

# IMPACT OF LIVE MEALWORM FEEDING AND PROBIOTIC ENRICHMENT ON THE HEALTH PERFORMANCE AND FATTY ACID COMPOSITION OF GIANT FRESHWATER PRAWN, *Macrobrachium rosenbergii*

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**Abstract:** The aquaculture industry is beset by rising feed costs and supply challenges, making the discovery of more sustainable feed sources essential. Insects, processed via entotechnology may be one possible solution to this problem while *Bacillus* probiotics pasta's potential to enhance species' health. This study investigates the effects of feeding probiotic-enriched live mealworms to giant freshwater prawns (*Macrobrachium rosenbergii*). A 17-week trial tested five feed types: Commercial Prawn Feed (CPF), CPF with live mealworm (CPF+MW), mealworm (MW), probiotic-enriched mealworm (PMW), and CPF with probiotic-enriched mealworm (CPF+PMW). GFP fed 50% or 100% live mealworms, with or without probiotics, showed similar monounsaturated fatty acid (MUFA) deposition, suggesting optimal MUFA retention. Arachidonic acid (ARA) levels in prawns exceeded that of the feed while live mealworm feeding did not impact docosahexaenoic acid (DHA) levels. These findings indicate selective retention of ARA and limited bioconversion of linoleic and alpha-linolenic acids into ARA and DHA. Additionally, MW and CPF+PMW-fed prawns had higher phenoloxidase activity, CPF+MW and PMW-fed prawns exhibited better survival in the nitrite stress test, suggesting improved immunity and stress tolerance. In conclusion, live mealworms are a viable feed alternative for GFP, supporting immune response and nutrient retention, though probiotic enrichment did not offer additional benefits under the conditions tested.

Keywords: *Bacillus subtilis*, *Macrobrachium rosenbergii*, probiotic enrichment, sustainability, *Tenebrio molitor*.

## Introduction

*Macrobrachium rosenbergii* or Giant Freshwater Prawns (GFP) are native to Southeast Asia and can command a market price ranging between RM68.00 and RM90.00 a kilogramme (kg) (Pillai *et al.*, 2022). In Malaysia, the aquaculture production of GFP has reached 189 tonnes, valued at RM15.5 million in 2023 (DOF, 2023). Although the hatchery technology of the giant freshwater prawn has been established at a commercial level since 1980, the dependence on live feed during the larval and post-larval stage causes the high feeding cost that highly impedes the commercial production of this species (Barros & Valenti, 2003).

Feeding costs are one of the main operating expenses of a modern farming system and the aquaculture industry is growing quickly due in part to the widespread use of aquafeed; any reduction in feed prices can result in significant savings for the sector (Kutty, 2005; Kim *et al.*, 2013). To ensure the sustainability of aquaculture, it is imperative to investigate local alternative sources of protein and energy due to the unpredictability and rising costs of feed components (Rana *et al.*, 2009; Khanjani *et al.*, 2023). Plant-based protein meals and oils have been identified as substitutes for fishmeal and fish oil but there are setbacks in terms of land

use, anti-nutritional factors, and deforestation (Sales & Glencross, 2011; Sabaté & Soret, 2014; Teoh & Ng, 2016). Recently, the use of insects as a more sustainable alternative source of protein has drawn great interest in the scientific world (van Huis & Oonincx, 2017; Hua *et al.*, 2019) and insects have been proven to be feasible alternatives for aquafeed (Chemello *et al.*, 2020; Oonincx *et al.*, 2020; Gałęcki *et al.*, 2021; Murawska *et al.*, 2021; Chong *et al.*, 2022), especially for carnivorous species (Sharifinia *et al.*, 2023a; 2023b).

Entotechnology has been recently reported to be a sustainable way to process organic waste and produce food for humans, animals, or fish. Insects have been reported to have the ability to reduce faecal coliform and Salmonella germs, as well as organic waste by mean between 50% and 75%, depending on the species and type of waste (Johnston, 2017). In terms of cultivation, mealworm production requires little management and less laborious skills (Tan *et al.*, 2018). Mealworms are nutritionally rich and versatile insect sources that are prized for having higher protein and fat levels than earthworms (Parolini *et al.*, 2020) and black soldier fly larvae (Barragan-Fonseca *et al.*, 2023).

The nutrient composition of mealworms can be influenced by factors such as life stage, environment, substrate, and diet (Siemianowska *et al.*, 2013; Finke, 2015; Khosravi *et al.*, 2018; Jeong *et al.*, 2022). Specifically, the larvae of the yellow mealworm (*Tenebrio molitor*) are one of the most raised insect species for food purposes and can break down and consume various agricultural and food industry materials (Bordiean *et al.*, 2020). Manipulating their diet and substrate allows for the customisation of the nutritional content, making them a valuable resource for sustainable feed in various industries (Ramos-Elorduy *et al.*, 2002; Oonincx *et al.*, 2015; Berezina, 2017; Mancini *et al.*, 2019).

Probiotic bacteria added to the food is one method that has been driven by EU regulations on the prophylactic use of antibiotics to prevent losses from disease in the aquaculture industry (Thakur *et al.*, 2016). Probiotics from the

*Bacillus* strain have been shown to benefit freshwater fish and crustaceans by preventing diseases and improving feed utilisation efficiency, growth performances, and water parameters (Hai, 2015; Nathanailides *et al.*, 2021).

These naturally occurring bacteria exert their beneficial effects on the host by modifying the intestinal microbiota, secreting antibacterial substances, competing with pathogens to prevent their adhesion to the intestine, competing for nutrients necessary for pathogen survival, and producing an antitoxin effect (Cruz *et al.*, 2012). Yet, there are no scientific studies on feeding live mealworms enriched with probiotics to GFP. This study investigates the potential of live mealworm feeding, with and without probiotic enrichment, as a sustainable feeding strategy and its effects on the health performance and Fatty Acid (FA) composition of GFP.

## Materials and Methods

The authors did not require ethical approval for a research project involving the use of invertebrate animals from the Universiti Tunku Abdul Rahman.

### Culture of Mealworm

In this study, mealworms were used as feed for GFP after being bred and cultured (modified from Bordiean *et al.*, 2022). Initially, 2 kg of live mealworms were purchased from a local aquarium shop in Kampar and distributed into four plastic containers (30 cm x 15 cm x 5 cm; W x L x H). The mealworms were then segregated into other same-size containers as the population increased to avoid overcrowding.

The mealworms were cultured under shaded conditions (light: dark; 12 h: 12 h) with food sources (potatoes, carrots, and lettuce) and moist cotton pads to maintain humidity. The larvae developed into several stages and were sorted to reduce cannibalism. Pupae and adult beetles were separated into another container containing wheat bran as substrate and hydration sources, including potatoes, carrots, and lettuce.

The reared mealworms that were approximately 2 cm in length were harvested and weighed before being used as feed material for the GFP feed trial. The mealworms were continuously reared until the last week of the GFP feeding trial.

### **Probiotic Enrichment of Mealworm**

The probiotic enrichment was carried out based on the modified protocol of Dadvar *et al.* (2023). A commercial probiotic, IG Guard A was purchased from ADBIOTECH Company Limited. The probiotic powder contained  $2.0 \times 10^{10}$  cfu  $\text{kg}^{-1}$  *B. subtilis*, 120 g  $\text{kg}^{-1}$  egg powder, and carrier distillers dried grain (wheat flour and corn flour) up to 1 kg. As shown in Table 1, starch was the predominant nutrient in the probiotic powder.

Then, groups of mealworms each weighing  $154 \pm 0.5$  mg were selected for *B. subtilis* enrichment. The selected mealworms were starved for two days and placed in a plastic container (1.73 cm x 1.18 cm x 0.56 cm) before being given 10 g of IG Guard A. The mealworms were kept in the container for three hours and their feeding patterns were observed to confirm the consumption of the probiotic powder.

The mealworms were then left in the container for 24 hours to allow for intake of the probiotic. The next day, the mealworms were harvested and fed to the experimental Post Larvae (PL) according to the feeding regime.

### **Culture of Giant Freshwater Prawn**

PL day 15 of GFP was obtained from Pusat Penetasan Udang Galah, Kg. Acheh, Malaysia

and acclimatised for seven days upon arrival at the Aquaculture Facilities, Universiti Tunku Abdul Rahman (4.245073886171705, 100.657248451251).

The PL were fed twice daily with a commercial prawn diet (Dindings Freshwater Prawn Feed 2203) to satiation during the conditioning. At the commencement of the feeding trial, groups of 20 PL with an initial body weight of  $0.45 \pm 0.2$  g (mean  $\pm$  SE) was randomly selected and distributed into each of the 15 tanks [159 L capacity; 18 inch (W) x 30 inch (L) x 18 inch (H)].

The PL were starved for 24 hours before being transferred to experimental tanks, which were equipped with a recirculating freshwater system and 20 Polyvinyl Chloride (PVC) pipes (diameter 25 mm; length 45 mm) were placed at the tank bottom to provide refuge for the PL.

Five experimental dietary treatments were used in the feeding trial: Commercial prawn feed (CPF, control), commercial prawn feed and live mealworms (CPF+MW), live mealworms (MW), live mealworms enriched with probiotics (PMW), and commercial prawn feed and live mealworms enriched with probiotics (CPF+PMW). CPF+MW and CPF+PMW were prepared in the ratio of 1 g of commercial feed to 1 g of mealworms or probiotic-enriched mealworms, respectively. All the experimental diets were weighed on a wet-weight basis and converted to dry weight for data calculation.

Each diet was randomly assigned to triplicate groups of prawns. The PL were fed three times daily at 09:00, 13:00, and 17:00 hour to apparent satiation (about 15% of body

Table 1: Nutrient content of probiotic powder from ADBIOTECH Co., Ltd.

<b>Composition</b>	<b>Value (%)</b>
Moisture	$\leq 28.0$
Crude protein	$\geq 5.0$
Crude ash	$\leq 3.0$
Crude fat	$\leq 7.0$
Crude fibre	$\leq 10.0$
Starch content	$\leq 50.0$

weight per day) for the initial weeks and then twice daily at 09:00 and 17:00 hours to apparent satiation (about 8% of body weight per day) by week 4.

Faeces were siphoned from each tank every day after the first feeding and all PL were batch-weighted weekly to monitor growth performance. The feeding trials were conducted for 17 weeks and total feed consumption was recorded daily.

### **Sample Collection**

After 17 weeks of feeding trial, the PL had grown to be approximately 10 cm long and were henceforth referred to as prawns. A final sampling was conducted for body weight measurement and various sample collections. Five prawns from each tank were euthanised and stored in a freezer (-20°C) for subsequent determination of whole-body FA composition. Then, another five prawns were collected for phenoloxidase activity (PO) assay. Finally, five prawns were left in their respective experimental tanks for subsequent nitrite stress tests.

### **Nitrite Stress Test**

After the feeding trial ended, five prawns were kept in their initial experimental tanks, which were added with 21 mg L<sup>-1</sup> of sodium nitrite for five days (modified from Li *et al.*, 2019). During the nitrite stress test, prawns were fed with their respective diets according to their designated tanks twice daily until apparent satiation. The concentration of nitrite in the culture water was monitored for five days. The percentage of mortality was recorded for each group up to 120 hours and survival was calculated.

### **Haemolymph Collection and Phenoloxidase Activity Assay**

PO activity of prawns was measured according to Liu and Chen (2004) with slight modifications. To obtain the haemolymph, syringes were coated with an anticoagulant (30 mM trisodium citrate, 0.34 M sodium chloride, pH 7.55, and glucose were added until 30% was reached), inserted in

the ventral sinus, and then 40 µL of haemolymph were added to 360 µL of the anticoagulant.

The collected haemolymph was then centrifuged at 700 x g for 20 minutes. The supernatant was removed and the pellets were gently re-suspended with 200 µL cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.1 M tri-sodium citrate, pH 7.0), centrifuged a second time, and re-suspended with 200 µL cacodylate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.01 M calcium chloride, 0.26 M magnesium chloride, pH 7.0).

Each aliquot (100 µL) was incubated with 50 µL trypsin (1 mg mL<sup>-1</sup>) for 30 minutes at room temperature, followed by adding 50 µL of L-3,4-dihydroxyphenylalanine (L-DOPA) (1 mg mL<sup>-1</sup>). Five minutes later, 800 µL cacodylate buffer was added and then measured at an optical density of 490 nm. Control (100 µL of cell suspension, 50 µL of cacodylate buffer, and 50 µL of L-DOPA) was used to correct for background interference, which replaced 50 µL trypsin for cacodylate buffer. PO activity (unit of optical density at 490 nm) was expressed as dopachrome formation in 40 µL of haemolymph.

### **Fatty Acid Analysis**

Total lipids were extracted from the samples using a modified method based on Folch *et al.* (1957). The weighed samples were soaked overnight in a chloroform/methanol mixture (2:1, v/v), homogenised, and filtered. The filtrate was emulsified with distilled water and allowed to separate into two clear layers. The lipid layer was carefully collected and quantified after solvent evaporation using a rotary evaporator, followed by a stream of nitrogen gas to ensure the complete removal of residual solvents.

FAs were esterified into methyl esters using the saponification-esterification method (AOAC, 1990) with modification. FA Methyl Ester (FAME) was resolved and analysed by using gas-liquid chromatography (Shimadzu GC-2010) equipped with a flame ionisation detector and Shimadzu GC solution software.

The FAME was then separated on a BPX5 with a 5% Phenyl Polysilphenylene-siloxane column (30 m x 0.25 mm ID, 0.25 µm film thickness, SGE, GC column). The column temperature was set at 140°C for the first 2 minutes, increased to 225°C at 2°C min<sup>-1</sup>, then, increased to 240°C at 1°C min<sup>-1</sup> and held for 20 minutes.

An SPL-2010 injector was used and the split ratio was set as 1:100. Injector port and detector temperatures were 250°C. Helium was used as carrier gas. FAs were identified by comparing retention time with known standards (Supelco 37 Component FAME Mix, Bellafonte, PA, USA) (Ti *et al.*, 2019).

### Statistical Analysis

All of the data were subjected to the Shapiro-Wilk test for normality test and subjected to a one-way ANOVA using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). When a significant difference was observed, Duncan's Multiple Range test was used to compare means. Treatment effects were considered at  $P < 0.05$  level of significance and results were presented as mean  $\pm$  standard error.

### Results and Discussion

In the present study, feeding GFP live mealworms, with or without probiotic enrichment, effectively replaced commercial feed without significantly affecting growth performance. Detailed results on the effects of live mealworm feeding, including probiotic enrichment, on growth, feed utilisation, survival, and proximate composition on GFP will be described elsewhere.

Over the 17 weeks experimental period, prawns exhibited a more than 400% increase in body weight, with no significant differences observed (ranging from 421.88% to 529.34%). Feeding preference influenced the results, with prawns in the CPF+MW and CPF+PMW groups showing a tendency to prefer mealworms over commercial feed and the highest feed consumption in wet weight recorded in prawns fed with MW.

Probiotic enrichment did not significantly affect the Feed Conversion Ratio (FCR), which remained within the normal range (1.8-3.1) while CPF-fed prawns had the highest FCR ( $3.72 \pm 0.32$ ). The crude lipid content in prawns did not align with that of the experimental feeding materials, suggesting that dietary FA composition plays a role in influencing lipid digestibility in prawns (Glencross *et al.*, 2002).

### Nitrite Stress Test and PO Activity

In the present study, no significant difference was observed among all experimental prawns in nitrite stress test, although numerically, the highest survival after nitrite stress test was recorded in PMW-fed prawns (73.33%) (Table 2). There is limited study on the effect of nitrite toxicity on GFP. Meanwhile, research conducted on whiteleg shrimp suggested that safe nitrite level was to be less than 0.45 mg L<sup>-1</sup> while 96 h lethal concentration (LC<sub>50</sub>) at 2 g L<sup>-1</sup> salinity was determined to be 9 mg L<sup>-1</sup> (Gross *et al.*, 2004).

In any case, individual Total Ammonia Nitrogen (TAN) and body mass correlations revealed that 1.6 mg L<sup>-1</sup> nitrite-nitrogen (NO<sub>2</sub>-N) stressed the *M. amazonicum* (Amazon river prawn) (Hayd *et al.*, 2014). It is suggested that feeding GFP with live mealworms did not have a negative impact on its tolerance to nitrite toxicity and the use of live mealworms enriched with probiotics tended to have a beneficial effect on the GFP's tolerance to nitrite stress.

Prawns fed with CPF+PMW had the highest PO activity while the prawns fed with MW showed the lowest PO activity, although no significant differences were observed among the treatment groups (Table 2). According to Motte *et al.* (2019), the PO activity of whiteleg shrimp increased linearly when dietary fishmeal was replaced with the mealworm feed of more than 50%. Previous studies suggested that the immune-boosting effects of mealworms have resulted from one or a mix of ingredients, including chitin, polysaccharides, nucleotides, antimicrobial peptides, and melanin pigments (Kan *et al.*, 2008; Ido *et al.*, 2015).

Furthermore, the PO activity of whiteleg shrimp was improved when fed with a high dose of *B. subtilis*, which included a concentration of  $10^8$  (Tseng *et al.*, 2009). Besides, a probiotic mixture (*Lactobacillus fermentum*; *L. pentosus*; *Saccharomyces cerevisiae*; *B. subtilis*) improved probiotic effectiveness for whiteleg shrimp (Wang *et al.*, 2019). In contrast to the nitrite stress results, entirely feeding with live probiotic-enriched mealworm did not result in a positive impact on the PO activity of GFP.

### Fatty Acid Composition of Feed

The major FAs present in the experimental feeds were oleic acid (18:1n-9), 18:2n-6, palmitic acid (16:0), and arachidic acid (20:0) (Table 3). Previous studies reported that the main FAs found in mealworms were 18:1n-9, 18:2n-6, and 16:0 as wheat bran was given to mealworms (Alves *et al.*, 2016; Jeon *et al.*, 2016). On the other hand, Zheng *et al.* (2013) reported that 18:2n-6 was the main FA found in mealworms, followed by 18:1n-9, 16:0, and stearic acid (18:0).

The difference was because decayed vegetables were used as feed substrates for mealworm in a study by Zheng *et al.* (2013). Moreover, the 18:1n-9 concentration reported by Alves *et al.* (2016) was similar (52.78%) to the MW in the present study (53.87%), however, the reported 18:2n-6 amount (11.45%) was lower when compared with the MW (20.31%). The FA composition of MW and PMW was shown to vary from each other. This might be due to the

probiotic-enriched mealworm consuming the egg powder contained in the IG Guard A. The nutrient composition of mealworms is affected by their diet and the substrate they are cultivated on, as shown in a study by Mancini *et al.* (2019).

In addition, the FA composition of mealworms was found to be influenced by their diet (Bordiean *et al.*, 2022). This observation highlights the impact of mealworms' diet on the nutritional value of the mealworms themselves. The CPF had significantly the highest SFA concentration (48.15%), compared with all other diets. Interestingly, the SFA concentration in MW (21.62%) and PMW (21.96%) did not differ significantly. This result suggests that the probiotic enrichment did not change the SFA concentration.

Meanwhile, a significantly different MUFA concentration was observed in the MW (55.75%) and PMW (49.40%) indicating that the mealworms used in the PMW may have been deprived of MUFA-containing ingredients during their overnight exposure to the probiotic powder for enrichment. It is worth noting that mealworms have a limited capacity to retain food in their gut of typically not more than two days (Chung *et al.*, 2013). The two-day fasting period of the mealworms before probiotic enrichment may have led to the complete emptying of their gut, resulting in the removal of nutrients, including the FAs derived from wheat bran.

This could explain the difference in MUFA concentrations between the MW and PMW diets. Furthermore, the n-3 PUFA was

Table 2: Growth performance, survival, total feed intake, feed conversion ratio, and PO activity of the experimental prawns after 17 weeks of feeding trial and survival of prawns after nitrite stress test<sup>1</sup>

Feeding Materials <sup>2</sup>	CPF	CPF+MW	MW	PMW	CPF+PMW
Total weight gain (%)	517.52 ± 65.58	478.84 ± 28.42	505.06 ± 84.14	421.88 ± 6.07	529.34 ± 54.37
Survival after nitrite stress test (%)	53.33 ± 17.64	60.00 ± 11.55	46.67 ± 29.06	73.33 ± 26.67	20.00 ± 11.55
PO activity (L mol <sup>-1</sup> cm <sup>-1</sup> )	0.16 ± 0.05	0.13 ± 0.03	0.34 ± 0.24	0.10 ± 0.00	0.45 ± 0.34

<sup>1</sup> Values are presented as mean ± standard error of triplicate groups of experimental prawns. Different superscripts in the same row indicate significant differences at  $P < 0.05$ .

<sup>2</sup> Feeding materials: CPF = commercial prawn feed (control); CPF+MW = commercial prawn feed + mealworm; MW = mealworm; PMW = probiotic mealworm; CPF+PMW = commercial prawn feed + probiotic mealworm.

Table 3: Fatty acid composition of experimental feed materials (% of total fatty acids)<sup>1</sup>

Fatty Acid (%)	CPF	CPF+MW	MW	PMW	CPF+PMW
11:0	9.83 ± 0.34 <sup>c</sup>	1.38 ± 0.04 <sup>a</sup>	1.97 ± 0.10 <sup>b</sup>	1.61 ± 0.02 <sup>b</sup>	1.21 ± 0.03 <sup>a</sup>
12:0	14.92 ± 0.64 <sup>b</sup>	ND	ND <sup>a</sup>	0.01 ± 0.00 <sup>a</sup>	0.01 ± 0.01 <sup>a</sup>
13:0	2.63 ± 0.20 <sup>b</sup>	0.05 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>	0.01 ± 0.01 <sup>a</sup>
14:0	3.03 ± 0.46 <sup>b</sup>	0.05 ± 0.00 <sup>a</sup>	0.09 ± 0.01 <sup>a</sup>	0.03 ± 0.01 <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>
16:0	15.21 ± 0.10 <sup>a</sup>	17.87 ± 0.03 <sup>d</sup>	17.57 ± 0.06 <sup>c</sup>	17.98 ± 0.02 <sup>d</sup>	16.82 ± 0.28 <sup>b</sup>
16:1	0.13 ± 0.01	ND <sup>a</sup>	0.11 ± 0.01	ND <sup>a</sup>	0.04 ± 0.00
18:0	0.10 ± 0.00 <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
18:1n-9 (OA)	21.41 ± 0.13 <sup>a</sup>	50.98 ± 0.39 <sup>c</sup>	53.87 ± 0.13 <sup>c</sup>	47.79 ± 0.03 <sup>b</sup>	53.16 ± 0.09 <sup>d</sup>
18:2n-6 (LA)	10.88 ± 0.17 <sup>a</sup>	20.51 ± 0.09 <sup>b</sup>	20.31 ± 0.02 <sup>b</sup>	21.61 ± 0.05 <sup>c</sup>	22.92 ± 0.08 <sup>d</sup>
20:0	2.16 ± 0.02 <sup>d</sup>	1.88 ± 0.02 <sup>c</sup>	1.56 ± 0.02 <sup>b</sup>	1.67 ± 0.09 <sup>b</sup>	0.01 ± 0.00 <sup>a</sup>
18:3n-6 (GLA)	0.28 ± 0.00 <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
20:1n-9	0.14 ± 0.04 <sup>b</sup>	0.77 ± 0.03 <sup>d</sup>	0.19 ± 0.02 <sup>bc</sup>	0.26 ± 0.03 <sup>c</sup>	ND <sup>a</sup>
18:3n-3 (ALA)	0.46 ± 0.05 <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
20:2	0.15 ± 0.01 <sup>a</sup>	0.20 ± 0.03 <sup>a</sup>	0.17 ± 0.02 <sup>a</sup>	0.65 ± 0.28 <sup>b</sup>	0.01 ± 0.00 <sup>a</sup>
22:0	0.11 ± 0.06 <sup>b</sup>	0.06 ± 0.03 <sup>b</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
20:3n-6	0.69 ± 0.10 <sup>b</sup>	1.03 ± 0.01 <sup>bc</sup>	0.01 ± 0.01 <sup>a</sup>	1.32 ± 0.31 <sup>c</sup>	0.05 ± 0.01 <sup>a</sup>
22:1n-9	0.33 ± 0.02 <sup>c</sup>	0.23 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>a</sup>	0.19 ± 0.09 <sup>b</sup>	0.07 ± 0.02 <sup>ab</sup>
20:3n-3	ND <sup>a</sup>	0.17 ± 0.09 <sup>b</sup>	ND <sup>a</sup>	0.30 ± 0.03 <sup>c</sup>	ND <sup>a</sup>
20:4n-6 (ARA)	ND <sup>a</sup>	0.60 ± 0.00 <sup>c</sup>	0.01 ± 0.00 <sup>a</sup>	0.34 ± 0.17 <sup>b</sup>	0.09 ± 0.02 <sup>a</sup>
22:2	0.16 ± 0.02 <sup>c</sup>	0.09 ± 0.02 <sup>b</sup>	0.05 ± 0.00 <sup>ab</sup>	0.54 ± 0.01 <sup>d</sup>	0.03 ± 0.02 <sup>a</sup>
24:0	0.03 ± 0.02	ND	ND	ND	0.01 ± 0.00
20:5n-3 (EPA)	1.25 ± 0.13 <sup>b</sup>	0.13 ± 0.02 <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>
24:1n-9	0.33 ± 0.01 <sup>b</sup>	0.01 ± 0.01 <sup>a</sup>	ND <sup>a</sup>	ND <sup>a</sup>	0.02 ± 0.01 <sup>a</sup>
22:6n-3 (DHA)	0.50 ± 0.01 <sup>c</sup>	0.20 ± 0.04 <sup>b</sup>	0.01 ± 0.00 <sup>a</sup>	ND <sup>a</sup>	0.16 ± 0.02 <sup>b</sup>
∑ SFA	48.15 ± 0.83 <sup>c</sup>	21.69 ± 0.17 <sup>b</sup>	21.62 ± 0.22 <sup>b</sup>	21.96 ± 0.08 <sup>b</sup>	18.44 ± 0.16 <sup>a</sup>
∑ MUFA	24.39 ± 0.21 <sup>a</sup>	52.21 ± 0.59 <sup>c</sup>	55.75 ± 0.16 <sup>c</sup>	49.40 ± 0.10 <sup>b</sup>	54.54 ± 0.09 <sup>d</sup>
∑ PUFA	14.38 ± 0.09 <sup>a</sup>	23.01 ± 0.12 <sup>c</sup>	20.56 ± 0.04 <sup>b</sup>	24.76 ± 0.75 <sup>d</sup>	23.25 ± 0.09 <sup>c</sup>
∑ n-3 PUFA	2.22 ± 0.10 <sup>d</sup>	0.59 ± 0.07 <sup>c</sup>	0.01 ± 0.00 <sup>a</sup>	0.31 ± 0.04 <sup>b</sup>	0.16 ± 0.02 <sup>ab</sup>
∑ n-6 PUFA	12.16 ± 0.19 <sup>a</sup>	22.42 ± 0.11 <sup>c</sup>	20.54 ± 0.04 <sup>b</sup>	24.45 ± 0.75 <sup>d</sup>	23.09 ± 0.10 <sup>c</sup>
∑ n-3/n-6	0.18	0.03	0.00	0.01	0.01

<sup>1</sup>Values are presented as mean ± standard error of triplicate of each feeding materials. Different superscripts in the same row indicate significant differences at P < 0.05.

<sup>2</sup>FA percentages that are counted but not shown in Table 10 are 4:0, 6:0, 8:0, 10:0, 15:0, 15:1, 17:0, 17:1, 20:0, 22:0, 23:0.

significantly lowest in MW (0.01%) while CPF (2.22%) recorded the highest n-3 PUFA. As expected, the CPF comprised significantly highest eicosapentaenoic acid (EPA) (1.25%) and docosahexaenoic acid (DHA) (0.50%) because the CPF was a commercially available

diet that was specifically formulated to meet prawns' nutrient requirements.

Nonetheless, the CPF+MW (0.20%) and CPF+PMW (0.16%) had significantly higher amounts of DHA compared with MW and PMW, where the DHA was from the CPF.

Undoubtedly, the mealworm lacks EPA and DHA (Jeon *et al.*, 2016; Adámková *et al.*, 2017; Ghosh *et al.*, 2017). Yet, manipulation of feed substrate for mealworms can greatly influence its FA composition, and providing the right type of feed can improve the proportion of PUFA to MUFA (Ooninx & Finke, 2021).

### Fatty Acid Composition of Prawns

The major FA composition from the present study in prawns fed with CPF in decreasing order were 18:1n-9, 16:0, 18:2n-6, 18:3n-3, 20:0, arachidonic acid (ARA, 20:4n-6) as shown in Table 4. Simultaneously, 100% replacement of CPF with MW showed a similar trend in

Table 4: Fatty acid composition of experimental prawns (% of total fatty acids) after 17 weeks of feeding trial<sup>1</sup>

Fatty Acid (%)	CPF	CPF+MW	MW	PMW	CPF+PMW
16:0	18.97 ± 2.10 <sup>b</sup>	17.45 ± 0.50 <sup>ab</sup>	14.72 ± 1.44 <sup>a</sup>	17.93 ± 0.83 <sup>ab</sup>	16.75 ± 0.35 <sup>ab</sup>
16:1	0.39 ± 0.39	0.41 ± 0.33	0.60 ± 0.52	0.07 ± 0.05	0.04 ± 0.04
17:0	0.91 ± 0.65	0.13 ± 0.04	0.16 ± 0.05	0.13 ± 0.04	0.11 ± 0.04
17:1	0.07 ± 0.02	0.01 ± 0.01	0.07 ± 0.03	0.05 ± 0.01	0.04 ± 0.01
18:0	0.01 ± 0.01	0.04 ± 0.04	0.00 ± 0.00 <sup>a</sup>	0.01 ± 0.01	0.00 ± 0.00 <sup>a</sup>
18:1n-9	30.13 ± 7.17 <sup>a</sup>	48.95 ± 2.76 <sup>b</sup>	47.77 ± 0.56 <sup>b</sup>	49.08 ± 1.69 <sup>b</sup>	49.20 ± 2.41 <sup>b</sup>
18:2n-6	16.66 ± 0.37	15.56 ± 1.09	17.08 ± 2.27	15.55 ± 1.88	17.64 ± 0.24
20:0	3.35 ± 1.15 <sup>b</sup>	0.45 ± 0.36 <sup>a</sup>	0.39 ± 0.39 <sup>a</sup>	0.63 ± 0.12 <sup>a</sup>	0.83 ± 0.19 <sup>a</sup>
18:3n-6	ND	2.67 ± 1.37	1.50 ± 1.50	3.33 ± 1.75	0.00 ± 0.00
20:1n-9	0.41 ± 0.14	0.16 ± 0.16	0.53 ± 0.32	0.24 ± 0.12	0.41 ± 0.17
18:3n-3	13.77 ± 5.25 <sup>b</sup>	1.98 ± 1.13 <sup>a</sup>	5.04 ± 0.91 <sup>a</sup>	2.06 ± 1.05 <sup>a</sup>	3.62 ± 0.20 <sup>a</sup>
20:2	0.15 ± 0.04	0.19 ± 0.08	0.16 ± 0.07	0.08 ± 0.06	0.24 ± 0.12
22:0	0.10 ± 0.10	0.10 ± 0.10	0.09 ± 0.09	ND	0.06 ± 0.06
20:3n-6	0.30 ± 0.03	0.56 ± 0.30	0.62 ± 0.32	0.14 ± 0.14	0.63 ± 0.45
22:1n-9	0.70 ± 0.13	0.48 ± 0.23	0.32 ± 0.14	0.54 ± 0.04	0.84 ± 0.32
20:3n-3	0.38 ± 0.20	0.52 ± 0.34	0.52 ± 0.34	0.23 ± 0.23	0.18 ± 0.18
20:4n-6	3.01 ± 0.49 <sup>b</sup>	2.01 ± 1.24 <sup>ab</sup>	0.20 ± 0.20 <sup>a</sup>	1.96 ± 0.44 <sup>ab</sup>	1.40 ± 0.21 <sup>ab</sup>
22:2	0.22 ± 0.15	0.36 ± 0.19	0.28 ± 0.14	0.12 ± 0.03	0.16 ± 0.07
24:0	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.03	0.03 ± 0.01	0.01 ± 0.01
20:5n-3	ND	ND	1.92 ± 1.25	ND	ND
24:1n-9	0.34 ± 0.15	0.13 ± 0.06	0.13 ± 0.04	0.14 ± 0.02	0.11 ± 0.03
22:6n-3	1.01 ± 0.20	0.71 ± 0.26	0.75 ± 0.30	0.68 ± 0.13	0.43 ± 0.22
∑ SFA	25.53 ± 2.47 <sup>b</sup>	21.13 ± 0.51 <sup>ab</sup>	17.77 ± 2.46 <sup>a</sup>	20.95 ± 0.89 <sup>ab</sup>	19.85 ± 0.52 <sup>a</sup>
∑ MUFA	33.54 ± 7.42 <sup>a</sup>	51.54 ± 2.62 <sup>b</sup>	50.23 ± 0.82 <sup>b</sup>	51.76 ± 1.55 <sup>b</sup>	52.13 ± 0.59 <sup>b</sup>
∑ PUFA	35.50 ± 5.91 <sup>b</sup>	24.56 ± 2.02 <sup>a</sup>	28.07 ± 1.50 <sup>ab</sup>	24.14 ± 1.40 <sup>a</sup>	24.28 ± 0.24 <sup>a</sup>
∑ n-3 PUFA	15.16 ± 5.38 <sup>b</sup>	3.20 ± 1.38 <sup>a</sup>	8.22 ± 1.23 <sup>ab</sup>	2.96 ± 0.74 <sup>a</sup>	4.23 ± 0.22 <sup>a</sup>
∑ n-6 PUFA	34.11 ± 5.79 <sup>b</sup>	23.34 ± 1.83 <sup>a</sup>	24.89 ± 1.80 <sup>a</sup>	23.23 ± 1.07 <sup>a</sup>	23.67 ± 0.30 <sup>a</sup>
∑ n-3/n-6	0.44	0.14	0.33	0.13	0.18

<sup>1</sup>Values are presented as mean ± standard error of triplicate groups of experimental prawns. Different superscripts in the same row indicate significant differences at  $P < 0.05$ .

<sup>2</sup>Feeding materials: CPF = commercial prawn feed (control); CPF+MW = commercial prawn feed + mealworm; MW = mealworm; PMW = probiotic mealworm; CPF+PMW = commercial prawn feed + probiotic mealworm.

the major FA composition of prawns, with decreasing order starting with 18:1n-9, 18:2n-6, 16:0, 18:3n-6, 18:3n-6, and 20:4n-6. Although the FA composition of prawns fed with either PMW or MW showed similarities, there were noticeable variations in the concentrations of 18:1n-9, 16:0, 18:2n-6, 18:3n-3, 20:0, and 20:4n-6.

Most bacteria generally have a basic FA composition consisting of straight-chain FAs such as myristic acid (14:0), 16:0, 18:0, and palmitoleic acid (16:1) (Kaneda, 1991). For prawns fed with PMW, the concentration of SFAs was numerically higher compared to those fed with MW, which is primarily due to the relatively higher concentration of 16:0 in the PMW-fed prawns. This observation aligns with a study conducted by Kolanchinathan *et al.* (2022), which identified heptadecanoic acid (17:0), 16:0, pentadecanoic acid (15:0), myristic acid (14:0), and tricosanoic acid (23:0) as the major SFAs present in *B. firmus* and *B. coagulans*.

It is important to note that the present study enriched mealworms with *B. subtilis*, which may explain the observed differences in the levels of 16:0 between PMW-fed and MW-fed prawns. As such, probiotic enrichment seemed to influence the FA composition of the prawns. Overall, the study indicates a potential connection between probiotic enrichment, mealworms, and the FA composition of prawns.

The present study showed no significant differences ( $P > 0.05$ ) in the total SFA content among the prawns fed with CPF, CPF+MW, and PMW. This suggests that prawns fed with the CPF+PMW, which had the lowest SFA concentration may retain an equal proportion of SFAs as contained in those fed with CPF. According to a study by Portella *et al.* (2013), the major SFAs found in GFP are 16:0 and 18:0. Previous research conducted on tiger shrimp has demonstrated that SFAs are the least digestible type of FA and their digestibility decreases as the carbon chain length increases (Glencross *et al.*, 2002). Therefore, the quantity of lipids in the diet and the specific FA composition

can influence their digestibility (Panini *et al.*, 2017b).

In the present feed study, prawns fed with PMW exhibited a higher concentration of 18:1n-9 compared to those fed with MW. Additionally, prawns fed with CPF+MW, MW, PMW, and CPF+PMW had significantly higher concentrations of 18:1n-9 (ranging from 40.69% to 43.31%), compared to prawns fed with the CPF (22.27%). These findings indicate that the inclusion of MW and probiotic enrichment in prawn diets can influence the FA composition, particularly the level of 18:1n-9. Interestingly, prawns fed with CPF showed the lowest amount of total MUFA (33.54%), compared with prawns fed with other diets.

Prawns fed with either 50% or 100% live mealworm regardless of probiotic enrichment or not showed a significantly equal proportion of total MUFAs suggesting maximum deposition of total MUFAs in prawns despite varied dietary MUFA. A previous study on whiteleg shrimp showed that increasing fishmeal substitution with mealworm meal resulted in a linear increase in MUFA, particularly the 18:1n-9 (Panini *et al.*, 2017a).

The concentration of 18:2n-6 in prawns remained consistent across all feed treatments, ranging from 15.55% to 17.64%. In contrast, prawns fed with the CPF exhibited the highest (3.35%) concentration of 20:4n-6 while those fed with MW had the lowest (0.20%) concentration. However, the concentration of 20:4n-6 was similar in prawns fed with CPF+MW, PMW, and CPF+PMW diets, ranging from 1.40% to 2.01%. Interestingly, the 20:4n-6 concentration in prawns was higher than that of the experimental feed, suggesting that prawns may selectively retain this EFA during the feeding trial. Furthermore, previous research has shown that the concentration of 20:4n-6 in GFP is influenced by the maturity of prawns, with more matured prawns exhibiting higher levels of 20:4n-6 (Cavalli *et al.*, 2001).

Similarly, a study investigating the use of mealworm meal as a fishmeal substitute in the diet of whiteleg shrimp also reported similar

results for 20:4n-6 (Panini *et al.*, 2017b). These findings highlight the dynamic nature of FA composition in prawns and suggest that both dietary factors and prawn physiology contribute to the observed variations. Prawns fed with CPF had the highest concentration of 18:3n-3 (13.77%), significantly different from other experimental groups (1.98%-5.04%) despite the absence of 18:3n-3 in the 50% or 100% live mealworm feeds. Indeed, the 18:3n-3 in prawns is resultant of the diets and initial prawn body.

Besides, CPF had significantly the highest DHA content among the experimental feeds, yet the DHA content resulted in prawns was not significantly different from each other. This suggests that prawns may have undergone limited bioconversion of 18:2n-6 and 18:3n-3 to ARA and DHA, respectively (González-Félix *et al.*, 2002). Another noteworthy observation was that probiotic enrichment did not result in significant differences in the total n-6 and n-3 PUFA concentrations of prawns fed with 50% or 100% live mealworm.

### Conclusions

In this study, feeding GFP with live mealworms demonstrated no adverse effects and may improve stress tolerance and immunity. Probiotic-enriched mealworms showed potential benefits in enhancing tolerance to nitrite stress. However, a diet consisting solely of probiotic-enriched mealworms did not improve PO activity in GFP.

Feeding prawns with between 50% and 100% of live mealworms, regardless of probiotic enrichment, resulted in a similar proportion of total MUFAs, indicating maximum MUFA deposition. The ARA concentration in prawns was higher than in the feed while live mealworm feeding had no significant effect on DHA content, suggesting selective retention of ARA and limited bioconversion of 18:2n-6 and 18:3n-3 into ARA and DHA.

In conclusion, combining live mealworms with commercial feed is a viable feeding strategy for GFP while probiotic enrichment of

mealworms does not offer additional benefits to prawn immune response and FA composition.

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### Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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