

Growth and Nutritional Quality in Giant Freshwater Prawn, *Macrobrachium rosenbergii* through Live Mealworm Feeding with Probiotic Enrichment

(Pertumbuhan dan Kualiti Nutrisi dalam Udang Galah, *Macrobrachium rosenbergii* melalui Pemakanan Ulat Hidup dengan Pengayaan Probiotik)

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ABSTRACT

To address the gap in sustainable aquaculture, a 17-week feeding trial was conducted to evaluate locally-sourced mealworms, with and without *Bacillus subtilis* probiotic enrichment, as an alternative feed material for giant freshwater prawn (*Macrobrachium rosenbergii*), assessing their effects on growth, feed utilization, and nutritional composition. Five experimental diets were tested: commercial prawn feed (CPF), CPF combined with live mealworm (CPF+MW), mealworm alone (MW), probiotic-enriched mealworm (PMW), and CPF combined with probiotic-enriched mealworm (CPF+PMW). Triplicate groups of 20 prawns were randomly assigned to each diet. Weight gain among the groups ranged from 421.88% to 529.34%, with no significant differences observed ($P > 0.05$). Prawns fed CPF exhibited a significantly higher feed conversion ratio (FCR) (3.72 ± 0.32 , $P < 0.05$), indicating less efficient feed utilization and leading to increased production costs. While CPF (45.01%) and MW (52.44%) diets differed significantly in crude protein content, the prawns fed CPF (59.24%) and MW (60.78%) showed similar crude protein levels. These results suggest that live mealworms are a viable alternative to commercial feed for GFP, maintaining growth performance and nutritional quality. Furthermore, combining live mealworms with commercial feed proves to be an effective feeding strategy, though enrichment with *B. subtilis* did not provide additional benefits for prawn growth or FCR.

Keywords: *Bacillus subtilis*; probiotic enrichment; sustainable feed; *Tenebrio molitor*

ABSTRAK

Untuk menangani jurang dalam akuakultur mampan, suatu kajian pemakanan selama 17 minggu telah dijalankan untuk menilai ulat tempatan dengan dan tanpa pengayaan probiotik *Bacillus subtilis* sebagai bahan makanan alternatif untuk udang galah (*Macrobrachium rosenbergii*), dengan menilai kesannya terhadap pertumbuhan, penggunaan makanan dan komposisi nutrisi. Lima diet uji kaji telah diuji: makanan udang komersial (CPF), CPF digabungkan dengan ulat hidup (CPF+MW), ulat sahaja (MW), ulat yang diperkaya dengan probiotik (PMW) dan CPF digabungkan dengan ulat yang diperkaya dengan probiotik (CPF+PMW). Tiga kumpulan udang sebagai peniga sebanyak 20 ekor secara rawak diberikan bagi setiap diet. Keputusan menunjukkan peningkatan berat dalam kalangan kumpulan berbeza antara 421.88% hingga 529.34%, tanpa perbezaan yang signifikan diperhatikan ($P > 0.05$). Udang yang diberi makan CPF menunjukkan nisbah penukaran makanan (FCR) yang lebih tinggi secara signifikan (3.72 ± 0.32 , $P < 0.05$), menunjukkan penggunaan makanan yang kurang cekap dan menyebabkan peningkatan kos pengeluaran. Walaupun diet CPF (45.01%) dan MW (52.44%) berbeza dengan ketara dalam kandungan protein kasar, tetapi udang yang diberi CPF (59.24%) dan MW (60.78%) menunjukkan tahap protein kasar yang serupa. Keputusan ini mencadangkan bahawa ulat hidup adalah alternatif yang sesuai untuk makanan komersial bagi GFP, mengekalkan prestasi pertumbuhan dan kualiti nutrisi. Selain itu, menggabungkan ulat hidup dengan makanan komersial terbukti sebagai strategi pemakanan yang berkesan, walaupun pengayaan *B. subtilis* tidak memberikan manfaat tambahan kepada pertumbuhan atau FCR udang.

Keywords: *Bacillus subtilis*; pemakanan mampan; pengayaan probiotik; *Tenebrio molitor*

INTRODUCTION

Macrobrachium rosenbergii, commonly known as the giant freshwater prawn (GFP), is native to Southeast Asia and commands a market price ranging from RM68 to RM90 per kilogram (Pillai et al. 2022). In Malaysia, GFP aquaculture production reached 189.1 metric tonnes, valued at RM15.5 million in 2023 (DOF 2023). Although hatchery technology such as water treatment, recirculating system and flowthrough system for this species has been commercially established since the 1980s, the reliance on live feed during the larval and post-larval stages leads to high feeding costs, significantly hindering large-scale production (Barroso et al. 2014). Feeding costs constitute the primary operational expenses in modern farming systems, and the rapid expansion of the aquaculture industry is partly attributed to the widespread use of aquafeeds (Turchini, Torstensen & Ng 2009). Aquafeeds are aquaculture feeds, formulated to meet the specific nutritional needs of various aquatic species at different life stages, ensuring an optimal balance of essential nutrients for their growth and development (Turchini, Torstensen & Ng 2009). A reduction in feed prices could lead to significant savings for the aquaculture sector (Kim et al. 2015). To ensure the sustainability of aquaculture, it is essential to explore local alternative sources of protein and energy due to the rising costs of conventional feed components (Khanjani et al. 2023; Rana, Siriwardena & Hasan 2009).

Plant-based protein meals and oils have been identified as potential substitutes for fishmeal and fish oil. They come with challenges such as land use concerns, anti-nutritional factors, and deforestation (Sales & Glencross 2011; Sabaté & Soret 2014; Teoh & Ng 2016). Insects have emerged as viable alternatives to fishmeal due to their sustainability and nutritional benefits (Gałęcki et al. 2021; Hua et al. 2019; Murawska et al. 2021; Ooninx et al. 2020; Sharifinia et al. 2023a; Sharifinia et al. 2023b; van Huis & Ooninx 2017), as they efficiently convert food waste, have a short life cycle, and are widely available across various geographic regions (Danks 2006; Rumpold & Schlüter 2013; van Huis 2013).

Food waste significantly impacts global food security, and Malaysia is no exception. Reports show that Malaysians discard approximately 4,046 tonnes of edible food daily, which is enough to provide three meals a day for three million people (Mohideen 2022). Entotechnology utilizes scientific and technical expertise to harness insects' potential for societal benefits in medicine, agriculture, food, and biotechnology. Recent research highlights it as a promising solution for managing organic waste and sustainably producing food for humans, animals, or fish (Hénault-Ethier 2017; López-Almonte et al. 2024). For instance, insects have been shown to effectively reduce fecal coliforms and Salmonella, as well as cut organic waste by 50-75%, depending on the species and type of waste (Chavez & Uchanski 2021). Entotechnology uses various insect species, with mealworms (*Tenebrio molitor*)

being particularly efficient for organic waste management and food production due to their ease of production, low resource demands, and minimal labor requirements (Paris et al. 2024; Patel 2019). Nutritionally, mealworms are valued for their high protein and fat content, surpassing that of earthworms (Parolini, Ganzaroli & Bacenetti 2020) and black soldier fly larvae (Barragán-Fonseca et al. 2023).

The nutrient composition of mealworms is affected by factors such as their life stage, environment, substrate, and diet (Finke 2015; Jeong et al. 2022; Khosravi et al. 2018; Siemianowska et al. 2013). In particular, the larvae of the yellow mealworm are among the most commonly farmed insects for food purposes and are capable of breaking down and consuming a variety of agricultural and food industry byproducts (Bordiean et al. 2020). By adjusting their diet and substrate, it is possible to tailor their nutritional content, making mealworms a valuable resource for sustainable feeding practices across various industries (Berezina 2017; Mancini et al. 2019; Ooninx et al. 2015; Ramos-Elorduy et al. 2002).

Probiotic bacteria, especially *Bacillus* strains, are gaining attention as a sustainable aquafeed strategy to reduce antibiotics in aquaculture, offering benefits like disease prevention, improved FCR, growth, and water quality for freshwater fish and crustaceans (Hai 2015; Nathanailides et al. 2021). Various *Bacillus* species have been isolated from the gut of GFP, with *Bacillus subtilis* shown to be a highly effective coloniser in the gut of GFP post-larvae (PL) (Karthik, Bhavan & Manjula 2018; Seenivasan et al. 2012). These beneficial bacteria act on the GFP by altering intestinal microbiota, secreting antibacterial compounds, competing with pathogens for adhesion sites and nutrients, and producing antitoxins (Cruz et al. 2012). Despite these advantages, research on the impact of feeding live mealworms enriched with probiotics to GFP remains limited. This study aims to explore the impact of live mealworm feeding on GFP with a dual focus: assessing both growth performance and feed efficiency, as well as evaluating the nutritional content. Additionally, it will evaluate whether probiotic-enriched mealworms provide further benefits in these parameters.

MATERIALS AND METHODS

Figure 1 presents an overview of the experimental procedure for this study.

CULTURE OF MEALWORM

In this study, mealworms were used as live feed for GFP following a breeding and culturing process adapted from Bordiean et al. (2022). Initially, 2 kg of live mealworms were purchased from a local aquarium shop in Kampar, Perak, Malaysia, and distributed into four plastic containers (30 cm × 15 cm × 5 cm; W × L × H). As the mealworm population is observed to increase, they were then transferred to additional containers of the same

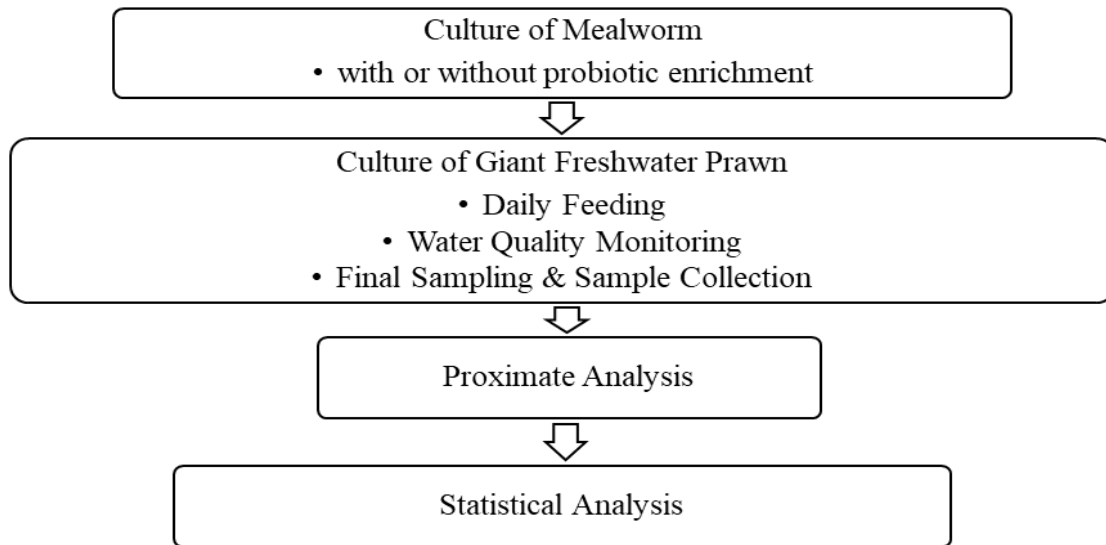


FIGURE 1. Flowchart outlining the experimental procedure for the present study

size to prevent overcrowding, maintaining a density of 1 larva/cm². The mealworms were cultured in a shaded environment with a 12-h light and 12-h dark cycle, with hydration provided through potatoes, carrots, lettuce, and moist cotton pads. Pupae and adult beetles were separated and placed into another container with wheat bran as substrate, along with additional hydration sources, including potatoes, carrots, lettuce, and moist cotton pads. Mealworms of approximately 2 cm in length were harvested, weighed, and used as live feed for the GFP feeding trial. The mealworms were continuously reared until the final week of the GFP feeding trial.

PROBIOTIC ENRICHMENT OF MEALWORM

Probiotic enrichment was performed following a modified protocol from Dadvar et al. (2023). A commercial probiotic, IG Guard A, was obtained from ADBIOTECH Co., Ltd. The probiotic powder contained 2.0×10^{10} CFU/kg of *Bacillus subtilis*, 120 g/kg of egg powder, and carrier distillers dried grains (wheat flour and corn flour) up to a total weight of 1 kg. Mealworms, each weighing approximately 154 ± 0.5 mg, were selected for *B. subtilis* enrichment. They were first starved for two days, then placed in a plastic container (1.73 cm \times 1.18 cm \times 0.56 cm) and given 10 g of IG Guard A. The mealworms were kept in the container for three hours, during which their feeding behavior was monitored to ensure they consumed the probiotic powder. The mealworms were then left in the container for an additional 24 hours to allow for complete intake of the probiotic (Chung et al. 2013), after which they were harvested and used as feed for the GFP feeding trial following the specified feeding regime.

CULTURE OF GIANT FRESHWATER PRAWN

Post-larvae day 15 (PL₁₅) of GFP were sourced from Pusat Penetasan Udang Galah (PPUG) in Kg. Aceh, Malaysia and were acclimated for seven days upon their arrival at the Aquaculture Facilities, UTAR. During acclimation, the PLs were fed twice daily with a commercial prawn diet (Dindings Freshwater Prawn Feed 2203) until satiation. At the start, groups of 20 PLs, each with an initial body weight of $0.44 \text{ g} \pm 0.00$, were randomly assigned to each of the 15 tanks (45.72 cm W \times 76.20 cm L \times 45.72 cm H). The tanks, equipped with a recirculating freshwater system, contained 20 PVC pipes (25 mm diameter \times 45 mm length) at the bottom to offer refuge for the PLs (Chong, Teoh & Wong 2022). Five experimental dietary treatments were used in the feeding trial: (1) commercial prawn feed (CPF, control), (2) commercial prawn feed with live mealworms (CPF+MW), (3) live mealworms alone (MW), (4) live mealworms enriched with probiotics (PMW), and (5) commercial prawn feed with probiotic-enriched mealworms (CPF+PMW). The CPF+MW and CPF+PMW diets were prepared in a 1:1 ratio, with 1 g of commercial feed to 1 g of mealworms or probiotic-enriched mealworms, respectively. All experimental diets were weighed on a wet-weight basis and converted to dry weight for calculating the FCR. Each diet was randomly assigned to triplicate groups of prawns. The PLs were fed three times daily at 09:00, 13:00, and 17:00 h to apparent satiation (approximately 15% of body weight per day) (Ng et al. 2017) during the initial weeks, and then twice daily at 09:00 and 17:00 h (approximately 8% of body weight per day) starting from week four (Ng et al. 2017). All PL were batch-weighed weekly to monitor growth performance. The feeding trial was conducted for 17 weeks, with total feed

consumption recorded daily. After the 17-week feeding trial, the PL had grown to approximately 10 cm in length and was henceforth referred to as prawn. A final sampling was conducted to measure body weight and collect various samples. The final body weight of all prawns was recorded, and five prawns from each replicate tank were euthanized and stored in a freezer at -20 °C for subsequent analysis of whole-body proximate composition. Prawn growth performance was assessed by measuring the percentage of weight gain. Additionally, the survival rate of the prawns was calculated. Feed intake and FCR were also determined. These parameters were evaluated using the following formulae:

$$\text{Weight gain (\%)} = \frac{\text{final body weight} - \text{initial body weight (g)}}{\text{initial body weight (g)}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{final prawn number}}{\text{initial prawn number}} \times 100$$

$$\text{FCR} = \frac{\text{total dry feed (g)}}{\text{wet weight gain (g)}} \times 100$$

The total production cost for each treatment was recorded to evaluate the feasibility of using live mealworms, including those enriched with probiotics, in commercial GFP farming.

WATER QUALITY MONITORING

Throughout the 17-week feeding trial, water pH and dissolved oxygen were monitored daily, using a digital pH tester (Extech Instruments, Taiwan) and an Eutech Basic Portable DO Meter DO 6+ (Thermo Scientific, Singapore), respectively. Additionally, water hardness, ammonia (NH₃), nitrite (NO₂⁻), and nitrate (NO₃⁻) levels were measured weekly using the Freshwater Master Test Kit (API®, USA). To maintain optimal water quality, a weekly water change was carried out, with 70% of the water being replaced in each tank to stabilize pH levels between 7.0 and 8.0 and hardness between 30 and 100 mg/L. If water hardness dropped below the desired range, calcium chloride was added to restore it.

PROXIMATE ANALYSIS

Proximate analysis of the treatment diets and prawn whole-body samples was conducted following standard AOAC methods (AOAC 1997) with modifications as outlined by Teoh and Loo (2022). In summary, dry matter content was determined using the oven-drying method, where samples were dried at 103 °C until a constant weight was achieved. Crude protein (CP) was determined using the Kjeldahl method, which involved digesting the sample with concentrated H₂SO₄ and a catalyst, followed by distillation

with water and 32% NaOH. The resulting distillate was titrated with 0.1 N HCl, and the volume of HCl used was recorded. Ash content was determined by incinerating the samples in a furnace at 550 °C for 5 h. For crude fibre analysis, pre-dried, defatted samples were sequentially digested by boiling with 0.13 M H₂SO₄ and 0.23 M NaOH, followed by washing with distilled water using a Fibrebag system for filtration. The digested samples were then ashed at 550 °C for 4 hours and weighed. Crude lipid (CL) was extracted using a modified Folch, Lees and Sloane Stanley (1957) method, where weighed samples were soaked overnight in a chloroform/methanol solution (2:1), homogenized, filtered, and emulsified with distilled water, allowing it to separate into two distinct layers: An upper water-rich layer and a lower lipid-rich chloroform layer. The CL was collected and dried at 40 °C until a constant weight was achieved. Finally, the nitrogen-free extract (NFE) was calculated after the proximate analysis using the formula: NFE = % dry matter – (% ash + % CP + % CL + % crude fiber) (Teoh & Loo 2022).

STATISTICAL ANALYSIS

All data (growth, survival rate, FCR, and proximate composition) were checked for normality using the Shapiro-Wilk test and then analyzed using one-way ANOVA in SPSS 20.0 (SPSS Inc., Chicago, IL, USA). When significant differences were detected, post hoc Duncan's Multiple Range Test was applied for mean comparisons between treatments. All of the statistical probability values were considered significant at $P < 0.05$, and the results are presented as mean ± standard error.

RESULTS AND DISCUSSION

GROWTH PERFORMANCE AND SURVIVAL OF PRAWN

After the 17-week feeding trial, the prawns exhibited weight gain ranging from 421.88% to 529.34% (Table 1), with no significant differences observed among the treatment groups ($P > 0.05$). Prawns fed with CPF+PMW and CPF+MW showed weight gains of 529.34% ± 54.37 and 478.84% ± 28.42, respectively, indicating that a combined diet of CPF and live mealworms could serve as a feeding strategy to reduce reliance on aquafeeds. The addition of probiotics may further enhance growth performance. Choi et al. (2018) found that replacing 50% of dietary fishmeal with mealworms optimally promoted shrimp growth without adverse effects. The use of live mealworms in the present study may account for the differing results compared to studies that used mealworm meal. In another study where the bacterial strain is directly fed, Kolanchinathan et al. (2017) reported growth improvements in tiger shrimp after a 15-day feeding period with live cells of *Bacillus firmus* at a concentration of 3.01×10^9 CFU/g. The administration of probiotics, particularly *Bacillus* spp., has been suggested to

enhance growth and immune function in cultured species by secreting lipase, which stimulates the production of essential fatty acids (El-Dakar & Goher 2004; Gullian, Thompson & Rodriguez 2004). However, when the prawns were indirectly administered the bacterial strain, the lack of significant differences among treatments in this study was observed. This outcome is likely due to reduced bacterial delivery and uptake through indirect administration, leading to suboptimal colonization and immune interaction (Hoseinifar et al. 2024). Lower bacterial concentrations, environmental factors like waterborne dispersion, and reduced bacterial adhesion to the prawn's gut may have limited the probiotic effects, resulting in minimal differences among treatment groups (El-Saadony et al. 2022; Kulkarni et al. 2021). In addition, the survival rate of the experimental prawns ranged from 75.00% to 85.00% (Table 1), with relatively higher survival observed in prawns fed live mealworms, regardless of probiotic enrichment. Mortality was primarily attributed to aggressive and cannibalistic behavior. Due to their territorial nature, GFP often attack each other, especially targeting prawns that have just molted, resulting in injuries that frequently lead to death (Seidel, Schaefer & Donaldson 2007). This suggests that partially replacing CPF with live mealworms, whether probiotic-enriched or not, does not compromise and may even enhance the growth and survival performance of GFP.

WATER PARAMETERS OF EXPERIMENTAL TANKS

NO₂⁻ was not detected in all the tanks and there were no significant differences observed for DO, pH, NH₃ levels among the experimental tanks throughout the feeding trial (Table 2). The DO ranged from 7.34 - 7.45 mg/L and pH ranged from 7.39 to 7.44. The lowest NH₃ level (0.33 mg/L) was recorded in CPF+PMW tanks while the highest (0.50 mg/L) was detected in CPF, CPF+MW and MW tanks. This result suggests that probiotic enrichment could help to maintain a low NH₃ level in the culture tanks. Low NH₃ levels are crucial in prawn tanks, as prawns are highly

susceptible to ammonia toxicity, which can damage gills, weaken immunity, and increase mortality (Lin et al. 2022). High ammonia disrupts water quality, harms beneficial bacteria essential for waste breakdown, and triggers stress responses that hinder growth and molting (Romano & Zeng 2013). Maintaining low NH₃ supports a healthy tank environment, optimizes growth, and reduces the need for frequent water changes or chemical treatments, lowering operational costs. According to Kewcharoen and Srisapoom (2019), adding *B. subtilis* with a concentration of $1 \times 10^3 - 1 \times 10^5$ CFU/mL to water culturing whiteleg shrimp could reduce the total NH₃ content, and feeding the shrimp with *Artemia* enriched with microencapsulated *B. subtilis* (10^9 CFU/g) also decreased the concentration of NH₃ and NO₂⁻ in the culture water (Nimrat et al. 2012). Furthermore, NO₃⁻ level was significantly higher in the CPF tank (26.67 ± 6.07 mg/L) potentially due to nutrient leaching of the CPF and relatively more feces produced by prawns fed with CPF. Algae growth was observed in CPF tanks, which can disrupt the microbiome balance and lead to mortality due to asphyxiation (Irungu et al. 2018), which partly explain the relatively lower survival rate in those fed with CPF.

FEED CONVERSION RATIO

FCR is crucial in aquaculture as it measures feed utilization efficiency, directly affecting production costs and sustainability. A lower FCR indicates less feed is needed for growth, enhancing profitability and reducing waste (Besson et al. 2016). In this study, prawns fed with CPF had significantly ($P < 0.05$) higher FCR values (3.72 ± 0.32) compared to those on the other diets (Table 1). However, no significant differences in FCR were observed among prawns fed the other four diets ($P > 0.05$). Although the FCR values for prawns fed CPF+MW and CPF+PMW were slightly higher than those for prawns fed MW and PMW, all FCR values remained within the normal range of 1.8 to 3.1, as suggested by Paul and Rahman (2016).

TABLE 1. Growth performance, survival, total feed intake and feed conversion ratio of the experimental prawns after 17 weeks of feeding trial. Values are presented as mean \pm standard error of triplicate groups of experimental prawns. Different superscripts in the same row indicate significant differences by the test of $p < 0.05$ using Duncan's multiple range test

Feeding materials ¹	CPF	CPF+MW	MW	PMW	CPF+PMW
Total weight gain (%)	517.52 \pm 65.58	478.84 \pm 28.42	505.06 \pm 84.14	421.88 \pm 6.07	529.34 \pm 54.37
Survival (%)	75.00 \pm 2.89	85.00 \pm 5.77	78.33 \pm 4.41	81.67 \pm 1.67	80.00 \pm 2.89
Total feed consumption (g) (ww) ²	129.87 \pm 2.21 ^{ab}	124.67 \pm 3.47 ^a	161.77 \pm 9.53 ^c	150.90 \pm 3.31 ^{bc}	127.71 \pm 6.20 ^{ab}
Total feed consumption (g) (dw) ³	117.02 \pm 1.99 ^c	75.74 \pm 2.10 ^b	50.78 \pm 2.99 ^a	47.37 \pm 1.04 ^a	77.58 \pm 3.77 ^b
FCR	3.72 \pm 0.32 ^b	2.19 \pm 0.01 ^a	1.63 \pm 0.31 ^a	1.63 \pm 0.40 ^a	2.24 \pm 0.32 ^a

¹Feeding materials: CPF = commercial prawn pellet (control); CPF+MW = commercial prawn pellet + mealworm; MW = mealworm; PMW = probiotic mealworm; CPF+PMW = commercial prawn pellet + probiotic mealworm

²Total feed consumption was calculated in wet weight (ww)

³Total feed consumption was calculated in dry weight (dw)

The results were influenced by the prawns' feeding preferences, with a tendency to favour mealworms over commercial feed in the CPF+MW and CPF+PMW groups. The highest feed consumption in wet weight was recorded in prawns fed with MW. This preference aligns with the natural diet of wild prawns, which includes mollusks, oligochaetes, insects, and other crustaceans (Chakraborty 2017). Such inherent feeding behaviors, including capture and consumption methods, may significantly influence FCR by affecting feed utilization efficiency (Ahmed, Lodhi & Shukla 2021). In a study by Motte et al. (2019), replacing 50% of dietary fishmeal with mealworm meal improved the FCR of whiteleg shrimp from 1.59 to 1.20. Although previous research shows that direct probiotic supplementation improves prawn FCR (Kolanchinathan et al. 2022; Zokaeifar et al. 2012) and suggests that multiple probiotic strains enhance FCR and growth in penaeid shrimp, limited studies have explored their effects on *Macrobrachium* spp. (Toledo et al. 2019). In this study, probiotic enrichment of mealworms did not significantly impact prawn FCR, suggesting that the supplementation method may not have been optimal. Future research could examine higher probiotic concentrations, strain combinations, and extended feeding periods to optimize FCR and growth performance in GFP.

PROXIMATE COMPOSITION OF EXPERIMENTAL FEED MATERIAL

Significant differences were observed in the dry matter, ash, crude fiber, CP, and CL contents among the experimental feed materials used in this study (Table 3). The CPF had the lowest CP ($45.01\% \pm 0.48$) and CL ($14.62\% \pm 0.17$) contents, while MW showed the highest values, with CP at $52.44\% \pm 0.19$ and CL at $31.55\% \pm 0.59$. PMW exhibited a similar CL content to MW. The nutritional composition of mealworms is reflective of their food source; mealworms fed wheat bran displayed higher CP levels compared to those fed rye bran (Bordiean et al. 2022). Importantly, the type of dietary protein source often holds more significance than its quantity (Rumbos et al. 2020). In this study, while

the CP concentrations differed between MW ($52.44\% \pm 0.19$) and PMW ($49.63\% \pm 0.52$), both diets maintained similarly high CL contents ($31.55\% \pm 0.59$ and $32.50\% \pm 0.33$, respectively), which were significantly higher than the other three diets. This suggests that the CL content in mealworms remains consistent even with probiotic enrichment. Dreassi et al. (2017) also found that feeding mealworms different food, such as wheat bran, rye bran, and rapeseed meal, resulted in similar CL content in the larvae. The ash content was approximately $9.28\% \pm 0.42 - 9.67\% \pm 0.62$ in CPF+MW, MW, and PMW, while CPF had the highest ash percentage at $14.70\% \pm 1.78$. Ash content in the feed represents inorganic compounds like calcium, essential for prawn shell formation during molting. Sufficient calcium ensures prawns receive the nutrients needed for successful molting and healthy growth (Khalid et al. 2023). Additionally, CPF+PMW had the highest crude fiber percentage ($7.04\% \pm 0.67$) among the five feed treatments.

PROXIMATE COMPOSITION OF PRAWN

The ash content ($3.32-4.87\%$) and crude fiber ($7.03-8.91\%$) were not significantly different across the experimental prawns (Table 3). However, prawns fed with CPF+MW exhibited the lowest dry matter content ($20.36\% \pm 4.83$), which was significantly lower than that in prawns fed with CPF+PMW ($26.96\% \pm 3.72$; $P < 0.05$). The dry matter content in prawns fed with CPF, MW, and PMW showed no significant differences ($22.09\% \pm 0.96$, $22.27\% \pm 2.79$, and $21.17\% \pm 1.71$, respectively). Notably, prawns fed with MW ($60.78\% \pm 1.05$) and CPF ($59.24\% \pm 1.98$) had significantly higher CP content. Interestingly, despite CPF having the lowest CP among the diets, prawns fed with CPF showed relatively high CP content in their bodies. A commercial feed specifically formulated to meet the nutritional requirements of prawns, including all ten essential amino acids (EAAs) such as methionine and lysine, which are deficient in mealworms (Nunes et al. 2014). Although mealworms lack methionine, supplementation of this EAA is only necessary when dietary protein levels

TABLE 2. Water parameters of experimental tanks throughout the feeding trial. Values are presented as mean \pm standard error of triplicates groups of experimental prawns. Different superscripts in the same row indicate significant differences by the test of $p < 0.05$ using Duncan's multiple range test

Feeding materials ¹	CPF	CPF+MW	MW	PMW	CPF+PMW
DO (mg/L)	7.45 ± 0.45	7.43 ± 0.04	7.37 ± 0.05	7.35 ± 0.04	7.34 ± 0.02
pH	7.44 ± 0.08	7.42 ± 0.07	7.43 ± 0.03	7.39 ± 0.03	7.43 ± 0.05
Ammonia (mg/L)	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.42 ± 0.08	0.33 ± 0.08
Nitrite (mg/L)	0.00	0.00	0.00	0.00	ND
Nitrate (mg/L)	26.67 ± 6.07^b	10.00 ± 0.00^a	5.00 ± 0.00^a	6.67 ± 1.67^a	10.00 ± 0.00^a

¹Feeding materials: CPF = commercial prawn pellet (control); CPF+MW = commercial prawn pellet + mealworm; MW = mealworm; PMW = probiotic mealworm; CPF+PMW = Commercial prawn pellet + probiotic mealworm

fall below 20–30% (Motte et al. 2019). Specifically, GFP requires all ten EAAs, though their precise proportions remain unclear (Sarman et al. 2018). Furthermore, the highest CL content was observed in prawns fed with CPF (14.19% ± 3.58), which was significantly higher than those fed with MW (7.90% ± 4.76), PMW (6.89% ± 1.54), and CPF+MW (3.99% ± 0.55), but not significantly different from those fed with CPF+PMW (11.09% ± 2.22). The CL content in prawns did not correspond to that of the experimental diets (MW, PMW, and CPF+MW), indicating that the dietary fatty acid composition may influence CL digestibility in prawns (Glencross et al. 2002). This inconsistency may stem from selective absorption or metabolism of specific fatty acids influenced by the prawn's physiological needs (Kumar et al. 2018). Studies show that prawns may preferentially retain certain lipids to maintain membrane fluidity, support growth, or adapt to stress (Tocher & Glencross 2015). Additionally, interactions between dietary lipids and digestive enzymes affect fatty acid absorption (Xie et al. 2020), and species-specific metabolic pathways may adapt to different lipid sources (Chen et al. 2015; Ding et al. 2022). Additionally, a previous study on tiger shrimp demonstrated that dietary phospholipids could significantly enhance CL digestibility and emulsification of neutral lipids (Glencross, Smith

& Williams 1998). It is suggested that adding dietary phospholipids could improve CL digestibility in prawns, enhancing lipid metabolism, nutrient absorption, and growth. Future research on this approach may optimize prawn diets, improving feed utilization efficiency and supporting sustainable aquaculture.

EXPERIMENTAL FEED PRODUCTION COST

In this study, PMW feed was the most expensive at RM53.09 per kg, followed by mixed feeds of CPF+PMW at RM23.26 per kg and CPF+MW at RM6.34 per kg (Table 4). Panini et al. (2017) highlighted the high initial cost of mealworms compared to commercial prawn feeds but noted that large-scale mealworm farming could reduce costs over time. Grau, Vilcinskis and Joop (2017) supported this, showing that economies of scale and the use of organic waste as feed make mealworm production more viable as scale increases. Though costly for small-scale prawn farms, mealworm farming has sustainability benefits, converting waste into high-protein feed and reducing environmental impact (Colombo et al. 2023; Ooninx et al. 2015). On-site mealworm production can cut transportation costs and carbon emissions, offering a sustainable feed option for aquaculture (Rumbos et al. 2020).

TABLE 3. Proximate composition of experimental feed materials and the prawns after 17 weeks of feeding trial. Values are presented as mean ± standard error of triplicate of samples. Different superscripts in the same row indicate significant differences by the test of $p < 0.05$ using Duncan's multiple range test

Feeding materials ¹	CPF	CPF+MW	MW	PMW	CPF+PMW
<i>Feed</i>					
Dry matter (%)	90.11 ± 0.47 ^c	57.99 ± 0.33 ^c	32.24 ± 0.43 ^a	41.26 ± 1.24 ^b	64.62 ± 1.16 ^d
Ash (%)	14.70 ± 1.78 ^b	9.67 ± 0.62 ^a	9.28 ± 0.42 ^a	9.38 ± 0.35 ^a	12.57 ± 1.36 ^{ab}
Fibre (%)	2.27 ± 0.30 ^a	3.32 ± 0.12 ^a	4.03 ± 0.72 ^a	2.70 ± 0.63 ^a	7.04 ± 0.67 ^b
Crude protein (%)	45.01 ± 0.48 ^a	47.01 ± 0.24 ^b	52.44 ± 0.19 ^d	49.63 ± 0.52 ^c	47.44 ± 0.70 ^b
Lipid (%)	14.62 ± 0.17 ^a	27.29 ± 2.39 ^c	31.55 ± 0.59 ^d	32.50 ± 0.33 ^d	19.78 ± 0.21 ^b
NFE ² (%)	23.40	12.71	2.70	5.79	13.17
<i>Prawn</i>					
Dry matter (%)	22.09 ± 0.96 ^{ab}	20.36 ± 4.83 ^a	22.27 ± 2.79 ^{ab}	21.17 ± 1.71 ^{ab}	26.96 ± 3.72 ^b
Ash (%)	4.97 ± 0.94	3.32 ± 1.01	3.57 ± 0.92	3.84 ± 1.52	4.67 ± 1.46
Fibre (%)	7.03 ± 1.28	7.93 ± 1.70	7.79 ± 1.30	8.21 ± 1.40	8.91 ± 3.00
Crude protein (%)	59.24 ± 1.98 ^b	48.93 ± 1.75 ^a	60.78 ± 1.05 ^b	50.40 ± 1.25 ^a	50.12 ± 1.28 ^a
Lipid (%)	14.19 ± 3.58 ^c	3.99 ± 0.55 ^a	7.90 ± 4.76 ^{ab}	6.89 ± 1.54 ^{ab}	11.09 ± 2.22 ^{bc}
NFE ³ (%)	14.57	35.83	19.96	30.66	25.21

¹Feeding materials: CPF = commercial prawn pellet (control); CPF+MW = commercial prawn pellet + mealworm; MW = mealworm; PMW = probiotic mealworm; CPF+PMW = Commercial prawn pellet + probiotic mealworm

²Nitrogen free extract (NFE) = 100 – (crude protein + crude lipid + crude fibre + ash)

TABLE 4. Cost (RM/kg) of experimental feed for giant freshwater prawn¹

Feeding materials ²	CPF	CPF+MW	MW	PMW	CPF+PMW
Component					
Mealworm ³	Nil	5.30	13.75	12.83	5.43
Wheat bran ⁴	Nil	0.42	1.09	1.02	0.43
Probiotic powder ⁵	Nil	Nil	Nil	39.24	16.80
Commercial prawn feed ⁶	1.30	0.62	Nil	Nil	0.64
Total ⁷	1.30	6.34	14.84	53.09	23.26

¹Total cost of individual feed used was calculated for the 17 weeks of feed trial

²Feeding materials: CPF = commercial prawn pellet (control); CPF+MW = commercial prawn pellet + mealworm; MW = mealworm; PMW = probiotic mealworm; CPF+PMW = Commercial prawn pellet + probiotic mealworm

³Mealworm cost (RM85/kg) was calculated, the total amount of mealworm used as feed in ww (kg) × purchase price (RM/kg)

⁴Wheat bran cost RM4.50/kg, an equal amount of wheat bran was used as the substrate for MW and PMW. For 1 g of mealworm in ww, 1.5 g of wheat bran was used approximately

⁵Probiotic cost (RM120/kg) was calculated, amount of probiotic powder used in PMW or CPF+PMW in ww (kg) × purchase price (RM/kg)

⁶Commercial prawn feed (RM 10/kg) was calculated as the amount of CPF used as feed in ww (kg) × purchase price (RM/kg)

⁷Total cost of feed was calculated based on total feed consumption of prawns from experimental groups

The feed/ hydration agents cost for mealworms was not included in the calculation because the products used were collected from the local market free of charge

CONCLUSION

The study demonstrated that using live mealworms as an alternative to commercial feed for GFP did not compromise prawn growth performance or survival. Notably, feeding prawns with live mealworms significantly improved their FCR and enhanced water quality, particularly by reducing nitrate concentration. The CL and CP content in the prawns did not align with that of the experimental feeds (MW, PMW, and CPF+MW), suggesting that nutrient digestibility is more influenced by fatty acid and amino acid compositions than by CL and CP content. This highlights the need to optimize fatty acid and amino acid profiles in future feed formulations to enhance nutrient absorption and utilization in prawns. However, enriching live mealworms with probiotics did not yield significant improvements in prawn growth performance, survival, or nutritional value, despite the higher costs compared to commercial feed and live mealworms alone. Therefore, the study concludes that a combination of live mealworms and commercial feed is a viable feeding strategy for GFP, while probiotic enrichment of mealworms may not offer substantial benefits to prawn productivity. Adopting live mealworms alongside commercial feeds can make prawn farming more sustainable and cost-effective, improving feed efficiency while reducing costs and supporting optimal growth.

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