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Mysid meal as a dietary replacement for fishmeal in the diets of Pacific white shrimp *Penaeus vannamei* (Boone, 1931) postlarvae

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The current study evaluates the nutritional and feed value of mysid meal (MM) as a substitute for fishmeal (FM) in the Pacific white shrimp (*Penaeus vannamei*) postlarvae diet. Five experimental diets were formulated by replacing 0 (MM0), 25 (MM25), 50 (MM50), 75 (MM75), and 100 % (MM100) of dietary FM with MM. These experimental feeds were fed to *P. vannamei* postlarvae in a 60-day feeding trial. Results revealed that MM could entirely substitute 100 % FM in the white shrimp diet. Furthermore, results showed that 75 % FM replacement with MM elicited a growth-enhancing effect and improved feed nutrient utilization. No significant treatment effects were detected in the survival, total feed intake, and biochemical body composition of *P. vannamei*. The observed improvement in shrimp growth in terms of weight gain (WG), specific growth rate (SGR), and nutrient retention were positively correlated with the substitution level of FM by MM. The feed conversion ratio (FCR) was negatively correlated with the substitution of MM and with the growth indices including WG and SGR. In conclusion, 100% of the FM (40% in the control diet) can be substituted by dietary MM without affecting the survival, growth, feed utilization, and biochemical carcass composition of *P. vannamei*. Polynomial regression analysis of SGR indicates that 65.50% of MM is optimum to replace FM in the diet of *P. vannamei* to attain maximum growth.

KEYWORDS

alternative protein feedstuff, fishmeal replacement, growth enhancement, nutrition, zooplankton-based meal

Introduction

While capture fisheries yields have decreased over the past decades, aquaculture exhibited rapid growth compared to other food-producing sectors to meet the surging food demand for fish and fish products (FAO, 2016, 2020). With the continued growth of aquaculture for decades, there has been an associated expansion of shrimp culture across the world due to the advancement of technologies on intensive high-density shrimp culture systems and the increasingly high market demands and export value of this commodity (Ayisi et al., 2017; Cummins et al., 2017; Li et al., 2022; Yildirim-Aksoy et al., 2022).

However, global farmed shrimp production relies heavily on wild-sourced fishmeal (FM) as a primary protein source for formulated feeds (Cummins et al., 2017; Sánchez-Muros et al., 2020; Li et al., 2023). FM is considered the most expensive macro-ingredient for the formulation of marine shrimp diets due to its favorable nutrient composition, high-quality protein (60–72% CP), complete essential amino acid (AA) and fatty acid profiles, a good source of vitamins and minerals, and a high digestibility (Cruz-Suárez et al., 2009; Lemos et al., 2009; Suárez et al., 2009; Riche, 2015). In recent years, the rapid development of aquaculture, coupled with the significant improvements in the shrimp culture industry, has caused more demand for FM (Li et al., 2022, 2023; Wang et al., 2023; Zheng et al., 2023). In 2016, the aquaculture industry utilized more than 70% of global FM production, and shrimp diets consumed about 31 % of FM for aquafeed production, making it the dominant consumer of ocean-derived FM (Tacon and Metian, 2015; Jannathulla et al., 2019).

Earlier reports on the dietary feed formulations for the Pacific white shrimp (*Penaeus vannamei*) showed that it typically comprises 25–50% FM inclusion, which accounts for the feed and production costs (Samocha et al., 2004; Amaya et al., 2007; Ye et al., 2012; Yun et al., 2017). In shrimp farming, the feed costs account for over 50% of the total production costs due to the high inclusion of dietary FM (Ayisi et al., 2017; Cummins et al., 2017). The rapid expansion of the aquaculture industry, coupled with the consequent increase in demand for FM, has caused a significant surge in FM prices and erratic supply of this crucial feed ingredient (Tacon and Metian, 2008; Duarte et al., 2009; Li et al., 2022; Zheng et al., 2023). Moreover, global FM production can often fluctuate unpredictably due to overfishing and climate-induced fluctuations (El Niño-Southern Oscillation events), making FM production and prices unpredictable (Naylor et al., 2009). The global demand for FM is anticipated to expand as the commercial production of farmed shrimp is projected to surge in the coming years. At some point, this projected demand for FM will likely intensify and eventually outstrip the forage fish populations that can be harvested from the ocean. Since it is unsustainable to extract these finite fish resources beyond their current capacity, finding cost-effective alternative non-fish-based ingredients that are nutritionally equivalent to FM, ideally of a lower trophic position, is required to ensure the sustainability of the aquaculture industry (Moren et al., 2006; Salas-Leiton et al., 2020).

Zooplankton has been proposed as a sustainable source of feed ingredients for aquaculture (Nicol and Endo, 1997, 1999; Melk et al., 2004) since its nutritional compositions are reported to be comparable to FM (Storebakken, 1988). The global productivity of zooplankton biomass typically produces hundreds of millions of tons, and their reproductive turnover rate is fast. Most of this large biomass is not used in commercial products, and only a tiny percentage is harvested (Hewitt et al., 2002; Huntington and Hasan, 2009; Naylor et al., 2009). There is a consensus belief that aquatic biomass that is not harvested and utilized is considered a wasted biological resource. The lack of comprehensive evaluation of these non-fish-based alternatives on shrimp limits their application in aquafeeds. Among possible alternatives under investigation, many underutilized species of zooplankton with large biomass from lower trophic levels, such as mysids, could offer a relatively untapped potential novel ingredient to support the needs of the growing aquaculture industry. Mysid has a high calorific value and is considered an excellent experimental organism for various bio-assays due to its abundance and ease of culturing (Eusebio et al., 2010; Biju and Panampunnayil, 2011). This zooplankton is abundant in the aquatic environment and is documented to have a short reproductive cycle (14–21 d) (Mauchline, 1980; Domingues et al., 1998). Mysid species are vastly underutilized, and their utilization as feed could be vital for the aquaculture industry. The mysid shrimp is characterized by its high protein (52–75% DM) content (Eusebio et al., 2010; Buen-Ursua et al., 2015). Apart from the high protein content, mysids have been receiving attention as a potential ingredient in aquafeeds due to their significant amount of total lipid and fatty acids, which is ideal for growth and metabolism (Izquierdo, 1996; Eusebio et al., 2010). In addition to their high nutritional quality, mysid is suitable for aquaculture as a live feed (Biju et al., 2009). Although information on its nutritional value and biological testing are not well documented relative to FM replacement in shrimp diets, mysids are considered an alternative live food for the nursery culture of marine fish (Eusebio et al., 2010).

Zooplankton-based meals have been suggested as an alternative protein source in fish diets (Virtue et al., 1995; Moren et al., 2006; Salas-Leiton et al., 2020). Zooplankton-based meals were shown to be suitable feed ingredients for *P. monodon* (Smith et al., 2005; Williams et al., 2005) *and P. vannamei* (Nunes et al., 2011, 2019; Soares et al., 2021; Ambasankar et al., 2022; Wei et al., 2022). In addition, Moren et al. (2006) documented that during the feeding trial of Atlantic cod (*Gadus morhua*), it showed no significant difference in growth, while Atlantic salmon (*Salmo salar*) exhibited improved SGR when fed diets where 40% of the FM protein was replaced with Arctic krill (*Thysanoessa inermis*) or amphipod (*Themisto libellula*) meal. Zooplankton biomass (consisting of rotifers, copepods, cladocerans, ostracods, and protozoans) meal was able to replace 100% of FM in the diet of seabass, *Dicentrarchus labrax* fingerlings without adverse effects on the growth performance and feed utilization (Hassan et al., 2020). In addition, Abo-Taleb et al. (2021) found that the optimal FM replacement level for gray mullet was 75% of *Daphnia magna* meal, demonstrating a quadratic regression trend in growth and feed utilization. Using zooplankton-based meals as a potential substitute for FM in *P. vannamei* diets could be a promising alternative. Based on our intensive literature reviews, there is no published study on the feed value of mysid meal (MM) as an FM replacement in shrimp diets. Hence, this study aims to evaluate the feed value of MM as an alternative ingredient for FM on the growth, survival, feed utilization, biochemical composition, nutrient retention indices, and survival of *P. vannamei* postlarvae.

Materials and methods

Description of the study area

The study was conducted at the Multispecies Hatchery Complex of the University of the Philippines Visayas (UP Visayas), College of Fisheries and Ocean Sciences (CFOS)—Institute of

TABLE 1 Proximate analysis of MM and sardine FM for formulating the experimental diets for *P. vannamei*.

	MM	FM
Proximate composition (% dry matter)		
Crude protein[a]	53.15 ± 0.07	57.02 ± 0.16
Crude lipid[a]	4.42 ± 0.12	9.58 ± 0.78
Crude fiber[b]	5.87 ± 0.02	0.04 ± 0.01
Ash[a]	20.92 ± 0.53	19.62 ± 0.07
NFE[c]	15.65 ± 0.33	13.73 ± 0.58

Values are mean ± SEM of three replicates.
[a]Analyzed values from the Fish Nutrition Laboratory, UP Visayas, CFOS-IA, Miagao, Iloilo, Philippines.
[b]Analyzed values from the DOST (Department of Science and Technology), RSTL (Regional Standards and Testing Laboratory), Region VI, La Paz, Iloilo City, Philippines.
[c]Nitrogen-free extract, computed by difference.

Aquaculture (IA) in Miag-ao, Iloilo, Philippines. The area was equipped with aeration and good water facilities.

Sample collection

The mysids, *Mesopodopsis* sp., was procured from local fishermen in Atabayan, Tigbauan, Iloilo, Philippines. The samples were transported and processed as mysid meal (MM) at the Fish Nutrition Laboratory of the UP Visayas, CFOS–IA. Briefly, the mysids were oven-dried at 60°C for 24h, pulverized to a particle size of 100 μm using a mechanical grinder (JML Nutri Blitzer), and refrigerated at −20°C until use for diet formulation.

Experimental feed formulation

The nutritional proximate composition analysis of MM, sardine FM, and experimental feeds are shown in Tables 1, 2. Five experimental diets (37% crude protein) were formulated according to the formulations of Bauer et al. (2012) with modifications to satisfy the nutritional requirements of the white shrimp postlarvae. The treatments consisted of five experimental diets with control (MM0), 25% (MM25), 50% (MM50), 75% (MM75), and 100% (MM100) MM replacement of FM by weight, respectively (Table 2).

All experimental feeds were prepared at the UP Visayas, CFOS–IA, Fish Nutrition Laboratory. All dry ingredients were pulverized using an electric mechanical grinder (JML Nutri Blitzer) and finely ground into powder using a 100 μm mesh sieve. The sieved feedstuffs were weighed and appropriately mixed before including soybean lecithin and fish oil. After that, water (0.45–0.50 L·Kg^{-1}) was poured gradually into the dry feedstuffs to form a moistened dough. The dough was thoroughly mixed and pelletized using a laboratory pelletizer with a 2-mm die. The formed pellet strands were collected and dried to a moisture content of <10% at 60°C. The dried pellets were subsequently crumbled into suitable sizes, wrapped in polyethylene zip-lock containers, and kept at −20°C until use as feed.

Experimental animal and maintenance

The *P. vannamei* postlarvae (PL10) were procured from a reliable commercial *P. vannamei* hatchery at Guimbal, Iloilo, Philippines. Prior to the start of the experiment, the shrimp were screened to be free from White Spot Syndrome Virus and pathogenic *Vibrio parahaemolyticus* by molecular analysis using nested PCR IQ 2000^{TM} Detection and Prevention System, Genereach Biotechnology Corp., Taiwan (Dangtip et al., 2015).

The experimental animals were then acclimated to laboratory conditions for seven days in a 5-ton capacity circular fiberglass tank and fed with commercial feed before the start of the experiment. Optimum water quality parameters for the ideal growth and survival of *P. vannamei* postlarvae were monitored and maintained for the duration of the feeding trial (ammonia: 0–0.25 ppm, dissolved oxygen: >5 ppm, salinity: 30–35 ppt, pH: 7.86–8.05, temperature: 23.3–28.5°C).

Feeding trial and sampling protocol

The study was conducted in an indoor, recirculating water system with a water flow rate of about 0.60–0.80 L·min^{-1} using 20 units of 60-L capacity flat-bottom rectangular plastic tanks in a completely randomized design for a 60-day culture period. The recirculating system comprises a reservoir (1 ton) connected to three biofilter tanks containing charcoal, gravel, and polyethylene fiber as filters and a 100-watt circulation water pump. The experimental containers were equipped with individual aeration and covered with a PVC lid with black mesh netting to reduce disturbance and prevent shrimp from escaping. After the acclimatization, 500 healthy and homogenous-sized *P. vannamei* postlarvae (0.003 ± 0.000 g; mean ± SEM) were randomly assigned to each previously prepared holding tank (25 shrimp·tank^{-1}). Shrimp samples were also collected from the stock tank and used for the initial biochemical analysis of the carcass.

The *P. vannamei* postlarvae were fed thrice daily (08:00, 12:00, and 16:00 h) with the respective dietary treatments at 15% of the body weight. The unconsumed feeds were collected one hour after each feeding and weighed after oven-drying at 60°C to determine the total feed intake (TFI). The TFI was determined by the difference in the quantity of uneaten diets from the total amount of supplied experimental feeds. Periodic sampling was conducted every 2 weeks, in which the experimental animals from each tank (4 tanks·treatment^{-1}) were weighed in bulk and counted to regulate the feeding ration. At the termination of the study, shrimps from each treatment group were collected, individually weighed, euthanized, and subjected to final biochemical body composition analysis. Upon the termination of the feeding trial, the growth indices and feed utilization efficiency parameters of the shrimps in response to the experimental diets were evaluated by the following biometrics (Hardy and Barrows, 2002; Bulbul et al., 2016; Wei et al., 2022).

$$\textbf{Percent Weight Gain (\%WG)} = \frac{\text{FBW (g)} - \text{IBW (g)}}{\text{IBW (g)}} x\ 100$$

TABLE 2 Ingredients and proximate nutritional profile (g·1,000 g^{-1}) of dietary treatments for *Penaeus vannamei* postlarvae containing different levels of FM replacement with MM.

Ingredients (%)	Dietary treatments				
	MM0	MM25	MM50	MM75	MM100
Fishmeal (FM)[a]	400.00	300.00	200.00	100.00	0.00
Mysid meal (MM)	0.00	100.00	200.00	300.00	400.00
Soybean meal	220.00	220.00	220.00	220.00	220.00
Copra meal	10.00	60.00	110.00	160.00	197.50
Squid meal	30.00	30.00	30.00	30.00	30.00
Corn starch	197.50	147.50	97.50	47.50	10.00
Yeast	50.00	50.00	50.00	50.00	50.00
Wheat gluten[b]	50.00	50.00	50.00	50.00	50.00
Fish oil[c]	17.50	17.50	17.50	17.50	17.50
Soy lecithin	5.00	5.00	5.00	5.00	5.00
Vit. premix[d]	10.00	10.00	10.00	10.00	10.00
Min. premix[e]	10.00	10.00	10.00	10.00	10.00
Proximate composition (% dry weight) basis					
Crude protein[f]	37.06 ± 0.05	37.22 ± 0.48	37.06 ± 0.37	37.75 ± 0.36	37.48 ± 0.25
Crude lipid[f]	8.17 ± 0.31	8.13 ± 0.25	8.14 ± 0.28	8.15 ± 0.29	8.16 ± 0.34
Crude fiber[g]	3.16 ± 0.45	3.46 ± 0.37	4.23 ± 0.39	4.69 ± 0.20	4.81 ± 0.12
Ash[f]	12.32 ± 0.35	13.15 ± 0.38	13.64 ± 0.35	13.87 ± 0.40	14.12 ± 0.39
NFE[h]	39.29	38.04	36.93	35.54	35.43

[a]Sardine fishmeal.
[b]Vital wheat gluten.
[c]Danish fish oil.
[d]Vitamin mix (1,000 g^{-1}): Alpha-tocopherol (20,000 IU), Cholecalciferol (200,000 IU), Retinol (1,200,000 IU), Biotin (40 mg), Ethoxyquin (500 mg), Folic acid (1,800 mg), Cobalamin (2,000 mg), Riboflavin (8,000 mg), Pyridoxine (5,000 mg), Thiamin (8,000 mg), Calcium Pantothenate (20,000 mg), and Niacin (40,000 mg).
[e]Mineral-mix (1,000 g^{-1}): cobalt (20 mg), selenium (200 mg), iodine (1,800 mg), copper (4,000 mg), zinc (40,000 mg), manganese (10,000 mg), and iron (40,000 mg).
[f]Analyzed values from the Fish Nutrition Laboratory, UP Visayas, CFOS-IA, Miagao, Iloilo, Philippines.
[g]Analyzed values from the DOST-RSTL, Region VI, La Paz, Iloilo City, Philippines.
[h]Nitrogen-free extract, computed by difference.

$$\textbf{Specific Growth Rate } (\textbf{SGR, \%day}^{-1}) = \frac{Ln\ FBW\ (g) - Ln\ IBW(g)}{60\ days}\ x\ 100$$

$$\textbf{Feed Intake } (\textbf{FI, g·shrimp}^{-1}) = \frac{Dry\ diet\ given(g) - Dry\ remaining\ recovered\ (g)}{Number\ of\ shrimp}$$

$$\textbf{Feed Conversion Ratio } (\textbf{FCR}) = \frac{Dry\ feed\ intake\ (g)}{FBW\ (g) - IBW\ (g)}$$

$$\textbf{Protein Efficiency Ratio } (\textbf{PER}) = \frac{FBW(g) - IBW\ (g)}{Protein\ intake\ (g)}$$

$$\textbf{Survival Rate } (\textbf{SR, \%}) = \frac{Final\ number\ of\ shrimp\ survived}{Initial\ number\ of\ shrimp\ stocked}\ x\ 100$$

$$\textbf{Nutrient retention } (\%) = \frac{CNC_{Final} - CNC_{Initial}}{Nutrient\ intake} x\ 100$$

$$\textbf{CNC}_{\textbf{Final}} = \frac{CNC\ (protein\ or\ lipid,\ g{\cdot}g^{-1}\ diet)\ x\ FBW\ (g)}{100}$$

$$\textbf{CNC}_{\textbf{Initial}} = \frac{CNC\ (protein\ or\ lipid,\ g{\cdot}g^{-1}\ diet)\ x\ IBW\ (g)}{100}$$

$$\textbf{Nutrient intake} = \frac{Total\ feed\ intake\ (g)\ x\ Feed\ nutrient\ content\ (protein\ or\ lipid,\ g \cdot g^{-1}\ diet)}{100}$$

Where:
FBW = Final body weight (g) of individual shrimp
IBW = Initial body weight (g) of individual shrimp
CNC = Carcass nutrient content
CP_{Final} = Final carcass protein
$CP_{Initial}$ = Initial carcass protein.

Biochemical composition analyses

The initial and final carcass composition (crude protein, crude lipid, ash) of the test animals, primary protein sources (FM and MM), and the experimental feeds were evaluated in triplicates following the methods of the AOAC (2000). The moisture was determined according to Method 934.01 (AOAC, 2000). Crude protein was evaluated by the Kjeldahl method following Method 981.10 (AOAC, 2000). Crude lipid was quantified according to Bligh and Dyer (1959). Ash content was determined following Method 942.05 (AOAC, 2000). The AA composition of MM was determined using the Shimadzu HPLC analysis system (LC-10A/C-R7A) at the Fish Nutrition Laboratory of the UP Visayas, CFOS–IA. The chemical score index (CSI) of the MM was quantified according to Traifalgar et al. (2019) using the essential amino acids (EAAs) of *P. vannamei* tissue protein as the reference (Forster et al., 2002). The essential amino acid index (EAAI) of the MM was also computed according to Teruel (2002).

$$\textbf{Chemical score}\ (\%) = \frac{\text{g essential amino acid in 100 g}^{-1}\ \text{mysid meal protein}}{\text{essential amino acid in 100 g}^{-1}\ \text{P. vannamei tissue protein}}\ x\ 100$$

$$\textbf{Essential Amino Acid Index}\ (\textbf{EAAI}) = \sqrt[10]{\frac{100a}{a_r}x\ \frac{100b}{b_r}\ldots x\frac{100j}{j_r}}$$

Where a, b, . . . j = EAAs in the mysid meal protein (% protein)
a_r, b_r, j_r = EAAs in *P. vannamei* tissue protein (% protein).

Statistical analyses

All data in each parameter was analyzed using the SPSS ver. 20.0 Software Statistical Application Program for Windows. Growth indices, feed utilization, biochemical carcass composition, and nutrient retention data of *P. vannamei* were tested for uniformity in variance and normality of distribution before subjecting the effects of different treatments to one-way ANOVA. The experimental data were presented as mean ± SEM. The Tukey's HSD *post hoc* test was run to determine the differences among the five dietary treatment means. Pearson correlation coefficient was also determined to measure the correlation among various parameters. The optimum replacement level of FM by MM in *P. vannamei* diets was done using a quadratic regression analysis. The statistically homogenous means were denoted by similar superscript letters.

Results

Proximate analysis and amino acid profile of mysid meal

Proximate nutritional analysis showed that the mysid meal (MM) contained a protein, lipid, fiber, ash, and carbohydrate content of 53.15 ± 0.07%, 4.42 ± 0.12%, 5.87 ± 0.02%, 20.92 ± 0.53%, and 15.65 ± 0.35%, respectively (Table 1). The nutritional value of this ingredient was found to be high, exhibiting an overall chemical score index (CSI) of 38.85 with an EAAI value of 0.89. This ingredient has an overall EAA/NEAA ratio of 0.90 (Table 3). Compared with the EAA profile of *P. vannamei* tissue protein, each AA content of MM exhibited higher chemical score values except for the leucine, the most limiting AA of this ingredient. In addition, arginine was identified as another limiting AA in MM.

Growth response and feed utilization indices

The survival, growth indices (FBW, %WG, and SGR), and feed utilization (FCR and PER) efficiency of *P. vannamei* postlarvae fed with various dietary treatments are shown in Table 4. The shrimps fed with treatment MM75 significantly obtained the best FBW, %WG, SGR, PER, and FCR among all treatments (Table 4). However, the FBW, %WG, SGR, PER, and FCR values in treatment MM75 were statistically the same ($P > 0.05$) as those obtained in treatments MM25, MM50, and MM100. The *P. vannamei* fed with treatment MM100 (FM-free diet) exhibited growth comparable to that of the MM0 (100% FM-based diet). In addition, the FBW, %WG, SGR, FCR, and PER of shrimps were statistically the same ($P > 0.05$) in treatments MM25, MM50, MM75, and MM100. Moreover, no significant treatment effects were detected in the TFI ($P = 0.338$) and survival ($P = 0.098$) of white shrimps for all the dietary treatments (Table 4). The FBW, % WG, SGR, PER, PR, and LR of *P. vannamei* revealed a significant positive correlation for all the various treatments (Table 5). However, the FCR ($r = -0.56$, $P = 0.01$) of *P. vannamei* showed a significant negative association with the various substitution levels of FM by MM. Furthermore, the FBW, %WG, and SGR of *P. vannamei* revealed a significant positive correlation with the PER, protein retention (PR), and lipid retention (LR) (Table 5). However, FBW, %WG, and SGR of *P. vannamei* revealed a significant negative correlation with the FCR ($P < 0.05$). Quadratic polynomial analysis indicates that a 65.50 % substitution level of FM with mysid meal is optimum to promote maximum growth in *P. vannamei* postlarvae (Figure 1).

Carcass nutrient composition

The whole-body proximate profile of white shrimps fed with various experimental feeds is shown in Table 6. The biochemical carcass composition (i.e., moisture, protein, lipid, and ash content) of *P. vannamei* was not significantly influenced by the increasing levels of dietary MM ($P > 0.05$).

Nutrient retention

Nutrient retention of Pacific white shrimp at the termination of the 60-day growth trial is presented in Table 7. The *P. vannamei*

TABLE 3 EAA profile of *Penaeus vannamei* tissue proteins and mysid meal (chemical score, CSI, EAAI, EAA/NEAA ratio).

Amino acid	Mysid meal (% protein)[1]	*P. vannamei*, EAA profile (% protein)[2]	EAA requirement for shrimp (% protein)	Mysid meal EAA chemical score (%)[3]
Histidine	2.49	1.62	0.80[a]	153.67
Isoleucine	5:00	2.65	1.00[a]	188.49
Leucine	1.82	4.69	1.70[a]	38.43
Lysine	3.79	4.84	1.64[b]	78.34
Valine	1.83	3.10	1.40[c]	58.95
Arginine	2.42	6.10	2.32[d]	39.58
Threonine	2.62	2.52	1.51[e]	104.01
Methionine +cystine	2.93	2.67		109.78
Phenylalanine +tryptophan	7.89	5.39		146.29
Mysid meal CSI[4]				38.83
EAAI				0.89
EAA/NEAA ratio				0.90

[1]Mysid meal EAA (% protein): Actual analyzed values from the Fish Nutrition Laboratory, UP Visayas, CFOS-IA, Miagao, Iloilo, Philippines.
[2]*P. vannamei* EAA (% protein): data derived from Forster et al. (2002).
[3] EAA chemical score = {[EAA amount (g) in 100 g mysid meal protein]/[EAA amount (g) in 100 g shrimp protein]} x 100.
[4]Mysid meal protein CSI = the most limiting AA exhibiting the lowest EAA chemical score (Traifalgar et al., 2019).
[a]Millamena et al. (1999).
[b]Xie et al. (2012).
[c]Teshima et al. (2002).
[d]Zhou et al. (2012).
[e]Zhou et al. (2013).

TABLE 4 Growth, feed utilization indices, and survival of *P. vannamei* postlarvae fed with various levels of MM as a replacement for FM after the 60-day feeding trial.

Treatments	FBW (g)	WG (%)	SGR (%)	TFI (g)	FCR	PER	SR (%)
CTRL	0.90± 0.02[b]	29,983.33 ± 778.77[b]	9.51 ± 0.04[b]	1.83 ± 0.05[a]	2.03 ± 0.05[a]	1.33 ± 0.05[b]	93.00 ± 1.41[a]
MM25	1.04 ± 0.03[ab]	34,400.00 ± 1,130.39[ab]	9.74 ± 0.05[ab]	1.92 ± 0.13[a]	1.86 ± 0.13[ab]	1.45 ± 0.13[ab]	90.00 ± 1.63[a]
MM50	1.13 ± 0.05[a]	37,566.67 ± 1,575.27[a]	9.88 ± 0.07[a]	1.97 ± 0.07[a]	1.75 ± 0.07[ab]	1.54 ± 0.07[ab]	91.00 ± 1.41[a]
MM75	1.15 ± 0.09[a]	38,233.33 ± 2,848.00[a]	9.91 ± 0.12[a]	1.90 ± 0.05[a]	1.67 ± 0.14[b]	1.59 ± 0.14[a]	94.00 ± 1.63[a]
MM100	1.03 ± 0.06[ab]	34,316.67 ± 1,839.64[ab]	9.73 ± 0.09[ab]	1.84 ± 0.03[a]	1.80 ± 0.12[ab]	1.49 ± 0.14[ab]	91.00 ± 1.41[a]
ANOVA *P value*	0.003	0.003	<0.001	0.338	0.015	0.044	0.098

Values (mean ± SEM from four replicate tanks) are significantly different when different lowercase alphabets appear in the same column ($P < 0.05$).

fed with the treatment MM75 exhibited the highest numerical value on protein retention (PR) among all treatments. However, the PR values in the treatment MM75 were not significantly different from those shrimps received with treatments MM25, MM50, and MM100 groups ($P > 0.05$). On the contrary, the lowest PR was exhibited in *P. vannamei* fed with the treatment MM0. However, the PR of *P. vannamei* fed with treatment MM0 was statistically the same ($P > 0.05$) from those shrimps fed with treatment MM25, MM50, and MM100 diets (Table 7). The highest lipid retention (LR) was significantly obtained in *P. vannamei* fed with the treatment MM75. However, there was no significant difference in the LR of shrimps fed with treatments MM0, MM25, and MM50 ($P > 0.05$). The LR of shrimps fed with treatment MM0 was significantly lower than those of shrimps fed with treatments MM25 and MM50 ($P < 0.05$). The PR ($r = 0.46$, $P = 0.04$) and LR ($r = 0.73$, $P = 0.00$) of *P. vannamei* exhibited a significant positive correlation with the different dietary treatments (Table 5). However, the FBW, % WG, and SGR of *P. vannamei* showed a significant positive correlation with the PR and LR (Table 5; $P < 0.05$).

Discussion

The nutritional value of mysid meal (MM) has not been previously evaluated as a dietary protein source ingredient for *P. vannamei*. This study is the first to document the nutritional utilization of MM as a good-quality protein source in the *P. vannamei* diet based on several biological performance indices and biochemical parameters. Amino acid analysis of MM indicates a

TABLE 5 Correlation analysis of *Penaeus vannamei* fed with various levels of MM as an alternative for FM after the 60-day feeding trial.

Parameters	MMRL	FBW (g)	%WG	SGR	TFI (g)	FCR	PER	PR
MMRL								
FBW	**r = 0.48 *P* = 0.03**							
%WG	**r = 0.48 *P* = 0.03**	**r = 1.00 *P* = 0.00**						
TFI	**r = 0.02 *P* = 0.92**	**r = 0.46 *P* = 0.04**	**r = 0.46 *P* = 0.04**					
SGR	**r = 0.50 *P* = 0.02**	**r = 1.00 *P* = 0.00**	**r = 1.00 *P* = 0.00**		**r =0.46 *P* = 0.04**			
FCR	**r = −0.55 *P* = 0.01**	**r = −0.85 *P* = 0.00**	**r = −0.85 *P* = 0.00**	**r = −0.86 *P* = 0.00**	**r = 0.06 *P* = 0.80**			
PER	**r = 0.49 *P* = 0.03**	**r = 0.85 *P* = 0.00**	**r = 0.85 *P* =0.00**	**r = 0.85 *P* = 0.00**	r = −0.06 *P* = 0.78	**r = −0.99 *P* = 0.00**		
PR	**r = 0.46 *P* = 0.04**	**r = 0.83 *P* = 0.00**	**r = 0.83 *P* = 0.00**	**r = 0.83 *P* = 0.00**	r = −0.06 *P* = 0.79	**r = −0.96 *P* = 0.00**	**r = 0.96 *P* = 0.00**	
LR	**r = 0.73 *P* = 0.00**	**r = 0.72 *P* = 0.00**	**r = 0.72 *P* =0.00**	**r = 0.71 *P* = 0.00**	r = −0.13 *P* = 0.59	**r = −0.87 *P* = 0.00**	**r = 0.84 *P* = 0.00**	**r = 0.89 *P* = 0.00**

Values are mean ± SEM from four replicate tanks.
Significantly different results are highlighted in bold ($P < 0.05$). MMRL, mysid meal replacement level; *P*-value, *r* – Pearson correlation coefficient.

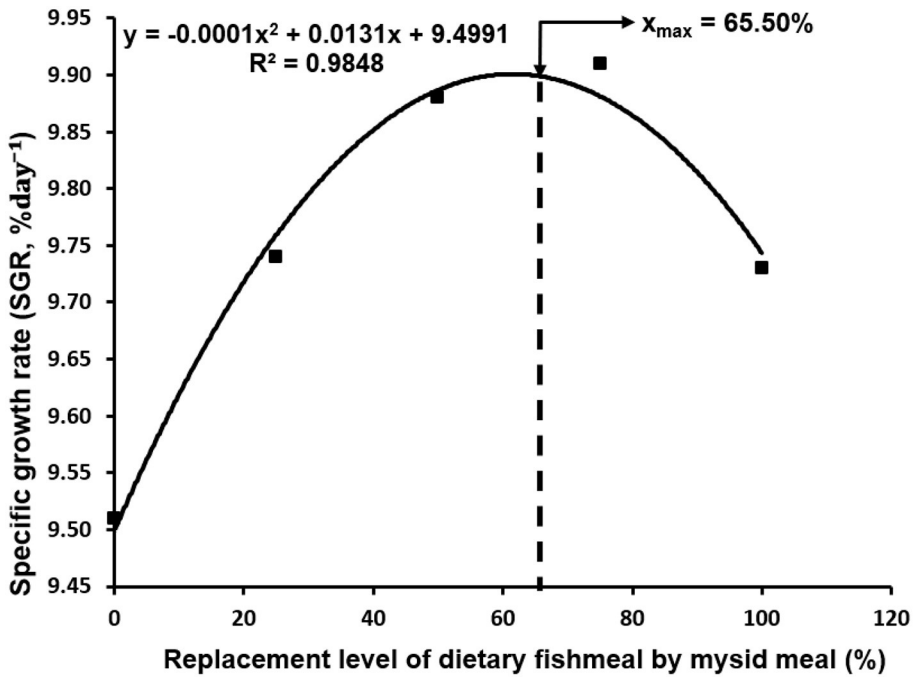

FIGURE 1
Relationship between specific growth rate (SGR, %day^{-1}) and replacement level of dietary fishmeal by mysid meal after the 60-day feeding trial of *Penaeus vannamei* based on quadratic regression analysis. Each point represents the mean of four replicate treatment groups of shrimps. Replacement levels derived with the second-order polynomial (quadratic) model for SGR indicated that the optimal level of dietary mysid meal was 65.50%.

complete and well-balanced AA content with a nutritional value closely similar to or surpassing the AA composition of the *P. vannamei* muscle protein. Moreover, the EAA profile of MM satisfies the ideal dietary EAA requirement of the penaeid shrimp (Table 3). Most EAA content of MM exhibits a higher chemical score closer to or above 100 %. This indicates that these AAs present in MM are sufficient to satisfy the EAA requirement of *P. vannamei* for optimum growth and development. Based on its CSI, the most limiting AA of MM is leucine (38.33%) followed by arginine (39.58%). This implies that MM can only supply 38.33% of leucine and 39.58% required by the *P. vannamei* and that the difference must be supplemented in the diet. Dietary supplementation of

TABLE 6 Biochemical carcass composition of white shrimps fed with various levels of MM as an alternative for FM after the 60-day feeding trial.

Treatments	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
MM0 (Control)	6.40 ± 0.33^{a}	62.46 ± 0.22^{a}	4.52 ± 0.37^{a}	15.81 ± 0.34^{a}
MM25	5.39 ± 1.23^{a}	63.22 ± 0.10^{a}	4.67 ± 0.25^{a}	16.19 ± 0.70^{a}
MM50	4.42 ± 0.57^{a}	62.62 ± 0.43^{a}	4.47 ± 1.01^{a}	16.97 ± 0.09^{a}
MM75	5.74 ± 0.02^{a}	62.53 ± 0.21^{a}	5.08 ± 0.66^{a}	16.89 ± 0.70^{a}
MM100	5.95 ± 0.24^{a}	62.50 ± 0.20^{a}	5.19 ± 0.23^{a}	16.56 ± 0.34^{a}
ANOVA *P value*	0.157	0.131	0.704	0.197

Values (mean ±SEM from four replicate tanks) are significantly different when different lowercase alphabets appear in the same column ($P < 0.05$).

TABLE 7 Nutrient retention of *Penaeus vannamei* fed with various levels of MM as an alternative for FM after the 60-day feeding trial.

Treatments	Protein retention (%)	Lipid retention (%)
MM0 (Control)	19.30 ± 0.79^{b}	6.32 ± 0.26^{c}
MM25	20.67 ± 1.06^{ab}	6.98 ± 0.36^{bc}
MM50	21.97 ± 0.66^{ab}	7.12 ± 0.22^{bc}
MM75	23.46 ± 1.73^{a}	8.81 ± 0.65^{a}
MM100	21.22 ± 1.57^{ab}	8.09 ± 0.60^{ab}
ANOVA *P value*	0.047	0.001

Values (mean ±SEM from four replicate tanks) are significantly different when different lowercase alphabets appear in the same column ($P < 0.05$).

either natural of synthetic leucine will ensure that growth will be at the optimum and will not be limited with the deficiency of this essential amino acid. In addition, MM contains high crude protein (53.15% DM) comparable to FM protein, and this ingredient has an EAAI value of 0.89, considered a good-quality feed protein source for aquafeeds. The EAAI is another critical parameter to assess the nutritional quality of protein of potential feed ingredients (Peñaflorida, 1989). In addition to identifying the limiting AA, this index has been considered when evaluating the nutritional value of proteins because other necessary AAs may also affect the quality of proteins (Hepher, 1988). Peñaflorida (1989) graded feedstuffs following the report of Oser (1959), which classifies potential protein ingredients with an EAAI value of <0.70 as inadequate protein, about 0.80 as suitable protein, and 0.90 as good-quality protein. The MM used in the present study has slightly lower EAAI values than Peruvian FM (0.92), Herring FM (0.95), shrimp meal (0.98), and squid meal (0.96) (Peñaflorida, 1989). However, the EAA/NEAA ratio of MM in the current study is 0.90, which is comparable to the 0.9-1.09 range in FM-based diets reported by Ween et al. (2017) and Kim et al. (2018), as cited by Sobczak et al. (2021). This result demonstrates that MM is classified as a good-quality protein that could provide adequate EAAs to meet the requirement of *P. vannamei* to achieve optimum growth.

The growth performance indices (FBW, % WG, and SGR), feed utilization efficiency parameters (FCR and PER), and survival of *P. vannamei* fed with MM-based diets were similar in all treatment groups at the end of the feeding trial. Shrimp fed with complete replacement of FM (MM100) showed similar growth performance with the control treatment (MM0), receiving the 100% FM-based diet. However, the shrimp fed with increasing levels of MM-based ingredients (MM25, MM50, and MM75) showed a similar trend with the MM100 treatment toward better growth performance and feed utilization. These results suggest that MM can substitute up to 100 % of the FM, whose content accounted for 40% in the FM-based control treatment, without causing any adverse effects on the biological performance of *P. vannamei*. This current finding is the first to document the complete substitution of FM with MM in the *P. vannamei* diet. A similar result was documented by Wei et al. (2022), who found that the zooplankton-based feed ingredient (i.e., krill meal) can completely replace dietary FM without adverse effects on the growth and feed utilization of *P. vannamei*. Previous research findings also have proved that FM could be substituted entirely by krill meal in the diets of various farmed species such as Atlantic cod (Moren et al., 2006; Tibbetts et al., 2011), Atlantic salmon (Olsen et al., 2006), Atlantic halibut (Tibbetts et al., 2011) and triploid rainbow trout (Wei et al., 2019). A similar observation was documented by Hassan et al. (2020), who reported that replacing FM (control diet containing 35% FM) with zooplankton biomass meal up to 100 % significantly enhanced growth performance and feed utilization of seabass fingerlings. Similarly, Kader et al. (2012a) reported that the dehulled soybean meal supplemented with fish soluble, krill meal, and squid meal could completely replace FM in juvenile red sea bream diets without significantly affecting fish performance. Our findings add to these earlier works that MM could completely replace FM in the white shrimp postlarval diet.

The growth-promoting effects of MM-based diets have been significantly observed in shrimp feed with 75% FM replacement. Analysis by polynomial quadratic equation indicates that 65.50% replacement of FM with MM is optimum to elicit a maximum growth response of *P. vannamei*. Similar to our present results, Abo-Taleb et al. (2021) documented the best growth and feed utilization indices in gray mullet larvae when fed with diets containing 75% substitution of FM *by Daphnia magna* meal. Similarly, the growth-stimulating effects of krill meal have also been reported in studies done in *Salmo salar* (Hatlen et al., 2017), *Procambarus clarki* (Gao et al., 2020), *Penaeus monodon* (Smith et al., 2005), *P. vannamei* (Nunes et al., 2011) and *Larimichthys crocea* (Wei et al., 2019). The underlying physiological mechanisms of the growth-promoting effects of zooplankton-based ingredients in fish and crustaceans are not fully understood and remain to be elucidated in future investigations. The enhanced growth performance observed in aquatic animals fed with plankton-based diets has been primarily associated with enhanced palatability,

improved feed intake (FI), and adequate supply of highly available and well-balanced nutrients (Suresh and Nates, 2011; Nunes et al., 2019; Shan et al., 2019). The marked superiority in shrimp growth fed with MM75 over that of the control treatment (MM0) is presumptive evidence for a growth factor in MM. Williams et al. (2005) reported an unknown growth factor in zooplankton-based meals that was present in the insoluble protein component of the meal. This growth factor, associated with zooplankton-based meals, was hypothesized as a protein that activates the neurosecretory hormones of the X-organ-sinus gland complex, which modulates several metabolic processes (i.e., molting, protein synthesis, glucose metabolism, osmoregulation, and reproduction) in crustaceans (Huberman, 2000; Udomkit et al., 2004; Williams et al., 2005).

The proximate whole-body composition analysis of *P. vannamei* indicated that the carcass protein, lipid, moisture, and ash contents were unaffected by FM's replacement level with MM. These findings suggest that MM supplies well-balanced and readily available nutrients and that accelerated growth does not impair the composition of the muscle tissue (Fricke et al., 2023). However, in the current study, dietary MM replacement levels significantly influenced body protein and lipid retention. Protein and lipid retention in treatments fed with MM-based diets are significantly higher than those groups receiving 100% FM-based diets. These high protein and lipid retention must have also contributed to the better growth response in shrimp fed with MM75. The significant enhancement of PR could be attributed to the better AA profile of MM, and the improvement of PR can be linked with the enhancement of protein synthesis (Hernández et al., 2011). In crustaceans, it has been observed that a lower PR is associated with a higher rate of protein catabolism (Bulbul et al., 2016), decreased feed, and inefficient feed utilization (Yue et al., 2012).

PR is considered an important index for the sub-optimal supply and utilization of amino acids (Sanchez-Lozano et al., 2011; Kader et al., 2012b). In this study, the PR values varied between 18.68 and 22.70%, which was relatively high compared to 8.50 to 13.2% reported by Alam et al. (2002) for kuruma shrimp. The result of the study was closer to the findings of Bulbul et al. (2013), who documented a PR ranging from 18.50 to 22.68 % for kuruma shrimp using a combination of plant protein meals to replace FM. Significantly highest PR value was observed in shrimp fed with the MM75 group, indicating better utilization of MM protein by *P. vannamei* in this treatment. This result was supported by a significant growth improvement of *P. vannamei* fed with the MM75 group among treatments. Therefore, the increase in PR would be one of the contributory factors for the significant growth enhancement and improved feed utilization in shrimp fed with the MM75 group. Although there was limited information on the relationship between PR and growth performance, Deng et al. (2006), Sanchez-Lozano et al. (2011), and Kader et al. (2012b) indicated a positive correlation between higher retention of protein and faster growth performances in marine fish species.

The relatively high survival and no significant differences observed in all dietary treatments indicate that the experimental animals are healthy and in good nutritional condition, suggesting that MM is a suitable dietary ingredient to satisfy the nutritional requirement of the white shrimp (Alvarez et al., 2007; Suárez et al., 2009). The significant growth improvement of *P. vannamei* can also be attributed to the high-quality nutrient composition of MM with high crude protein content comparable to FM and complete AA profile (Nordgarden et al., 2003; Espe et al., 2006). The nutritional value of MM as a complete substitution of FM to support the growth and development of *P. vannamei* is considered unprecedented. The findings of this study suggested that MM could be a viable alternative for FM in the *P. vannamei* diet, and this feed ingredient could minimize the pressure on capturing dwindling natural fish stocks for aquaculture feed use.

Conclusion

The current study concludes that MM can successfully replace up to 100% of the FM (40% in the control feed) with no deleterious effects on survival, growth performance, feed utilization, biochemical composition, and nutrient retention of the shrimp. The dietary MM could also elicit growth-promoting effects if utilized at 65.50% replacement of FM in *P. vannamei* post-larval diets.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Visayas. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MA: Conceptualization, Investigation, Methodology, Visualization, Writing—original draft. RT: Conceptualization, Investigation, Methodology, Supervision, Writing—original draft, Writing—review & editing. LL: Conceptualization, Methodology, Writing—original draft. SN: Conceptualization, Methodology, Writing—original draft. MN: Conceptualization, Methodology, Writing—original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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