Evaluating Black Soldier Fly and Bloodworm Larvae Meals as Sustainable Protein Sources for Growth and Survival of Red Hybrid Tilapia (*Oreochromis* spp.) in the Nursery Phase

ABSTRACT

Aims: This study aimed to assess the effects of using insect larvae meals, such as BSF and BW larvae, as alternative feeds to commercial pellets on the growth performance and survival of *Oreochromis* spp. at the nursery stage.
Study design: This study followed the randomized control treatment with three different diet.
Place and Duration of Study: This study was carried out at the Fisheries Research Institute GlamiLemi (FRIGL) in Negeri Sembilan, Malaysia, within 35 daysfrom July to August 2023.
Methodology:A total of 360 red hybrid tilapia (*Oreochromis* spp.) fingerlings weighing 0.62 ± 0.06 g were randomly distributed into 12 rectangular aquarium tanks (250mm) at a stocking density of 30 fish per tank. They were fed with commercial pellets (CP), Black Soldier Fly (BSF) larvae, and Bloodworm (BW). The feed amount was calculated at 10% of the total body weight per tank, with feeding conducted three times daily. The experimental setup was performed in triplicate. For the analysis of growth performances and fish survival, sampling was conducted weekly.
Results:BSF larvae meal had high lipid and protein content, measuring 35.35 ± 0.21% and 29.67 ± 0.98%, respectively. BW larvae meal also showed high lipid and protein content, with 28.23 ± 1.33% and 27.94 ± 0.52%, respectively. Fish fed with BSF and BW larvae exhibited significantly higher survival rates, 93.3 ± 3.3% and 86.7 ± 14.5%, respectively, compared to the control group.Meanwhile, the group fed with the commercial pellet showed the lowest survival rate, just 74.4 ± 12.6%. Fish fed with the BW larvae recorded the highest weight gain, 1.07 ± 0.16 g, followed by fish fed with BSF larvae, 0.66 ± 0.11 g, and then fish fed with commercial pellets, 0.41 ± 0.09 g.
Conclusion: Both BSF and BW larvae exhibited significant growth response properties to tilapia fingerings compared to commercial pellets. The findings from this study are promising and should be further verified at the farm scale.

Keywords: Aquaculture, black soldier flies, bloodworm, growth performance, larvae meals, Oreochromis spp.

1. INTRODUCTION

Aquaculture plays a vital role in Malaysia and globally, contributing significantly to food security and economic development. In Malaysia, it's recognized as a key sector in the country's economic growth trajectory. Over the years, aquaculture has shown substantial growth, increasing its share of national fish production from 7% in 1992 to nearly 13% in 2003, with projections indicating the potential to surpass capture production in the future [1]. Aquaculture production in Malaysia has seen a remarkable rise from less than 80,000 metric tonnes in 1992 to over 427,000 metric tons in 2017, valued at MYR3 billion [2]. However, despite its promising trajectory, the aquaculture industry in Malaysia faces challenges, resulting in recent production declines.

Tilapia, *Oreochromis spp.*, holds significant importance in Malaysia's aquaculture industry, contributing around 90% of total tilapia production [3]. Demand for tilapia is projected to almost double from 4.3 million to 7.3 million tons between 2010 and 2030, according to the World Bank [4].Concerns regarding tilapia production in Malaysia predominantly revolve around the sustainability and environmental impact of using fishmeal in tilapia feed, given the concerns about overfishing and the depletion of wild fish stocks, crucial for fishmeal production. This raises concerns about the long-term sustainability of the aquaculture industry, particularly in regions like Malaysia, where tilapia farming is a significant economic contributor.

The current issue surrounding fishmeal sources in Malaysia and Asia primarily revolves around challenges related to its supply for aquaculture and animal feed industries. Fishmeal, a critical ingredient, particularly in the poultry feed industry, faces challenges such as overfishing, declining fish stocks, and environmental concerns, impacting its availability [5]. This underscores the need for sustainable management practices and responsible consumption to ensure the long-term availability of fishmeal for aquaculture.

Insect larvae meal presents a promising alternative to traditional fishmeal in aquafeeds, offering benefits such as sustainability, cost-effectiveness, and nutritional value [6]. Species like the black soldier fly (BSF) and bloodworm (BW) show potential as substitutes for fishmeal, providing essential amino acids and improving the health of aquatic species. Black soldier fly(*Hermetiaillucens*) larvae meal, emerges as a promising alternative, offering rich protein and nutrient content, and the potential to reduce reliance on fish meal and fish oil, thereby promoting sustainable aquaculture practices [7].Bloodworms or known as Chironomidae larvae, earn their name from their worm-like cylindrical body structure and distinctive red hue resulting from the presence of hemoglobin [8].Bloodworms stand out as an excellent choice for ornamental fish nourishment due to their ability to fulfill the dietary requirements of freshwater fish [9],boasting a rich protein content of 52.11% and 4.50% fat [10],thereby promoting fish development [11].Therefore, the objective of this study was to assess the effects of using insect larvae meals, such as BSF and BW larvae, as alternative feeds to commercial pellets on the growth performance and survival of *Oreochromis spp.* at the nursery stage.

2. MATERIALS AND METHODS

2.1 Proximate Analysis

BSF larvae, sourced from BioloopSdnBhd, and BW larvae, cultivated at FRIGL, were used (Fig. 1). The moisture and ash percentages of the larvae meals were analyzed, along with their proximate compositions. Additionally, the levels of crude protein and crude lipid were assessed using the Kjeldahl method and the Soxhlet extraction method, respectively (AOAC, 2000).

Fig. 1. A) Bloodworm larvae (Chironomidae), B) Black Soldier Fly larvae (*Hermetiaillucens*), and C) Commercial pellet

2.2 Feeding Trials

This study was carried out at the Fisheries Research Institute GlamiLemi (FRIGL) in Negeri Sembilan, Malaysia, within 35 days (d) from July to August 2023. For the feeding trials, a total of 360 red hybrid tilapia (*Oreochromis*spp.) fingerlings weighing 0.62 ± 0.06 g were obtained from the Tilapia Unit at the Fisheries Research Institute GlamiLemi (FRIGL), Negeri Sembilan. Prior to the trial, the fish were stocked for three days in circular tanks at the Hatchery Complex, FRIGL, for acclimatization. At the start of the experiment, the fingerlings were starved for 24 hours, weighed, and then randomly distributed into 12 rectangular aquarium tanks (250mm) at a stocking density of 30 fish per tank. They were fed with commercial pellets (CP), BSF, and BW. BSF matured larvae were provided in dried form, while BW was given fresh.The fingerlings, approximately 1 inch in size, were graded and selected. The feed amount was calculated at 10% of the total body weight per tank, with feeding conducted three times daily. The experimental setup was performed in triplicate. Water depth in the tanks was maintained at 45 cm throughout the experimental period, while dissolved oxygen (DO) was kept at ≥4 mg/L.

2.3 Growth Performances and Survival Analysis

For the analysis of growth performances and fish survival, sampling was conducted weekly. Water levels were lowered, and all fish in each tank were captured using a scoop net and placed in labeled aerated buckets for individual sampling to prevent mix-ups. Weight measurements were taken using an electronic balance with an accuracy of 0.01g. All measurements were recorded, and feeding was adjusted accordingly. Experimental tanks were inspected daily, and weight changes and any mortalities were recorded. The weight measurements were used to calculate the specific growth rate (SGR), weight gain (WG), and average growth rate (AGR). Calculations were done as follows:

Weight gain (WG, g) = Final Body Weight (FBW, g) – Initial Body Weight (IBW, g)

Specific growth rate (SGR) = 100 × (Ln FBW – Ln IBW)/time

Average Growth Rate (AGR) = Mean Weight Gain/Number feeding days

2.4 Statistical Analysis

The data were then analyzed using one-way analysis of variance (ANOVA) to compare growth performances and survival data from different larvae meal groups using GraphPad Prism 9. Values with *P*= .05 were considered significant.

3. RESULTS

Based on the proximate analysis, BSF larvae meal possessed a lipid content of 35.35 ± 0.21%, protein content of 29.67 ± 0.98%, a moisture content of 58.34 ± 2.01%, and an ash content of 10.3 ± 0.03%, while BW larvae meal showed a lipid content of 28.23 ± 1.33%, a protein content of 27.94 ± 0.52%, a moisture content of 92.12 ± 0.11%, and an ash content of 6.81 ± 0.47%. Table 1 shows the proximate composition of BSF and BW larvae meals. In comparison, BW larvae meal exhibited higher ash content and moisture content compared to BSF larvae meal. However, BSF larvae meal showed higher lipid and protein content compared to BW larvae meals. When comparing with the commercial pellet, both BSF and BW larvae meals exerted higher lipid content; however, they exhibited slightly lower protein content compared to the commercial pellet.

Table 1: Proximate composition of experimental feeds based on a percentage of dry matter (%)

Proximate parameters	Commercial pellet (CP)	Larvae meals	
		Bloodworm (BW)	Black Soldier Fly (BSF)
Ash (%)	11.18 ± 0.86	6.81 ± 0.47	10.30 ± 0.03
Moisture (%)	4.82 ± 0.15	92.12 ± 0.11	58.34 ± 2.01
Lipid (%)	3.51 ± 0.01	28.23 ±1.33	35.35 ± 0.21
Protein (%)	32.88 ± 0.81	27.94 ± 0.52	29.67 ± 0.98

Based on the findings from feeding trials, tilapia fingerlings fed with BW larvae meal and BSF larvae meal exhibited an increasing trend in weight gain from day 0 to day 35. Fish that were fed the BW larvae meal, showed larger increments in weight change after 21 d of culture compared to fish that were fed with the CP and BSF larvae meals, respectively (Fig. 2).

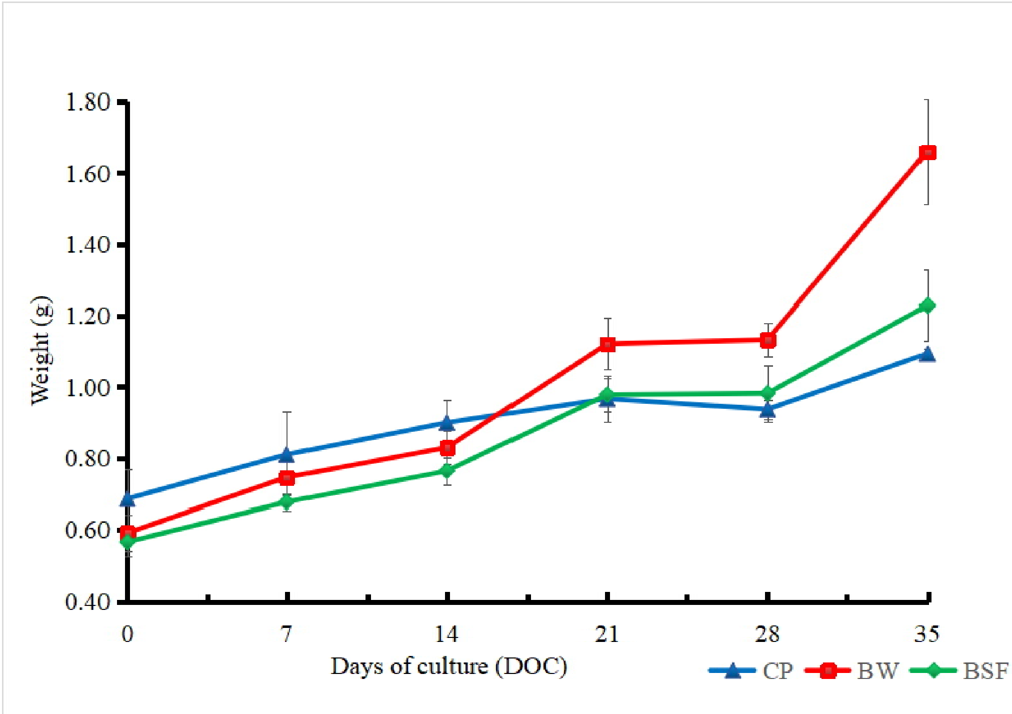

Fig. 2. The growth of tilapia, *Oreochromis* spp., fed with insect larvae meals at the nursery stage.

Fig. 3 shows the survival percentage of fish fed with experimental diets after 35 d of culture. The fish fed with BSF larvae exhibited a significantly higher survival percentage, 93.3 ± 3.3%, compared to fish fed with the commercial pellet (CP) control group. Survival data from fish fed with BW larvae showed 86.7 ± 14.5%, which was higher than the CP control group. Meanwhile, the group fed with the commercial pellet showed the lowest survival rate, just 74.4 ± 12.6% (Fig. 3). The survival rate of Oreochromis spp. fingerlings was generally high (90-95%), with BSF larvae meal showing very low mortality after 35 d of culture, and a significant difference ($p < 0.05$) was detected in the CP control group. Survival data from fish fed with BW showed 86.7% ± 14.5, which was higher than the CP control group.

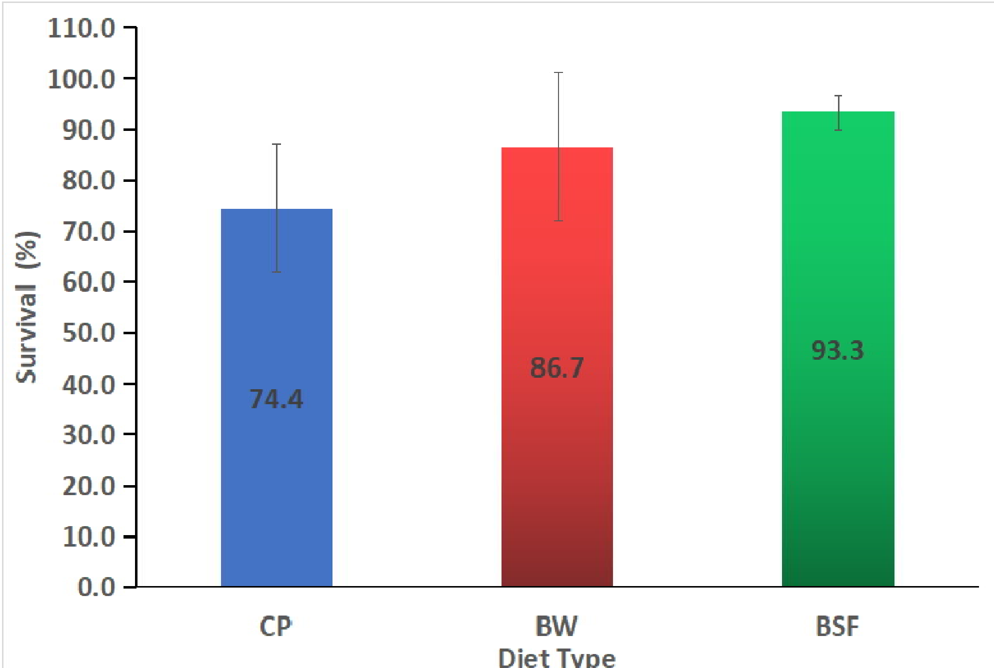

Fig. 3. The survival of tilapia, *Oreochromis* spp., fed with insect larvae meals at the nursery stage (CP: Commercial pellet; BW: Bloodworms; BSF: Black Soldier Fly)

Table 2 presents the growth performance of fish fingerlings fed with experimental diets during the nursery stage for 35 d. Fish fed with the BW larvae recorded the highest weight gain, 1.07 ± 0.16 g, followed by fish fed with BSF larvae, 0.66 ± 0.11 g, and then fish fed with commercial pellets, 0.41 ± 0.09 g (Table 2). Regarding specific growth rate (SGR), fish fed with the BW larvae exhibited the highest SGR compared to fish fed with BSF and CP. The average growth rate (AGR) showed that fed with the BW larvae displayed the highest average growth rate compared to fish fed with CP and BW larvae. The SGR, AGR, and weight gain (WG) results were consistent, demonstrating that fish fed with BW larvae had the best growth performance. Fish that were fed the BW larvae meal, had larger increments in weight change after 21 d of culture compared to fish in the CP control group. The fish fed with the BW recorded the highest weight gain, 1.07 ± 0.16 g. Fish fed with BW larvae showed the highest specific growth rate compared to fish fed with CP control and BSF. The AGR showed that fish fed with BW larvae exhibited the highest average growth rate compared to fish fed with CP control and BSF.

Table 2: Growth performance of tilapia, *Oreochromis* spp. feed with insect larvae meals compared to commercial pellets at the nursery stage

Growth Parameters	Commercial pellet (CP)	Bloodworm (BW)	Black Soldier Fly (BSF)
IW (g)	0.69 ±0.08	0.59±0.05	0.57±0.04
FW (g)	1.09 ±0.01	1.66 ±0.15	1.23 ±0.10
WG (g)	0.41±0.09	1.07±0.16	0.66±0.11
SGR (%)	1.35 ± 0.36	2.95±0.35	2.21±0.34
AGR (g/day)	0.012±0.002	0.031±0.005	0.019±0.003

**IW: Initial weight, FW: Final weight, SGR: Specific Growth Rate, AGR: Average Growth Rate, WG: Weight gain.*

4. DISCUSSION

The findings showed that both BSF and BW had high protein content. A greater proportion of protein in larvae meal indicates a higher protein content compared to other nutrients like fats or carbohydrates. Protein is a vital nutrient for animals, including humans, as it plays essential roles in muscle growth and repair, enzyme production, and immune function [15]. Larvae meal with higher protein content can serve as a valuable feed ingredient for aquaculture species, livestock, or poultry, promoting growth and overall health [16]. Not only that, BSF and BW also possessed higher lipid content compared to the commercial pellets. Lipids perform various crucial functions in the body, including providing energy, forming cell membranes, and aiding in the absorption of fat-soluble vitamins. A higher lipid content in larvae meal implies it can offer more energy per unit weight of feed, which is beneficial for animals requiring a high-energy diet, such as growing animals or those experiencing periods of stress [17]. Moreover, a higher lipid content can contribute to a more balanced nutrient profile in larvae meal, particularly for species with specific dietary needs, enhancing overall palatability and digestibility [18].

Black soldier fly (BSF) larvae are abundant in nutrients, boasting up to 50% crude protein and up to 35% lipids when measured on a dry weight basis [19]. This rich protein and lipid content positions BSF as a promising alternative protein source for animal feed. Research indicates that BSF possesses an amino acid profile akin to fishmeal, featuring elevated levels of essential amino acids such as leucine, lysine, valine, and histidine [20]. Additionally, BSF larvae's lipid composition is noteworthy, characterized by a significant proportion of saturated fatty acids like lauric acid and myristic acid [21]. Overall, the nutritional makeup of BSF larvae, particularly their elevated protein and lipid levels, underscores their potential as a sustainable and nutritious feed ingredient, capable of replacing conventional protein sources like soybean meal and fishmeal. As for bloodworms (BW), particularly the Chironomid larvae, they exhibit a protein content ranging from 39.4% to 50.81% and a fat content falling between 1.70% and 4.18% [22]. These figures signify that BW serves as a commendable protein source and contains moderate levels of fat, rendering them a valuable nutritional choice for various aquatic animals.

The findings of this experiment indicated that tilapia fingerlings responded positively to the BW and BSF larvae meals. The BW and BSF larvae meals consistently showed higher growth and survival rates than the commercial pellets. The positive growth performance of fish from BW and BSF larvae meals is related to the nutritional content of the larvae meals. Both BW and BSF larvae meals exhibit higher protein and lipid contents compared to fish in control commercial pellets. The protein content of BSF larvae meals may be contributed by the high levels of essential amino acids like leucine, lysine, valine, and histidine. Additionally, the lipid content of BSF is notable, with a high proportion of saturated fatty acids like lauric acid and myristic acid.

The findings of this study were in parallel with the previous findings indicating that both BSF and BW are potential growth promoters of tilapia fish at nursery stage. BSF larvae meal can enhance the specific growth rate (SGR) and feed conversion ratio (FCR) of farmed aquatic animals [23]. Research shows that trout and salmon fed a BSF diet grow as quickly as those fed traditional fishmeal [23]. Substituting fishmeal with BSF larvae meal at levels up to 75% does not negatively impact fish growth [24]. However, the optimal inclusion level varies based on factors such as fish species, life stage, and the nutritional composition of the BSF meal. BSF larvae are a cost-effective, protein-rich, and high-quality alternative to fishmeal, with a similar amino acid profile and abundant beneficial nutrients [19]. Replacing fishmeal with BSF larvae reduces dependence on wild fish stocks for aquafeed, promoting sustainability in aquaculture. Additionally, BSF can be reared on organic waste, helping to reduce waste volume. However, increase in hepatosomatic index (HSI), visceral somatic index (VSI), and crude lipid content of BSF may negatively impact fish health [25]. It is suggested that BSF protein can replace up to 50% of dietary fishmeal protein without adversely affecting growth and nutrient utilization [26].

Bloodworms are highly beneficial for fish growth, serving as a protein-rich live food that fish favor for optimal development and enhanced coloration. Previous study indicated that bloodworms significantly boost fish growth and pigmentation [22], making them a valuable food source for ornamental fish. They are nutrient-dense, with a protein content ranging from 52.11% to 60.15%, and an average energy density of 2.46 kJ g-1 [22]. Given the increasing demand in the ornamental fish industry, mass-producing bloodworms is crucial since natural sources may not consistently meet the required quality and quantity. Incorporating bloodworms into a fish's diet supports their health and growth, making them a preferred option for fish enthusiasts seeking to improve their fish's nutrition. However, a previousstudy suggested that weight gain in fish fed with bloodworm showed the least weight increment and bloodworm did not exhibit good growth response to angelfish, *PterophyllumScalare*[27].

5. CONCLUSION

In this study, both BSF and BW larvae exhibited significant growth response properties to red hybrid tilapia compared to commercial pellets. Therefore, further investigation and understanding of these larvae meals as growth promoters are warranted. The overall results of this study were likely influenced primarily by the nutritional quality of the tested diets. The findings from this study are promising and should be further verified at the farm scale by using a combination of both BSF and BW as a feed source to replace commercial diets. This could eventually lead to reduced reliance on commercial feeds.

ETHICAL APPROVAL

The Department of Fisheries Malaysia's (DoF) Fisheries Research Institute GlamiLemi's trained fisheries research officers completed all processes and operations. The Department of Fisheries Malaysia which withstands the position of government body in charge of the fisheries industry in Malaysia, created standard operating procedures for the handling and sampling of fish which applied accordingly in all procedures to meet its established standards. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Department of Fisheries Malaysia. The protocol was approved by the Committee on the Ethics of Animal Experiments, Department of Fisheries Malaysia (DOF) (Protocol Number: DOF/KAKH/03/2020).

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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