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Effects of caterpillar and mealworm meals in diets on the growth performance of white-leg shrimp in an integrated multi-trophic aquaculture system

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Abstract

Background and Objective: Due to its excellent nutritional content, insect meal is being investigated as a fishmeal substitute in aquafeed. The study examined the use of mealworm *Tenebrio molitor* and *Papilio demoleus* caterpillar meal for an integrated multi-trophic aquaculture (IMTA) system reared white-leg shrimp *Litopenaeus vannamei*.

Methodology: Four diets were created, including a commercial pellet (CP) as a control. The other three diets used ground commercial pellets as a base and added 1% caterpillar meal (CM), 1% mealworm meal fed whole wheat flour (MM), or 1% mealworm-fed beetroot meal (MFBM). The restricted caterpillar meal resources reduced insect meal incorporation. Each IMTA system raised 50 tails of juvenile white-leg shrimp as the main species at 5 g/m^2 in a one-ton, high-density polyethylene tank. White-leg shrimp tank water samples were taken every 15 days to analyze *in situ* water parameters and dissolved inorganic nutrients.

Main Results: Caterpillar larvae had a strong nutritional profile of crude protein (48.09 ± 0.29%), crude lipid (5.33 ± 1.34%), dry matter (22.26%), and crude ash (7.17%). This study found that mealworms have a decent nutritional profile of 41% crude protein and 45% crude lipid. White-leg shrimp fed CP demonstrated significantly better total weight gain (423.43%) and specific growth rates (5.40% per day) compared to the other diets. Water quality measurements were similar in all treatments ($P > 0.05$), indicating that white-leg shrimp growth was unaffected. The weight gain and specific growth rates of *K. alvarezii* and *P. viridis* did not significantly differ ($P > 0.05$) across the four treatments.

Conclusions: This study shows that mealworm and caterpillar meals can be a viable source of protein for the white-leg shrimp. This work is expected to provide a foundation for future investigations and an expanded understanding of potential target ingredients for the aquaculture industry.

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INTRODUCTION

Southeast Asian countries have engaged in shrimp farming for over a century. However, viral outbreaks have posed significant challenges, compelling many farmers to either switch to fish farming or cease operations altogether (Lin and Chen, 2003; Daniel, 2018). The introduction of specific pathogen-free (SPF) white-leg shrimp in 1999 offered as a sustainable alternative to tiger prawn farming (Chen and Chen, 1992; Lin and Chen, 2003). Despite this advancement, white-leg shrimp farming has environmental implications, including nitrogenous waste generation from uneaten feeds and feces, which can negatively impact water quality (Li *et al*., 2024).

To mitigate these effects, the integrated multi-trophic aquaculture system (IMTA) has emerged as a promising solution. IMTA combines species from different trophic levels, where the waste from one species serves as nutrients for others (Neori *et al*., 2004). For example, cultivated shrimp are fed commercial diets, while extractive species such as seaweed and green mussel utilize the organic and inorganic waste, respectively (Neori *et al*., 2004). By shifting from monoculture to IMTA, the shrimp aquaculture sector can achieve both ecological and economic benefits. One major challenge in aquaculture is the rising cost of feed, with fishmeal and soybean meal accounting for 60%–70% of total production costs (Choi *et al*., 2018). As fishmeal becomes increasingly scarce, research has focused on alternative protein sources with comparable nutritional profiles (Daniel, 2018). Insects, such as mealworm larvae and lime swallowtail larvae, have been identified as nutrient-rich candidates for aquaculture feed (Siemianowska *et al*., 2013). Although considered agricultural pests due to their rapid proliferation and crop risks (Siemianowska *et al*., 2013), these insects offer potential as sustainable feed sources for aquaculture. Conventional pest control methods, such as pesticides and enzyme inhibitors, have raised environmental and health concerns, highlighting the need for eco-friendly approaches. Rather than eradicating these species, their nutritional profiles present an opportunity to repurpose them in the aquaculture sector (Gu *et al*., 2001).

In this study, a biomimetic approach was applied to aquaculture feed design. The metamorphosis of organisms such as caterpillars and mealworms can transform into new organisms such as butterflies and beetles. The transformation entail was so amazing through the metamorphosis cycle (Gu *et al*., 2001). During metamorphosis, certain hormone-reactive biological processes and the entire organism adjust to its new way of existence. Steroid hormones include ecdysone and juvenile hormones that control the developmental change in insects from the juvenile to adult stages to assist with metamorphosis and molting (Liu *et al*., 2020). In shrimp, the hormone named ecdysone is also responsible for controlling the molting (Gu *et al*., 2001). The feed source and the fed species in this study belong to the same phylum. Thus, it is hypothesized that the phylogenetic affinities in their metabolites could augur well for the compatibility needed in the nutritional physiology of the shrimp.

While white-leg shrimp culture has expanded, and many studies have proved that mealworm meal can promote the growth performance of white-leg shrimp (Panini *et al*., 2017; Choi *et al*., 2018), there is a requirement for additional data to assess the influence of diets integrating caterpillar meals and mealworm meal on the growth performance of white-leg shrimp within the IMTA system. Furthermore, the water quality implications of cultivating white-leg shrimp alongside green mussels and seaweed as extractive species still need to be explored. This study evaluated the nutritional value of mealworms and *Papilio demoleus* caterpillars. The nutritional value of four different types of diets, including a commercial pellet (CP) as a control, caterpillar meal + CP (CM), mealworm meal + CP (MM), and mealworm-fed beetroot meal + CP (MFBM), was analyzed. The growth performance and survival rate of white-leg shrimp, green mussels, and seaweed in the IMTA system were also assessed concerning the different feed types. The impacts of the various pellet types on the water quality parameters were also evaluated. The data obtained from this research provides valuable insights into the nutrition of white-leg shrimp in the context of caterpillars and mealworms in the IMTA system. Moreover, it sheds light on the effect of different pellet types on the water quality conditions for the white-leg shrimp.

MATERIALS AND METHODS

Mealworm (*Tenebrio molitor*) Larvae Culture

The mealworms were initially purchased from a local shop and transferred into their designated cultured media. Whole wheat flour was the primary food source for mealworms, and beetroot powder was a dietary supplement. The first culture medium consisted of whole wheat. In contrast, the second culture medium consisted of whole wheat flour supplemented with 10% beetroot powder. The mealworms were cultured for three weeks and collected before transitioning into the pupa stage, as shown in Figure 1. To provide water, wet cotton filled with water was changed daily. After drying the larvae in the oven at 105°C, they were ground using a blender and stored in plastic bags at -20°C before use (Panini *et al*., 2017).

Caterpillar Collection

The *P. demoleus* larvae were initially collected from the wild from citrus plants such as lemon trees. Subsequently, citrus plants were planted at the Borneo Marine Research Institute of Universiti Malaysia Sabah to facilitate the caterpillar production and collection. The fifth instar was collected and put in the freezer until further use.

Experimental Diets

This experiment used four types of pellets: commercial shrimp pellets (CP) as a control. The commercial pellet was ground and supplemented with a 1% inclusion of the targeted ingredient (caterpillar meal, mealworm meal fed whole wheat, and mealworm meal fed beetroot meal). Subsequently, the pellets were processed to match the desired sizes for the target species (Table 1). The juvenile white-leg shrimp were fed differently using the four different formulated feeds at 5% of the body weight two times daily at 9 a.m. and 4 p.m.

Experimental Design for Land-Based Integrated Multi-Trophic Aquaculture System

This study was conducted at the IMTA Research Area of Borneo Marine Research Institute, Universiti Malaysia Sabah, Malaysia. The white-leg shrimp post-larvae (PL12) were obtained from Asia Aquaculture Sdn. Bhd. and transported to UMS. The white-leg shrimp post-larvae were then cultured until they grew to juvenile size (approximately 1 inch) before use for the experiment. One ton high-density polyethylene tank was used to rear 50 tails of juvenile white-leg shrimp as the main species at 5 g/m^2. At the same time, the green mussel was placed in a 250 L fiberglass tank. A ratio of 3:1 was used for the green mussel and shrimp, respectively (Sasikumar and Viji, 2015). The seaweed, *Kappaphycus alvarezii*, was placed in a rectangular polyethylene tank with a capacity of 500 L. For efficient nutrient uptake and lower nutrient levels, the biomass ratio of bivalve/seaweed was 1:0.8 (Sasikumar and Viji, 2015). IMTA systems were prepared in a triplicate set (three sets per treatment). Effluent from the white-leg shrimp tank was pumped into the green mussel tank using an Atman AT-104S water pump before being supplied to the seaweed tank. The water from the seaweed tank was then transferred back to the white-leg shrimp tank (Figure 2).

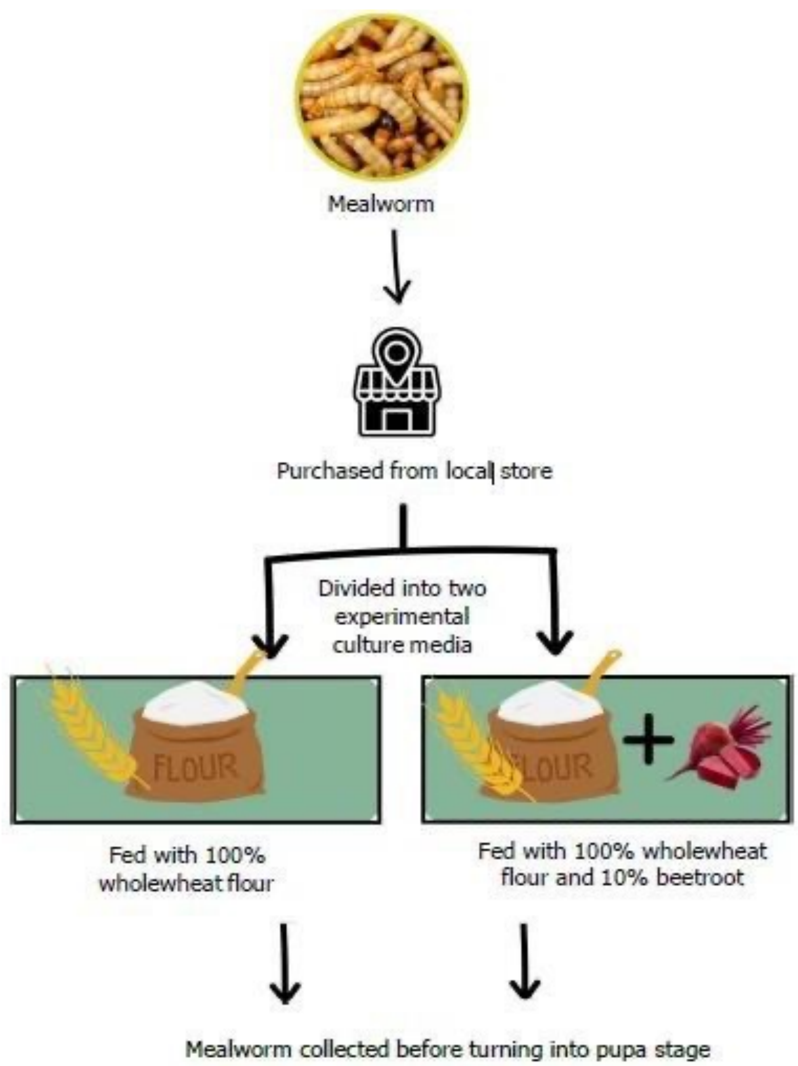

Figure 1 Schematic diagram of culturing mealworm with two different culture media

Table 1 Formulation (% dry weight) of the juvenile White-leg shrimp formulated feed with the inclusion of 1% targeted ingredients

Diets/Ingredients	CP	CM	MM	MFBM
Commercial pellet (CP)	100.00	86.25	86.25	86.25
Carboxymethylcellulose (CMC)	-	5.00	5.00	5.00
Fish oil	-	1.80	1.60	1.60
Alpha starch	-	5.98	6.38	6.38
Caterpillar meal (CM)	-	1.00	-	-
Mealworm-fed beetroot meal (MFBM)	-	-	1.00	-
Mealworm Meal (MM)	-	-	-	1.00

Note: CP = commercial pellet as a control, CM = caterpillar meal + CP, MM = mealworm, meal + CP, and MFBM = mealworm fed beetroot meal + CP.

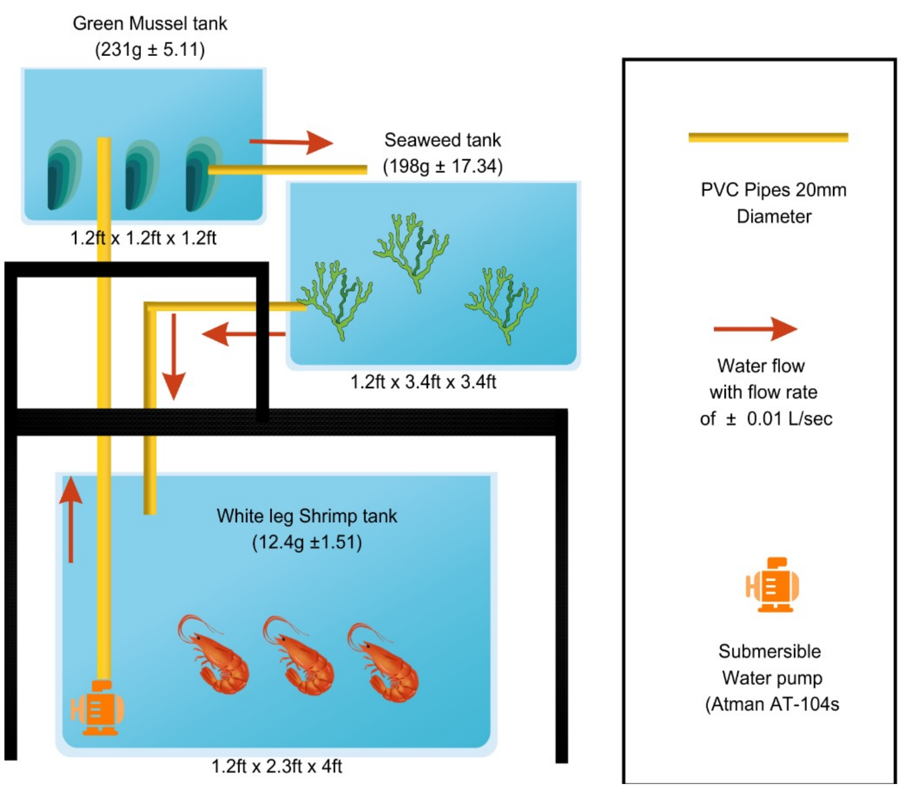

Figure 2 Schematic diagram of the recirculation system in the Integrated Multi-trophic Aquaculture system

Proximate Analysis

The proximate composition of the target ingredient-formulated diet was analyzed according to the AOAC (2023). Moisture content was measured by drying the samples to a constant weight in an oven at 105°C, method 934.01. Determination of protein inorganic nitrogen followed the Kjeldahl method 954.01. For lipid analysis, the procedure involved drying and extraction with petroleum ether using SoxtecTM 2043, method 954.02. Ash content was measured by putting 2 g of the sample into the crucible and heating it in the muffle furnace at 550°C for 5 hours. All of the samples were analyzed with three replicates.

Growth Performance of Shrimp, Seaweed, and Green Mussels and Feed Utilization of Shrimp

White-leg shrimp with an average initial body weight of 0.24 g was used in this study. The experiment was conducted in October 2022 for 30 days, and the experimental species was measured at every one-week interval. All the individuals in the tanks were measured. The weight gain, specific growth rates, and survival rates were calculated according to the formulas as stated below (Mok *et al.*, 2021):

$$\text{Weight gained (\%)} = \frac{\text{Final weight (g) - Initial weight (g)}}{\text{Initial weight}} \quad \text{--- [1]}$$

$$\text{Survival rates (\%)} = \frac{\text{Final number of shrimps}}{\text{Initial number of shrimps}} \times 100 \quad \text{--- [2]}$$

$$\text{Specific growth rates (\% per day)} = \frac{\text{In(final body weight)} - \text{In(initial body weight)}}{\text{Cultured period}} \times 100 \quad \text{--- [3]}$$

$$\text{Feed conversion ratio} = \frac{\text{Shrimp weight gained (g)}}{\text{Feed intake (g)}} \quad \text{--- [4]}$$

$$\text{Total feed intake} = \sum \frac{\text{Total daily dry feed consumed}}{\text{Total number of shrimp}} \quad \text{--- [5]}$$

Water Quality Parameter

The white-leg shrimp in this study was reared in seawater with a salinity of 30 ppt. YSI Multi-parameter, Pro Plus, USA, was used to record *in-situ* water quality parameters such as dissolved oxygen (mg/L), pH, temperature (°C), and salinity (ppt) daily. Every seven days, 500 mL water samples from each white-leg shrimp, seaweed, and green mussel tank were taken using a polyethylene bottle and brought to the chemical laboratory for further analysis. Standard procedures by APHA (2023) were followed to determine nitrogen-ammonia using the phenate method (APHA 4500-NH3 (F)), nitrogen-nitrite using the colorimetric method (APHA 4500 NO2 (B)), nitrogen-nitrate using the ultraviolet spectrophotometric method (APHA 4500-NO3 (B)), and phosphorus using the ascorbic acid method (APHA 4500-P (E)).

Statistical Analysis

The system was designed for the four treatments and in a triplicate set. The tanks, each for each treatment, were placed randomly. Data for specific growth rate (% per day), weight gain (%), survival rate (%), proximate composition (%), and water quality were analyzed using one-way analysis of variance (ANOVA) to test the significance of the difference ($P < 0.05$) between the diets. A post hoc test (Duncan) was conducted to determine if there was a significant difference between the treatments. SPSS software 26.0 was used for statistical analyses.

RESULTS AND DISCUSSION

Proximate Composition of Experimental Diets and Targeted Ingredients

The results of the proximate compositions of targeted ingredients in this experiment (Table 2) showed that mealworm and caterpillar meals can be a good source of nutrition for white-leg shrimp. Previous studies have shown that a diet based on insects may be less expensive than a fishmeal diet (Choi *et al.*, 2018). In this study, the mealworm larvae fed with 100% whole wheat crude protein was recorded at 41.87%, and the mealworm larvae fed with 90% whole wheat and 10% beetroot powder was recorded at 41.48%. The study found that the crude protein levels in this mealworm were like those reported in previous studies by Liu *et al*. (2020), 49.1% and 46.44%, respectively, but slightly higher than the value obtained by Siemianowska *et al*. (2013), which was 17.92%. While for the lipid recorded in this study was 45.34% and 45.14% for mealworm larvae fed whole wheat and mealworm larvae fed beetroot powder, respectively. Lipid reserves are usually at their highest in the final larval stage before metamorphosis. Another important factor is the different diets used to raise insects (Panini *et al*., 2017). In this study, this conclusion is supported by the fact that there were no significant differences between the two diets, as indicated in Table 3. Notably, Table 3 presents differences observed exclusively in the commercial pellet diet. According to research conducted by Liu *et al*. (2020), supplementing the diet of mealworms with fresh plant materials did not have a significant effect on their chemical composition. However, when carrots, oranges, and red cabbage were added to their diet, the weight of the mealworm larvae increased significantly. Even though mealworms can tolerate drought and absorb water from the air, they grow faster when they are provided with water or food with high moisture content. As for the caterpillar larvae, the crude protein recorded was at 48.09%, while the crude lipid recorded was at 5.33%. There is a need for more data regarding feeding caterpillar larvae to white-leg shrimp. However, numerous authors have investigated various types of caterpillars on *Clarias gariepinus* larvae. The silk caterpillar (*Bombyx mori*) could be an effective substitute for fishmeal in the African catfish diet. This study discovered that fish growth rates and feed utilization parameters were higher in fingerlings fed diets with mixed fishmeal and silkworms in 50:50 ratios. Anvo *et al*. (2017) evaluated the effectiveness of incorporating *C. butyrospermi* caterpillar meal as a partial or complete substitute for fishmeal in the diet of *C. gariepinus* larvae. They discovered that a 25% inclusion of the *C. butyrospermi* caterpillar in the *C. gariepinus* diet could improve growth performance for a profitable feed for catfish larvae.

Regarding the key ingredients, mealworm larvae are readily accessible and conveniently procured from local sources. In contrast, sourcing caterpillar larvae of *P. demoleus* can pose challenges due to their limited presence on citrus plants. Furthermore, the widespread use of pesticides on citrus plants further complicates locating these caterpillars. It is not environmentally friendly to harvest wild butterfly caterpillars. Their use on a limited scale is to gain insights into the nutritional profiles that can be compared with larvae of other insects that are a sustainable source. Additionally, during heavy rainy seasons, butterfly activities decrease, making it even more

Table 2 Proximate composition of targeted ingredients

Proximate composition	Experimental target ingredient		
	Caterpillar larvae	**Mealworm larvae fed with 100% wholewheat**	**Mealworm larvae fed with 90% wholewheat and 10% beetroot powder**
Crude protein (%)	48.09 ± 0.29^{b}	41.87 ± 0.26^{a}	41.48 ± 0.26^{a}
Crude lipid (%)	5.33 ± 1.34^{a}	45.34 ± 2.79^{b}	45.14 ± 0.26^{b}
Moisture (%)	77.73 ± 1.17^{b}	59.25 ± 0.62^{a}	59.35 ± 0.46^{a}
Dry matter (%)	22.26 ± 1.17^{a}	40.72 ± 0.62^{b}	41.38 ± 0.26^{b}
Crude ash (%)	7.17 ± 0.04	4.35 ± 0.16	4.44 ± 0.10

Note: Means within the same row followed by different superscript letters (a, b) are significantly different ($P < 0.05$). Values are presented as mean ± standard deviation.

difficult to find their eggs and larvae. This observation is supported by Yasmin *et al*. (2019), who discovered that the development of *P. demoleus* is slower at lower temperatures, while higher temperatures accelerate their developmental rate. Thus, if citrus plants are cultivated in large numbers in a controlled environment like a greenhouse, with regulated temperature and protection from predators, especially birds, the culture of *P. demoleus* caterpillar can be successful, and collecting the larvae becomes much more manageable.

Although the *P. demoleus* caterpillar is a significant pest that causes substantial damage by consuming large amounts of leaves from plants, especially wild and cultivated species (Jahnavi *et al*., 2018), this study shows it has potential as an alternative protein source in aquaculture nutrition. The caterpillar contains 48.09% protein, which is higher than the 41% protein content found in mealworm meals. Instead of focusing solely on pest control methods like natural enemies and biopesticides, more research is needed to explore the benefits this caterpillar could bring to the aquaculture and agriculture industries.

Table 3 Proximate composition of experimental diets

Proximate composition	Experimental diets			
	CP	CM	MM	MFBM
Crude protein (%)	44.91 ± 0.11^{b}	40.94 ± 0.15^{a}	40.98 ± 0.30^{a}	40.98 ± 0.26^{a}
Crude lipid (%)	5.62 ± 0.54	6.19 ± 0.40	6.10 ± 0.46	8.26 ± 0.46
Moisture (%)	8.63 ± 0.08^{d}	5.27 ± 0.14^{c}	3.59 ± 0.27^{a}	4.05 ± 0.35^{b}
Dry matter (%)	91.36 ± 0.08^{a}	94.71 ± 0.14^{b}	96.40 ± 0.27^{d}	95.93 ± 0.35^{c}
Crude ash (%)	11.05 ± 0.03	10.92 ± 0.25	10.54 ± 0.33	10.54 ± 0.50

Note: Means within the same row followed by different superscript letters (a, b, c, d) are significantly different ($P < 0.05$). Values are presented as mean ± standard deviation. CP = commercial pellet as a control, CM = caterpillar meal + CP, MM = mealworm, meal + CP, and MFBM = mealworm fed beetroot meal + CP.

Growth Performance and Feed Utilization of White-leg Shrimp in IMTA

This research offers convincing proof that both MM and CM can be a feasible source of protein in the diet of white-leg shrimp. Experimental diets were designed to be supplemented with *P. demoleus* caterpillar meals and two types of mealworm cultures. The specific growth rates and total weight gain of white-leg shrimp were significantly better in control compared to the other treatments (Table 4), which aligns with the higher crude protein content observed in the control, as it contained the highest protein content, 44.91%, compared to the other three treatments ($P < 0.05$). Juvenile and adult shrimp exhibited higher weight gain and percentage weight gain on a protein-based diet when given the 32% protein compared to the 16% protein diet. However, they displayed sensitivity to the source of protein (animal or plant origin) (Smith *et al*., 1985). In contrast, the small shrimp-like species used in this experiment demonstrated a pronounced sensitivity to changes in protein levels (Li *et al*., 2024). In this experiment, the protein level was between 44% and 40%, which was higher than the previous study by Lee and Lee (2018). A broken line analysis based on weight gained revealed that the optimal dietary protein levels would be 34.5% and 35.6% for small-sized shrimp with sizes of 0.6 to 5 g (Lee and Lee, 2018). Various factors, such as shrimp size, stocking density, culture system, species, and dietary protein sources, can influence the ideal dietary level necessary for the maximum growth of white-leg shrimp, as suggested by Lee and Lee (2018). Previous studies have explored the potential of using mealworm meals in the diet of white-leg shrimp. As stated by Panini *et al*. (2017), the performance of white-leg shrimp was not affected by replacing fishmeal with mealworm in the diets; however, the use of mealworm should be supplemented by methionine to meet the white-leg shrimp amino acid requirement.

Shrimp fed on diets with more than 25% fishmeal substitution for mealworms showed increased lipid content and decreased polyunsaturated fatty acids. Nevertheless, the quality of the white-leg shrimp fed on fishmeal substituted by mealworm was unaffected. In a previous research study conducted by Choi *et al*. (2018), it was found that replacing fishmeal with mealworms in the diet of white-leg shrimp had a positive impact on their growth performance and immune responses. The study concluded that a diet containing 50% mealworms was the most effective in promoting shrimp growth without any negative effects. The results of the study suggest that mealworm-based feed could be useful in enhancing both growth performance and immunity of shrimp. The growth performance indices demonstrated that the inclusion of caterpillar meals and mealworm meals in the diet does not harm the growth performance of white-leg shrimp.

Table 4 Growth performance of the white-leg shrimp, seaweed and green mussel

Parameters	**Experimental diets**			
	CP	**CM**	**MM**	**MFBM**
White-leg shrimp				
Average initial body weight (g)	0.24 ± 0.02	0.25 ± 0.05	0.25 ± 0.03	0.23 ± 0.01
Average final body weight (g)	1.29 ± 0.13	1.04 ± 0.20	1.01 ± 0.18	0.98 ± 0.10
Total weight gain (%)	423.43 ± 86.40^{a}	317.08 ± 57.92^{b}	299.29 ± 32.70^{b}	313.45 ± 25.57^{b}
Survival rates (%)	98.66 ± 2.30	96.00 ± 4.00	96.66 ± 3.05	94.66 ± 3.05
Specific growth rates (% per day)	5.40 ± 0.51^{a}	4.70 ± 0.45^{b}	4.60 ± 0.28^{c}	4.72 ± 0.20^{b}
Total feed intake (g per shrimp)	1.49 ± 0.05	1.30 ± 0.11	1.29 ± 0.18	1.33 ± 0.07
Feed conversion ratio (FCR)	1.45 ± 0.18	1.66 ± 0.21	1.72 ± 0.13	1.80 ± 0.31
Seaweed, *Kapapphycus alvareezi*				
Initial weight (g)	205 ± 10.4	204 ± 9.3	203 ± 5.8	200 ± 2.1
Final weight (g)	235 ± 11.3	232 ± 7.5	231 ± 10.3	228 ± 7.1
Total weight gain (%)	14.6 ± 0.4	13.7 ± 2.2	13.7 ± 3.5	13.8 ± 2.6
Specific growth rates (% per day)	0.45 ± 0.01	0.42 ± 0.10	0.42 ± 0.10	0.42 ± 0.07
Green mussel, *Perna viridis*				
Initial weight (g)	235 ± 0.5	229 ± 1.5	227 ± 2.8	234 ± 2.1
Final weight (g)	244 ± 1.0	242 ± 2.0	238 ± 2.0	244 ± 2.0
Total weight gain (%)	4.4 ± 0.2	5.3 ± 0.9	4.6 ± 0.9	4.5 ± 0.9
Specific growth rates (% per day)	0.14 ± 0.01	0.17 ± 0.03	0.15 ± 0.03	0.15 ± 0.02

Note: Means within the same row followed by different superscript letters (a, b, c) are significantly different ($P < 0.05$). Values are presented as mean ± standard deviation. CP = commercial pellet as a control, CM = caterpillar meal + CP, MM = mealworm, meal + CP, and MFBM = mealworm fed beetroot meal + CP.

Growth Performance of Seaweed and Green Mussel in IMTA

The study showed that the specific growth rates of *K. alvarezii* exhibited no significant difference ($P > 0.05$) between the treatments, with specific growth rates ranging from 0.42% per day to 0.45% per day. Although these rates were lower than the growth rate achieved in a Florida Pompano (*Trachinotus carolinus*) integrated culture, they indicated that the nutrient concentration in the IMTA system supported seaweed growth (Hayashi *et al*., 2008). Integrating seaweed into aquaculture systems has been suggested as a sustainable way to increase production (Neori *et al*., 2004). Seaweed prefers ammonium as a nitrogen source, producing oxygen through photosynthesis (Abreu *et al*., 2009). As the rate of photosynthesis increases, the seaweed's uptake of ammonia increases, resulting in higher growth rates (Hamilton *et al*., 2022). According to research by Hamilton *et al*. (2022), seaweed and seagrasses can potentially increase the calcification rates of bivalve species. The study found that calcification rates tended to increase during the daytime when macrophytes were actively photosynthesizing.

***In-situ* and *Ex-situ* Water Quality in IMTA**

Effective water quality management plays a crucial role in determining the success of aquaculture production, especially for white-leg shrimp. White-leg shrimp heavily rely on the quality of their aquatic environment to maintain their health and promote growth. Several key water quality parameters significantly impact their well-being. In this study, various factors affecting water quality and their implications on white-leg shrimp production were examined.

Temperature is a vital factor affecting the growth and metabolism of aquatic animals, including white-leg shrimp. Our study recorded water temperatures in the shrimp tank ranging from 27.5°C to 27.8°C (Table 5). Dissolved oxygen is a critical indicator of water quality, as it directly affects the oxidation and reduction processes of various substances in the aquatic environment. In our study, dissolved oxygen levels ranged from 5.00 ± 0.38 to 5.11 ± 0.57 mg/L, which aligns with the recommended range of 5.0–9.0 mg/L suggested by Maicá *et al*. (2014).

Salinity measurements in all treatments ranged from 33.1 to 34.3 ppt, well within the recommended range. According to Saoud *et al*. (2003), white-leg shrimp can thrive in waters with salinities ranging from 0.5 to 40 ppt. This is due to their osmotic regulation capabilities, which allow them to adapt to various salinity levels.

pH levels in the white-leg shrimp tank during the study ranged from 8.25 to 8.34. Supriatna *et al*. (2022) mentioned that the optimal pH value for shrimp is pH 7.0–8.5, with a tolerance of pH 6.5–9.0. pH is one of the key environmental factors that impact white-leg shrimp's survival and growth, as well as their physiological processes, such as metabolism (Suwardi and Suwoyo, 2021). Water with a pH lower than 5.0 can inhibit shrimp growth, reduce appetite, and create a stressful environment for the shrimp.

The phosphate content in the white-leg shrimp tank ranged from 0.26 to 0.30 mg/L during the experiment. This falls within the recommended range of 0.05 to 0.5 mg/L for the white-leg shrimp (Kasnir *et al*., 2014). Phosphate levels can significantly affect shrimp productivity, as it is a form of phosphorus utilized by both high- and low-level plants, such as microalgae. Aquatic plants and algae require phosphorus to grow, but having too much in rivers and lakes can lead to excessive amounts of algae, known as algal blooms.

Nitrate levels recorded in the study fell within the range of 5.13 to 5.30 mg/L, supporting the growth of natural food in the shrimp culture media (Suwardi and Suwoyo, 2021). Nitrate levels in the range of 0.1–4.5 mg/L are optimal for algae growth (Effendi *et al*., 2018), and the safety nitrate level for white-leg shrimp was reported to be 232 mg/L (Tsai and Chen, 2002). Lin and Chen (2003) reported that the safe levels of nitrite for white-leg shrimp were estimated to be 6.1, 15.2, and 25.7 mg/L at salinities of 15, 25, and 35 ppt, respectively. While these thresholds are considered safe regarding acute toxicity, they may not be optimal for shrimp growth, health, or long-term survival under intensive conditions. Sublethal nitrite exposure has been shown to increase hemolymph nitrite accumulation and disturb ionic regulation, potentially affecting shrimp metabolism and immune function (Chen and Chen, 1992). In this study, the nitrite-N concentrations recorded (2.48–2.87 mg/L) were significantly below the reported safety threshold of 25.7 mg/L for 35 ppt salinity. However, it remains crucial to assess whether prolonged exposure to these concentrations may contribute to chronic stress or affect shrimp growth and survival. Additionally, factors such as pH, temperature, and dissolved oxygen may influence nitrite toxicity, underscoring the need for future studies that explore the combined effects of these variables on shrimp health (Fourooghifard *et al*., 2018; Li *et al*., 2024). Further research is necessary to refine existing water quality thresholds and develop more precise management strategies for shrimp culture under different environmental conditions.

Table 5 Water quality parameters recorded in the tanks of white-leg shrimp during the experiment

Parameters	N	CP	CM	MM	MFMB
Dissolved oxygen (mg/L)	30	5.08 ± 0.46	5.11 ± 0.57	5.11 ± 0.48	5.00 ± 0.38
pH	30	8.26 ± 0.09	8.34 ± 0.20	8.25 ± 0.05	8.25 ± 0.05
Temperature (°C)	30	27.6 ± 1.23	27.8 ± 1.21	27.5 ± 1.26	27.8 ± 1.15
Salinity (ppt)	30	33.1 ± 2.02	34.3 ± 2.66	33.4 ± 1.95	32.2 ± 2.00
Ammonia-N (mg/L)	15	0.23 ± 0.46	0.26 ± 0.12	0.19 ± 0.06	0.24 ± 0.06
Phosphate-P (mg/L)	15	0.26 ± 0.95	0.26 ± 1.06	0.26 ± 0.58	0.30 ± 0.55
Nitrite-N (mg/L)	15	2.81 ± 0.53	2.48 ± 0.62	2.73 ± 0.33	2.87 ± 0.73
Nitrate-N (mg/L)	15	5.30 ± 0.07	5.13 ± 0.08	5.29 ± 0.09	5.22 ± 0.01

Note: CM = caterpillar meal + CP, MM = mealworm, meal + CP, and MFBM = mealworm fed beetroot meal + CP.

The concentration of ammonia in natural waters can vary from 0 to 2.64 mg/L. However, in shrimp cultivation systems that are intensive, the levels of ammonia can go beyond 40 mg/L (Tong *et al*., 2023). Ammonia is a prevalent toxicant in cultural systems, mainly produced by the excretion of cultured animals and the mineralization of organic waste materials. The ammonia level recorded in our study was within the safety range, where the threshold for ammonia in shrimp culture is reported to be 3.95 mg/L (Fourooghifard *et al*., 2018).

The water quality parameters did not restrict white-leg shrimp growth. Stocking culture and extractive species densities in the IMTA system resulted in enhanced shrimp production and excellent water quality. In a study conducted by Fourooghifard *et al*. (2018), 25 shrimp/m^2 and 400 g of seaweed resulted in the best performance of white-leg shrimp. Total ammonia, nitrite, and nitrate concentrations in this treatment were significantly lower. The highest total ammonia, nitrite, and nitrate concentration was observed in treatment with a combination of 50 shrimp/m² without seaweed. The safety level for rearing white-leg shrimp was estimated to be 3.95 mg/L for ammonia, 25 mg/L for nitrite, 232 mg/L for nitrate, and 0.05 to 0.50 mg/L for phosphate (Tsai and Chen, 2002; Lin and Chen, 2003; Fourooghifard *et al*., 2018). As the levels of nitrogen compounds in all treatments are below the safety level, the combinations of cultivated species and extractive species in the IMTA systems help to maintain appropriate water quality parameters.

CONCLUSIONS

The present study investigates the potential of using caterpillar meal and mealworm meal as alternative protein sources for white-leg shrimp farming. The study aims to evaluate these ingredients as part of the diet and improve the farming system by incorporating a cost-effective and environmentally friendly technology known as the Integrated Multi-Trophic Aquaculture (IMTA) system. It has been shown that the caterpillar has the highest crude protein content (48%), while mealworm larvae have the highest crude lipid content (45%). This study provides evidence that mealworm and caterpillar meals can be viable sources of protein in the diet of white-leg shrimp. Although the inclusion of caterpillar meals and mealworm meals in the diet did not result in significant improvements in shrimp growth performance compared to the control diet, the growth performance indices suggest that these alternative protein sources can sustain shrimp growth without causing detrimental effects. However, the control diet yielded better specific growth rates and survival rates, indicating that these ingredients have the potential as partial substitutes for fishmeal. Further studies are needed to optimize the inclusion levels of caterpillar and mealworm meals and assess their long-term effects on shrimp growth performance.

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