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Potential Use of Live Mealworm as A Sustainable Feed to Improve Productivity of the Giant Freshwater Prawn, *Macrobrachium rosenbergii*

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A 63 days feeding trial was conducted to evaluate the effect of live mealworm feeding on the productivity of giant freshwater prawn (GFP), *Macrobrachium rosenbergii*. Three dietary treatments were used in this study: Treatment 1 (T1), commercial prawn feed (control); Treatment 2 (T2), combination of commercial prawn feed and live mealworm; Treatment 3 (T3), live mealworm, were fed to randomly assigned duplicate groups of the GFP. Results showed that GFP in T3 recorded the highest body weight gain (295%), followed by those with T2 and the lowest in T1, despite not significantly different ($p>0.05$). GFP fed with T1 (88%) showed lower survival rate than those fed with T2 and T3 which may be ascribed to the inferior water quality. Besides, T3 resulted in better feed conversion ratio (1.66) despite no significant difference was observed. Noteworthy, the feeding cost of T1 (RM0.018 g of prawn^{-1}) was significantly lower as compared to T2 and T3, but live mealworm feeding could be more cost-effective for longer culture period. Therefore, live mealworm could be a potential alternative to commercial prawn feed for GFP farming.

Keywords: Giant freshwater prawn; *Macrobrachium rosenbergii*; Live mealworm; Sustainable feed; *Tenebrio molitor*

I. INTRODUCTION

Aquaculture is the fastest growing global food production industry. The giant freshwater prawn (GFP), *Macrobrachium rosenbergii*, is becoming an increasingly important aquaculture species throughout the tropics (Maliwat *et al.*, 2017). This species is popular in Asia for its good taste and ability to be integrated with farms such rice or fish production, as well as being an alternative to marine shrimp production that have been affected by various diseases (Kim *et al.*, 2013). GFP is indigenous to southeast Asian countries and it is commonly farmed in small scale and semi-intensive in countries such as Malaysia (Banu & Christianus, 2016).

This is largely due to the inherent nature of the species mainly the cannibalistic behaviour, longer larval development and slower growth rate as compared to the marine prawns (New *et al.*, 2009). It has been reported that feeding costs represent the major operational costs of a modern prawn farm, any savings in feed costs can translate to substantial savings for the industry (Kim *et. al.*, 2013; Kutty, 2005), therefore the development of a sustainable and health promoting feed for giant freshwater prawn is imperative.

On the other hand, large amounts of food and non-profitable side streams from industrial processes are currently wasted (Pillay, 2018), but this could be used as feed

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for insects, which can convert diverse waste streams into protein. Edible insects are therefore gaining attention among the research community (Thévenot *et al.*, 2018). The nutritional components of mealworms can be classified as "high in" and "source of" according to the thresholds for World Health Organisation and Food and Agriculture Organisation of the United Nations food labels (Grau *et al.*, 2017). In the past two years, several studies have shown that mealworm meal, to a certain extent, can be utilised as fishmeal alternative for marine shrimps and fish (Azagoh *et. al.*, 2016; Choi *et. al.*, 2018; Iaconisi *et. al.*, 2018; Ido *et. al.*, 2019; Panini *et. al.*, 2017a; Tan *et al.*, 2018).

Despite various nutrition studies and farming technologies on GFP have been carried out (Ballester *et. al.*, 2017; Banu & Christianus, 2016; Hosain *et. al.*, 2021; Miao *et al.*, 2017), yet the data surrounding the live mealworm feeding for optimising the overall productivity of GFP lacks clarity at best. This study was aimed to investigate the potential use of live mealworm feeding for GFP, and evaluate its effect on the growth performance, feed utilisation, survival rate and feeding cost of the prawns.

II. MATERIALS AND METHODS

The authors did not need an ethical approval for research project involving the use of invertebrate animals from the institute, University Tunku Abdul Rahman (UTAR).

A. Mealworm Culture

In this study, the mealworm was bred, cultured and used as diet for GFP. For starter, 1 kg of live mealworm larvae were bought from the local pet shop, these mealworm larvae were cultured and fed with oatmeal, vegetable stem, and fruit peel throughout the culture period until the mealworms metamorphosed into beetles. The beetles were then collected and placed in a breeding tray to breed and allowed to begin another life cycle. The mealworm larvae with body length of approximately 2 cm were harvested and weighed before use in the feeding trial of GFP.

B. Dietary Treatment

Three dietary treatments were used in the feeding trial of GFP: Treatment 1 (T1) (control), commercial prawn feed (CP BLANCA 7703, 36% protein); Treatment 2 (T2), combination of live mealworm and commercial prawn feed whereby live mealworm was fed in the morning session, while commercial prawn feed was fed in the evening session; Treatment 3 (T3), live mealworm. The live mealworm was harvested from own culture.

C. Experiment Tank Setup

Six experimental tanks [16 (H) × 44 (W) × 56 (H) cm] with 0.25 m^2 area each were prepared and equipped with sponge filter to provide aeration and filtration. All experiment tanks were filled with an equal amount of dechlorinated water and added with one teaspoon of calcium carbonate powder to increase the water hardness. Net and polyvinyl chloride (PVC) pipes were provided into each tank as shelter for the postlarvae (PL) to hide. All experiment tanks were covered with net to prevent the PL from escape.

D. Post-larvae Nursery Tank Setup

A 75 L plastic container was used as the nursery tank, filled up with dechlorinated water up to 70% tank volume and provided with aeration, and two teaspoons of calcium carbonate powder. Around 300 juveniles were collected from a local hatchery centre (Pusat Penetasan Udang Galah) at Kg. Acheh, Perak, Malaysia (GPS coordinate: 4°14'44"N, 100°39'28"E) and kept in the nursery tank upon arrival at the experimental site. The juveniles were then nursed for 45 days by feeding them with commercial prawn feed before the commencement of the feeding trial.

E. Prawn and Experimental Condition

After the nursing period of 45 days, 24 similar size and healthy GFP with an initial mean weight of 1.33 ± 0.07 g and initial mean length of 5.20 ± 0.06 cm were randomly selected, and distributed into a series of six experimental tanks. Each of the dietary treatments was randomly assigned to duplicate tanks of GFP for 63 days. Initial sampling was carried out by batch weighing all the GFP of each experimental tank. The GFP were fed twice daily, morning at 9 am and evening at 4 pm, until apparent satiation. The feed consumption was recorded daily.

F. Water Quality Monitoring

Throughout 63 days of feeding trial, the water parameter such as pH, carbonate hardness, ammonia (NH_3), nitrite (NO_2^-) and nitrite (NO_3^-) was measured with API® Freshwater Master Test on weekly basis to ensure a constant water quality (pH ranged from 7.0 to 8.5; carbonate hardness ranged from 30 to 150 ppm; ammonia below 0.3 ppm; nitrite below 2.0 ppm; nitrate below 10.0 ppm) for all the experimental tanks. Water was topped off to the fixed level (70% of the tank volume) and water change was performed once necessary.

G. Final Sampling and Proximate Analysis

At the termination of the feeding trial, all prawns were individually weighed and their total length were measured. While the prawn growth performance was analysed in regard to its weight gain, length gain and specific growth rate (SGR). The feed utilisation efficiency was determined by feed conversion ratio (FCR). The feeding cost was calculated on 1 g of GFP weight gain. The abovementioned parameters were measured based on the formulae below:

$$\text{Weight gain (\%)} = \frac{[\text{Final mean weight} - \text{Initial mean weight}](\text{g})}{\text{Initial mean weight (g)}} \times 100$$

$$\text{SGR(\%/day)} = \frac{[\ln(\text{final mean weight}) - \ln(\text{initial mean weight})](\text{g})}{\text{Days of culturing trial}} \times 100$$

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

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

$$\text{Feeding cost}\left(\frac{\text{RM}}{\text{g}}\text{ of prawn}\right) = \frac{\text{Feed given (g)} \times \text{cost of feed (RM/g)}}{\text{Wet weight gain (g)}}$$

whereby, cost of commercial pellet = RM0.0507 g^{-1}; cost of starter mealworm = RM0.0104 g^{-1}

The proximate composition (moisture, ash, crude protein and crude fibre) of the experimental diets were determined using standard AOAC methods (AOAC, 1997). The crude lipid of the experimental diets was determined with modified Folch *et al.* (1957) protocol, whereby the lipid was extracted from the dried sample with chloroform: methanol at a ratio of 2:1 (v/v). After the extraction, the mixture from the bottom layer of the separating funnel containing chloroform and extracted lipid was collected. The extracted lipid was obtained from the mixture by removing the chloroform with oven (37°C) until all the solutions is completely evaporated. The nitrogen-free extract (NFE) of the sample was calculated by subtracting the composition of ash, protein, lipid and fibre with the composition of dry matter.

H. Statistical Analysis

All data obtained were presented as mean ± standard error (SE), and subjected to Shapiro-Wilk test for normality test and a one-way analysis of variance (ANOVA) using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) to determine if there is significant difference occurred among the different dietary treatments. The means of the results were compared using Duncan's multiple range test and the treatment effects were considered at $p<0.05$.

III. RESULTS AND DISCUSSION

A. Growth Performance and Survival

In the present study, no significant differences ($p>0.05$) were observed among all the dietary treatments (Table 1) in regard to their growth parameters and survival rate. However, the percentage of body weight gain of GFP fed with T3 (295.94%) was the numerically highest, followed by those fed with T2 (272.57%) and T1 (265.83%). Likewise, the highest percentage of length gain was also observed in the groups fed with T3 (50.29%), followed by those fed with T2 (37.14%), and T1 (36.65%). Furthermore, the highest SGR was recorded in GFP fed with T3 (2.18% day^{-1}), followed by those fed with T2 (2.09% day^{-1}) and T1 (2.06% day^{-1}). Besides, GFP fed with

T1 had a relatively lower survival rate (87.50%), while no mortality was recorded in those fed with T2 or T3.

GFP fed with Diet B and Diet C, which comprised of live mealworm, had relatively higher body weight gain, length gain and SGR than those fed with Diet A, suggesting that live mealworm feeding is a viable feeding method as the growth performance and survival rate of prawn were not compromised. Similarly, a previous study on African catfish by Ng *et al.* (2001) showed that feeding with a combination of live mealworm and commercial pellet did not affect the fish growth performance. In the present study, all the dietary treatments met the protein requirement (35%) of GFP, and T3 had the significantly highest crude protein (40.18%), crude lipid (38.11%) and crude fibre (11.03%) contents among all the dietary treatments which contributed to the better growth performance of GFP in this study. Mealworms have been reported to be high in nutritional value and efficient in converting organic waste material into novel protein and lipid sources which could reach 49.1% and 35.2% in dry basis, respectively (van Huis, 2013). Koshio *et al.* (1993) showed that increase in dietary protein increased the weight gain of kuruma prawn (*Penaeus japonicus*). However, excess dietary protein may cause more waste which lead to water deterioration and increase the production cost (Teshima *et al.*, 2006). According to Panini *et al.* (2017a), the high lipid content in mealworm is due to the lipid reservation during the larva stage for energy source prior to its metamorphosis into adult beetle. From the study on the dietary lipid level of the oriental river prawn, *M. nipponense* by Li *et al.* (2020), the increase of dietary lipid content from 5.02% to 8.89% resulted in higher weight gain, SGR, and protein retention as well as protein efficiency in the early-stage of oriental river prawn.

Table 1. Growth performance of GFP fed with different dietary treatments*

	Dietary treatment†		
	T1	**T2**	**T3**
Total weight (g)	20.21 ± 0.68	18.90 ± 0.42	21.06 ± 2.06
Weight gain (%)	265.83 ± 11.93	272.57 ± 4.57	295.94 ± 18.96
Final length (cm)	7.23 ± 0.10	7.06 ± 0.14	7.76 ± 0.39
Length gain (%)	36.65 ± 2.21	37.14 ± 0.01	50.29 ± 5.69
SGR‡ (% $days^{-1}$)	2.06 ± 0.05	2.09 ± 0.02	2.18 ± 0.08
Survival (%)	87.50 ± 12.50	100.00 ± 0.00	100.00 ± 0.00

*Values are means ± SE of duplicate groups of prawn (n=2). No significant difference was detected among the dietary treatment.
†T1 = Commercial prawn pellet (control); T2 = Commercial prawn pellet + live mealworm; T3 = Live mealworm.
‡SGR = Specific growth rate

B. Feed Conversion Ratio and Feeding Cost

The lowest FCR was observed in prawn fed with T3 (1.67 ± 0.08), followed by those fed with T2 (1.72 ± 0.00) and T1 (1.76 ± 0.00), although no significant difference was recorded across all the dietary treatments (Table 2). In other words, the GFP fed with full live mealworm (T3) tends to improve its FCR. To a certain extent, this is in the agreement with a previous study (Panini *et al.*, 2017a) whereby mealworm meal-diet increased the feed utilisation efficiency of the whiteleg shrimp. Furthermore, Choi *et al.* (2018), Motte *et al.* (2019), and Panini 2017a; 2017b reported that mealworm meal-diets resulted in a relatively high apparent digestibility coefficient (ADC) of crude protein (76.1%) and essential amino acids (72 - 86%) in the whiteleg shrimp. It is, thus, suggested that full live mealworm feeding did not negatively affect the feed intake but in turn improved the feed utilisation efficiency of GFP and this needs further investigation.

Yet, significant difference was noted in feeding cost in the present study whereby the lowest feeding cost was recorded in T1 (RM0.018 g of $prawn^{-1}$) followed by T2 (RM0.057 g of prawn-1) and the highest feeding cost was recorded in T3 (RM0.084 g of prawn-1) (Table 2), indicating that feeding the GFP with commercial feed was the cheapest among all the diets. It is true that insect production is usually labour-intensive and time-consuming, and thus more expensive than common meat products, yet the production cost can be reduced with large scale or long-term production with automation and controlled production systems (IPIFF, 2018; Thévenot *et. al.*, 2018; van Huis *et al.*, 2013). While technological improvements are required to produce a consistently high-quality insect, the use of live mealworm feeding is expected to be more cost-effective compared to commercial prawn pellet in the long run.

C. Proximate Composition of Experimental Diets

In regard to proximate composition, significant differences were noted among all the dietary treatments. T1 had the highest dry matter content (94.34%) which was significantly higher than T2 (67.77%) and T3 (41.21%). Likewise, T1 contained the highest ash and nitrogen free extract (NFE), followed by T2 and T3, whereas T3 comprised of the highest crude protein (40.18%), crude lipid (38.11%), and crude fibre contents (11.03%). The crude protein, lipid, and fibre contents in T1 were the lowest among the experimental diets with the values of 35.87%, 5.60%, and 2.69%, respectively. T2 was in the middle range between T1 and T3 for each of the proximate compositions (Table 3).

Table 2. FCR and feeding cost of GFP after 63 days of feeding trial*

	Dietary treatment†		
	T1	**T2**	**T3**
FCR	1.76 ± 0.00	1.72 ± 0.00	1.67 ± 0.08
Feeding cost (RM g of prawn-1)	0.0183 ± 0.0005^a	0.0572 ± 0.0087^b	0.0845 ± 0.0039^c

* Values are means ± SE of duplicate groups of prawn (n=2). Different superscripts indicate significant differences at p<0.05.
† See Table 1 footnote.

Table 3. Proximate composition of experimental diet*

Nutrient composition (%)	**Dietary treatment†**		
	T1	**T2**	**T3**
Dry matter	94.34 ± 0.07^c	67.77 ± 0.02^b	41.21 ± 0.12^a
Ash	8.82 ± 0.04^c	6.03± 0.08^b	3.24 ± 0.15^a
Crude protein	35.87 ± 0.11^a	38.02 ± 0.26^b	40.18 ± 0.62^c
Crude lipid	5.60 ± 0.24^a	21.85 ± 0.24^b	38.11 ± 0.25^c
Crude fibre	2.69 ± 0.06^a	6.86 ± 0.20^b	11.03 ± 0.34^c
NFE‡	47.03	27.23	7.44

*Values are means ± SE of duplicate groups of prawn (n=2), NFE of diets was excluded in statistical analysis. Different superscripts in the same row indicate significant differences at p<0.05. With the exception for moisture content, all the proximate composition was expressed in dry matter basis.
†See Table 1 footnote
‡NFE = Nitrogen free extract

D. Water Quality

In the present study, the lowest ammonia level was observed in the tank given with T3 (mean value of 0 ppm), followed by T2 ranged from 0 – 0.5 ppm, while the highest ammonia level was observed in the tank given with T1 which fluctuated dramatically from 0 – 1.0 ppm throughout the experiment. Both nitrite and nitrate levels of tanks given T2 and T3 were constant at 0 ppm, while for T1, the nitrate and nitrite levels were fluctuated dramatically from 0 – 0.38 ppm and 0 – 2.5 ppm, respectively throughout the experiment. As such, the highest frequency of water remediation was recorded in tanks provided with T1 (8 times), which was significantly higher than T2 and T3 while no significant difference was observed between latter two treatment groups with the frequency of 2.5 times (Table 4).

Table 4. Water quality parameters and remediation frequency of the experimental tanks throughout 63 days of feeding trial*

	Dietary treatment†		
	T1	**T2**	**T3**
Total ammonia nitrogen (ppm)	0.45 ± 0.03^c	0.10 ± 0.00^b	0.00 ± 0.00^a
Nitrate, NO_3^-	0.75 ± 0.25^b	0.00 ± 0.00^a	0.00 ± 0.00^a
Nitrite, NO_2^-	0.12 ± 0.04^b	0.00 ± 0.00^a	0.00 ± 0.00^a
pH	7.60 ± 0.00	7.60 ± 0.00	7.60 ± 0.00
Water remediation frequency	8.00 ± 0.01^b	2.50 ± 0.01^a	2.50 ± 0.01^a

*Values are means ± SE of duplicate groups of prawn (n=2). Different superscripts in the same row indicate significant differences at $p<0.05$.
†See Table 1 footnote

As compared to commercial pellet, live mealworm feeding could lessen water deterioration as the significantly highest frequency of water remediation was observed in tank provided with T1 due to the fluctuation and highest concentration of ammonia, nitrite, and nitrate. From the study of Smith *et al.* (2002), around 15% of nitrogen (N) leached out from the prawn pellet every 2 hours, around 1.9% of N lost in 10 minutes and the deposition of N increase the ammonium concentration in water. On the other hand, live mealworm has an impermeable exoskeleton or chitin which could act as a protective barrier to prevent loss or leaching of nutrients into the water environment (Andersen, 2009). Throughout the experiment, the pH level of all tanks was constant at 7.6, and this was contributed by the input of the calcium carbonate which increased the buffering capacity to prevent pH fluctuation (González-Vera & Brown, 2017). For GFP fed with T2 and T3, no mortality was recorded in the present study, indicating that the intake of mealworm could lessen water deterioration which then contribute to improved health status of GFP. This is in line with a recent study by Bordiean *et al.* (2020) whereby partial intake as high as 25% of mealworm in diet over 6 weeks period could help to develop anti-inflammatory and immune system of some small fish species. In addition, Kumar *et al.* (2015) suggested that increase of chitin (determined as crude fibre) concentration in GFP diet has improved the immune repose and survival rate of GFP toward the white muscle disease.

IV. CONCLUSION

In brief, live mealworm feeding exerted some beneficial effects for GFP farming as it tended to improve the growth performance and the FCR of the GFP, while the survival rate of GFP was not compromised and water quality were significantly improved. However, the cost of live mealworm feeding was higher as compared to commercial prawn feed in the present study, which was a short-term culture. It is speculated that live mealworm feeding could be more cost-effective for a large scale and long-term application. In light of the above, live mealworm larvae, which can be cultured with food waste or agricultural by-products, could be used as a potential alternative to commercial prawn pellet for GFP and thus, leading to a more sustainable GFP farming.

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