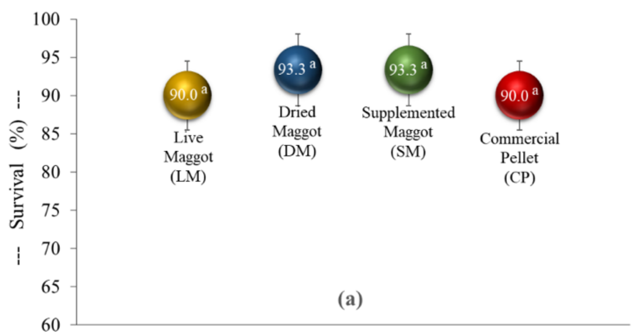
Figure 4. Protein content of experimental diets. Values represent the average value of n = 3. Different superscript letters indicate significant differences based on the results of the analysis of variance (α = 0.05).

The protein content of the maggots analyzed in this study fell within the range reported in previous studies, which is typically between 30 and 50% depending on the food substrate (Saputra and Lee, 2023). Information provided by the producer regarding the feed substrate used for maggot cultivation included vegetable and fruit waste. Meneguz *et al*. (2018) found that the protein content of live maggots fed on fruit and vegetable waste was 30.7% and 41.8%, respectively. Pérez-Pacheco *et al*. (2022) obtained a live maggot protein content of 15.7% in fruit waste feed substrate. Lan *et al*. (2022) reported a protein value of 38.5% for maggots cultivated with fruit and vegetable waste substrates. The quality and quantity of vegetables and fruit used as food substrates appear to influence the protein content in maggots.

The dry matter (DM) diet used in this experiment was roasted by the producer using sand at approximately 100°C for around 25 minutes. Rostika *et al*. (2022) reported that the protein content in DM roasted with sand at a temperature of 100 to 180°C for approximately 15 minutes was 35.1%, similar to the results in this study. However, DM and SM's protein content was significantly lower than LM's. The heating process used to prepare DM and maggot flour has been reported to reduce the nutritional composition of proteins due to changes in protein structure caused by the denaturation process. Meanwhile, the protein content of the CP tested ranged from 12% to 14%, as stated on the packaging label.

Survival and Growth

The main targets of aquaculture are to achieve high survival and growth rates, as they determine the production volume. In this study, tilapia were reared in an integrated multi-trophic system for four weeks with different maggot diets, and their survival and growth performances are shown in Figure 5.



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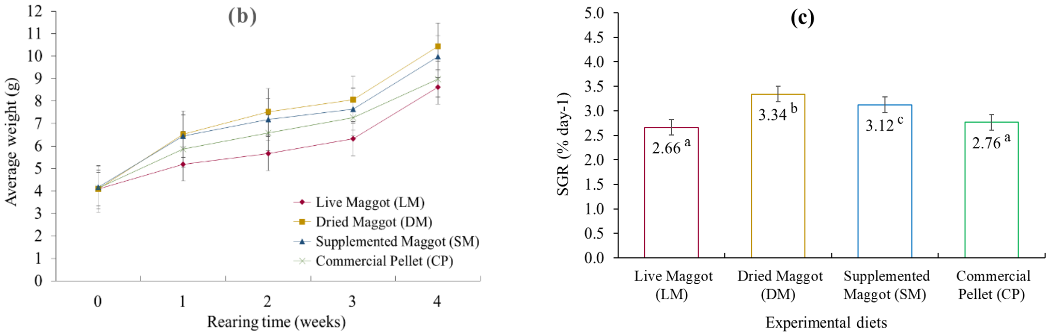

Figure 5. (a) Survival, (b) weekly growth, and (c) specific growth rate (SGR) of tilapia in integrated multi-trophic aquaculture based on treatment diet. Different superscript letters indicate significant differences based on the results of the analysis of variance (α = 0.05).

The survival rate of tilapia in all treatment diets was above 85%, and there were no significant differences ($P > 0.05$) between the treatment groups (Figure 4a). Previous studies have not provided scientific evidence regarding the performance of tilapia fed various forms of maggot feed in multi-trophic cultivation systems, making it difficult to judge whether the results in this study are better or worse. However, in monoculture systems, the substitution of maggot meal and fish meal has been widely published, with survival values ranging from 73 to 100% (Devic *et al*., 2018; Prajayati *et al*., 2020; Tippayadara *et al*., 2021). Similar results have been reported in other species, such as catfish, milkfish, and eels, with survival rates ranging from 80 to 95% (Fawole *et al*., 2020; Herawati *et al*., 2020). The survival rate in the comparison studies was quite high and did not indicate significant differences between treatment diets, which is consistent with the results of this experiment.

The observed mortality was not related to the experimental diet tested but was due to the aggressive behavior of tilapia. They forced their way into the lobster shelter and became trapped inside. Similar to the phenomenon reported by Chivambo *et al.* (2020), the survival obtained in this study indicated that all experimental diets were well accepted by tilapia as an omnivorous species (Wuertz *et al*., 2022), providing the necessary energy to meet the fish basal survival requirements. This explanation follows the concept of energy expenditure, which states that the energy assimilated from feed (net energy) is first allocated to the fish's basal metabolic needs (basal metabolism) and movement energy (voluntary activity) (Weidner *et al*., 2020). Feeding every day at a frequency of four times and a dose of 10% of the weight of the tilapia biomass seems to have met these two basic energy requirements.

During the experimental period, there was no significant difference in the growth of tilapia fish from the initial weight between the treatment diet groups ($P > 0.05$). However, after 28 days, there was a significant increase in weekly body weight for each treatment diet ($P < 0.05$) (Figure 4b). These results suggest that the energy available from each experimental diet exceeded the basal requirements, allowing for growth allocation. This weight gain was further confirmed by the SGR values of tilapia at the end of the experiment which were above 2.5% day^{-1} for each diet group(Figure 4c). In comparison, other studies that substituted maggot meal and fish meal in tilapia monoculture obtained SGR of 3.1% day^{-1} (Devic *et al*., 2018) and 1.3% day^{-1} (Tippayadara *et al*., 2021). The differences in results between these studies and our experiment may be attributed to variations in study methods, such as the aquaculture system applied and the duration and location of the study.

The SGR of tilapia fed the DM diet was significantly higher than those fed the SM,

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CP, and LM diets ($P < 0.05$). However, there was no significant difference ($P > 0.05$) in SGR between the LM and CP diets, indicating that the LM diet was not superior to CP under the conditions of this study. Additionally, the DM diet resulted in a higher SGR than the LM diet. Similar results were also found in tilapia-fed houseflies *(Musca domestica)*, with the SGR on dried houseflies (2.8% day^{-1}) being higher than on live houseflies (2.6% day^{-1}) (Alofa *et al.*, 2023).

Protein is a crucial nutrient for promoting fish growth (Herawati *et al.*, 2019). Among the diets tested, the LM diet had the highest protein content, followed by the DM, SM, and CP diets. However, the DM diet resulted in the highest SGR. It is worth noting that LM diets tend to sink, making them accessible to bottom-feeding freshwater lobsters, while the SM and CP diets float on the water surface, allowing surface-feeding tilapia to consume them. Some studies suggest that the high chitin content in maggots can hinder the digestibility and utilization of feed protein (Zulkifli *et al.*, 2022), but this response may not be universal across all fish species (Fischer *et al.*, 2021). Tilapia, in particular, has been found to have an advantage in degrading and digesting chitin through the activity of chitinolytic enzymes (Tippayadara *et al.*, 2021). This may explain the difference in SGR performance observed among the different feed diets in this experiment.

The study confirms that combining an alternative feed (maggots) with an aquaculture system (IMTA) has several benefits. For example, the nutritional profile of maggots can be adjusted based on the nutrition of the substrate. Additionally, the presence of freshwater lobsters, freshwater mussels, and paddy did not have any detrimental effects on tilapia. Visual observation of the tilapia fish during the experiment did not show any aggressive actions from the freshwater lobsters. There were beneficial effects in terms of waste utilization and water quality, as explained in the water quality section). Raising tilapia alongside freshwater lobsters, freshwater mussels, and paddy also leads to increased crop yields. Therefore, the results of this study can provide an option to address the complexities associated with the use of artificial feed in sustainable aquaculture development. This approach can also offer solutions to problems faced by farmers, especially small-scale farmers who are dominant in Indonesia.

Water Quality Parameters

The survival and growth of tilapia are greatly affected by variations in water quality, such as temperature, dissolved oxygen, pH, and ammonia (El-Hack *et al.*, 2022). It is important to note that in this experiment, the tilapia were reared using in an IMTA system with freshwater lobster, freshwater mussels, and paddy. The values for the four water quality parameters were similar in each experimental tank (Table 1). Additionally, all materials used were the same for all treatments, except for the type of diet. Therefore, it is reasonable to assume that the survival and growth rates of tilapia in this experiment were most influenced by the type of diet.

Table 1. Measurement values of water quality parameters during the experiment.

Parameters	Experimental diets			
	LM	DM	SM	CP
Temperature (°C)	25.2–27.8	25.4–27.6	25.2–27.9	25.4–27.8
Dissolved oxygen (mg L^{-1})	5.3–7.1	5.4–7.0	5.4–7.1	5.4–6.9
pH	6.4–7.0	6.5–7.0	6.4–7.0	6.5–6.9
Ammonia (mg L^{-1})	0.01–0.13	0.01–0.19	0.01–0.12	0.01–0.13

Note: LM = live maggot, DM = dried maggot, SM = supplemented maggot (SM), and CP = commercial pellets.

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The water temperature during the experiment was relatively stable, ranging from 20 to 30°C, which is suitable for tilapia (El-Hack *et al*., 2022). The shade of paddy leaves and floating beds seems to help reduce heat exposure in the water column (Li *et al*., 2019). Tilapia requires at least 5 mg L^{-1} of dissolved oxygen (El-Hack *et al*., 2022), and during the culture period, it never fell below this minimum limit. Continuous aeration and the photosynthetic ability of paddy (Srivastava *et al*., 2017) helped maintain dissolved oxygen levels. The pH values in this study tended to be acidic for all treatments but were within the safe range for tilapia. Although tilapia, a freshwater fish, can adapt to changes in water pH, it should still be maintained between 5 and 8 (El-Hack *et al*., 2022). The ammonia concentration was always less than 1 mg L^{-1} during the experiment, which is safe for fish, including tilapia (Lawson, 1995).

Proper water quality management is crucial for the successful cultivation of multiple species (Heriansah *et al*., 2022). The IMTA system used in this experiment is known for its ability to maintain favorable water quality conditions by minimizing the accumulation of waste (Thomas *et al*., 2021). For example, freshwater lobsters, which are bottom-feeding organisms (Chivambo *et al.*, 2020) were observed consuming leftover diets and sunken feces, in addition to the feed provided. Freshwater clams also play a role in reducing the pollution load of tilapia aquaculture waste through their filtering effect (Wedsuwan *et al*., 2016). Additionally, paddy, used as an absorber of inorganic nutrients in the floating beds, effectively absorbs dissolved nutrients from the medium through its roots (Srivastava *et al*., 2017). This demonstrates the advantage of the IMTA system in maintaining water quality for multi-species cultivation.

CONCLUSION

The study provides valuable information on different types of processed maggots that can be used in tilapia diets. It found that the protein content of maggots is influenced by the processing methods. The study also showed that diets including live maggots (LM), dried maggots (DM), supplemented maggots (SM), and commercial pellets (CP) did not significantly affect tilapia survival. However, dried maggots resulted in better growth performance. The study suggests that using maggot-based feed in an integrated multi-trophic aquaculture (IMTA) system has the potential to increase production and reduce the impact of feed waste. Further research, including field experiments, is needed to complement these findings.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest upon writing and publishing this manuscript. The manuscript has been approved by all authors for publication and it is not being submitted to any other journal.

AUTHOR CONTRIBUTION

HH discovered the research idea; HH, FF, NN, and MIA developed and designed the experiment. MIA collected data and handled nearly all of the technical work under the direction of HH, FF, and NN. All authors made significant contributions to this publication, including data processing, data analysis, and text preparation.

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