Functional Assessment of Fish Meal Replacement with Earthworm and Maggot Meals on the Growth and Nutritional Value of the Pacific Whiteleg Shrimp *Litopenaeus vannamei* (Boone, 1930)

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Abstract

Fish meal and other marine-derived proteins constitute approximately 25% of commercial shrimp feed formulations. In intensive and semi-intensive shrimp culture, prepared feeds are a primary cost driver, accounting for 30–40% of total production expenses. To enhance profit margins in this competitive market, replacing fish meal with alternative ingredients is a promising strategy. Ideal substitutes should be readily available, inexpensive, and nutritionally comparable. Studies on species such as freshwater prawns and banana shrimp have shown encouraging results using earthworm (*Oligochaeta*) and maggot (*Diptera*) meals as dietary replacements. This study demonstrates that earthworm and maggot meals can effectively replace fish meal in commercial diets for *L. vannamei*, leading to improved growth performance, enhanced feed utilization, and superior overall production outcomes.

Keywords: Fish meal replacement, Supplemental feeding, Maggot meal, Earthworm meal, Aquaculture, *Litopenaeus vannamei*

1. Introduction

The Pacific white shrimp, *Litopenaeus vannamei* (Boone, 1931), is naturally distributed in the tropical marine habitats of the eastern Pacific Ocean, off the coasts of the USA, Mexico, and Central America. It has been successfully introduced for aquaculture in countries such as India, China, and across Southeast Asia. As part of the macro-benthic fauna, this species prefers shallower regions with ample sunlight (FAO, 2006). Larvae and juveniles are typically found in estuarine and mangrove habitats, while adults live and breed in marine environments. Their development involves six nauplii, three zoeal, and three mysis stages before reaching the post-larval and adult stages. Females grow faster and are generally larger than males, reaching a maximum size of 230 mm with a carapace length of 90 mm (Bailey-Brock & Moss, 1992). The average weight ranges from 30 to 50 grams per individual. They possess a relatively long rostrum with 7–10 dorsal teeth and 2–4 ventral teeth (Pérez Farfante, 1969). The carapace has a translucent white coloration that can vary based on diet and water conditions (FAO, 2006). In their natural habitat, *L. vannamei* are bottom-dwelling omnivores and detritivores, with a diet consisting of phytoplankton, rotifers, and polychaetes. Early-stage post-larvae primarily consume phytoplankton, while their preference shifts to zooplankton during the mysis and later post-larval stages. Older juveniles and adults are bottom feeders, consuming polychaetes and zooplankton detritus (Kungvankij, 1986).

Globally, the demand for crustacean products is high and has increased annually (FAO, 2012). Aquaculture has met a significant portion of this demand, with production reaching 6.9 million tonnes, valued at an estimated 32.33 billion Euros (FAO, 2016). Over 62 crustacean species are farmed worldwide, with *L. vannamei* dominating production (FAO, 2012). Approximately 80% of all farmed shrimp products, amounting to over 3.18 million tonnes, are derived from *L. vannamei* (FAO, 2016). This species' rapid growth, high survival rate even at high stocking densities, efficient feed utilization, and disease tolerance have led to its widespread adoption in intensive culture systems (Wyban & Sweeney, 1991; Ponce-Palafox et al., 1997). Due to these advantageous traits, *L. vannamei* is a predominant choice for global aquaculture.

Although production costs for this species are relatively favorable, further optimization is possible through strategies like feed substitution. Feeds with a minimum of 20% crude protein are considered necessary for optimum shrimp growth (NRC, 2011). In most commercial feeds, fish meal and other marine-derived proteins constitute approximately 25% of the total feed weight (Davis, 2020). This has led to over 30% of global fish production and 17% of global shrimp production being used in commercial feeds (Tacon & Metian, 2008; Naylor et al., 2009). Formulated feeds are a primary cost driver in intensive and semi-intensive shrimp culture, accounting for 30–40% of total production expenses (Tacon, 2004). However, due to limited supplies and high demand for marine-derived ingredients, the economic viability of such feeds is becoming uncertain. Therefore, substituting fish meal in commercial feeds can enhance profit margins in this highly competitive market. Ideal substitutes should be readily available, low-cost, and nutritionally comparable.

Various alternative meals have been tested in shrimp feeds with varying success. Plant-derived meals from soybean, cottonseed, lupin, cowpea, and mung bean have been investigated (e.g., Lim & Dominy, 1990; Sudaryono et al., 1995; Davis & Arnold, 2000). Unconventional animal proteins, such as tadpoles, snails, and termites, have also been used to replace fish meal in various aquaculture species with relatively good results (Kader et al., 2010; Djissou et al., 2016a). Feeds incorporating earthworm and maggot meals have been developed for species including common carp (Pucher et al., 2014), red tilapia (Jabir et al., 2012), Nile tilapia (Ezewudo et al., 2015), and catfish (Aniebo et al., 2009; Ugwumba, 2010). The substitution of fish meal with earthworm and maggot meals has also shown promise in species like freshwater prawns (Habashy, 2012) and banana shrimp (Rachmawati et al., 2022). Furthermore, combination feeds incorporating both fish meal and maggot meal have yielded better outcomes than fish meal alone (Djissou et al., 2016b). Even for *L. vannamei*, the complete replacement of fish meal with soybean meal has demonstrated observable benefits (Olmos et al., 2011).

For the successful replacement of fish meal with unconventional protein sources, the substitutes must contain the ten essential amino acids required for growth (Médale et al., 2013). Numerous studies provide insight into nutrient digestibility for *L. vannamei* (Smith et al., 1985; Davis & Arnold, 1993) and comparative assessments of various feed substitutes (Akiyama et al., 1989; Brunson et al., 1997). The nutrient digestibility of different ingredients is a critical metric for formulating effective fish meal substitutes in commercial feeds.

The biochemical composition of maggots and earthworms has been characterized (Park et al., 2016; Parolini et al., 2020), including their essential amino acid profiles (Djissou et al., 2015). These nutritional values largely fall within the optimal range for penaeid shrimp (Yang et al., 2009; Rachmawati et al., 2022). Collectively, these studies suggest that earthworm and maggot meals can effectively replace fish meal in commercial feeds, potentially improving feed utilization and growth in cultured shrimp. This study aims to evaluate the effects of replacing fish meal with earthworm meal (*Lumbricus rubellus*) and black soldier fly larva (BSFL) meal

(*Hermetia illucens*) in formulated feeds for *L. vannamei* on growth performance, feed utilization, and protein digestibility.

2. Materials and Methods

2.1. Experimental Animals and Acclimation: Juvenile *Litopenaeus vannamei* shrimp were obtained from a local hatchery. Prior to the experiment, 300 animals were selected based on uniform size (110 mm ± 20 mm), weight (5.2 g ± 0.3 g), and the integrity of all body appendages. The selected shrimp were stocked into 15 tanks at a density of 20 individuals per tank. A random sample from the batch was tested for major pathogens using polymerase chain reaction (PCR) to ensure they were disease-free before the trial began. The growth trial was conducted over 42 days from July to August. Shrimp were weighed individually on a weekly basis.

2.2. Experimental System and Water Quality Management: The shrimp were cultured in 60-liter plastic crates (Supreme®) measuring 60 × 40 × 32 cm. Each tank held 20 shrimp. Each experimental diet was randomly assigned to three replicate tanks (n=3). The tanks were cleaned, sun-dried, and filled with saline water (25 ppt) to a depth of 25 cm. Continuous aeration was provided to maintain dissolved oxygen (DO) at optimal levels. A 10% water exchange was performed daily between 08:00 and 10:00 hours. Key water quality parameters—temperature, pH, and DO—were monitored twice daily (08:00 and 16:00 hours) using a multi-parameter meter (Lutron DO-5519SD). Salinity was measured with a refractometer. Total ammonia nitrogen (TAN) was measured every two weeks starting from day zero using the acidimetric titration method.

2.3. Diet Formulation and Feeding Regime: Five isonitrogenous and isoenergetic experimental diets were formulated. The control diet (Diet A) contained fish meal as the primary protein source. In Diets B through E, fish meal was completely replaced by varying ratios of earthworm meal (EWM) and maggot meal (MM; from Black Soldier Fly, *Hermetia illucens*), as follows:

- Diet A: 100% Fish Meal (Control)
- Diet B: EWM:MM = 1:1

- Diet C: EWM:MM = 1:2
- Diet D: EWM:MM = 1:3
- Diet E: EWM:MM = 1:4

All protein meals were commercially sourced with a minimum guaranteed protein content of 40%. The basal composition of all diets included soybean meal, corn meal, wheat flour, a 1:1 mixture of palm and fish oil, a vitamin-mineral premix, and carboxymethyl cellulose (CMC) as a binder. Diets were pelleted (1 mm diameter) using a laboratory feed extruder and air-dried. Shrimp were fed a fixed ration of 5% of their average body weight (ABW) per day, distributed over five feedings (08:00, 11:00, 15:00, 19:00, and 22:00 hours). Each tank received only one diet type for the entire experiment.

Table 1: Proximate composition (% dry matter) of major dietary ingredients

Ingredient	Protein	NFE	Lipid	CF	Ash
Fish Meal	48.33	5.18	15.65	3.27	27.57
Earthworm Meal	45.50	3.27	16.54	24.31	10.38
Maggot Meal	54.01	19.15	12.05	1.61	13.18
Soybean Meal	47.14	33.18	5.03	3.40	11.25
Corn Meal	13.21	0.95	28.52	56.86	0.46
Wheat Flour	10.99	61.03	1.85	14.52	11.61

2.4. Data Collection and Calculations: The average weight of shrimp in each tank was recorded at the start (initial) and end (final) of the experiment. Daily feed consumption was recorded. These data were used to calculate the following performance indices:

- Weight Gain (WG, g) = Final weight - Initial weight
- Relative Growth Rate (RGR, %/day) = [(Final weight - Initial weight) / (Initial weight × Experimental days)] × 100
- Feed Conversion Ratio (FCR) = Dry feed intake (g) / Weight gain (g)

- Feed Efficiency (FE, %) = [Weight gain (g) / Feed intake (g)] × 100
- Protein Efficiency Ratio (PER) = Weight gain (g) / Protein intake (g)
- Apparent Protein Digestibility (ADCp, %) = 100 × [1 - ((% Cr_2O_3 in feed / % Cr_2O_3 in feces) × (%Protein in feces / %Protein in feed))]
- Survival Rate (SR, %) = (Final number of shrimp / Initial number of shrimp) × 100

2.5. Statistical Analysis: The experiment followed a Completely Randomized Design (CRD). All data were checked for normality and homogeneity of variance. One-way Analysis of Variance (ANOVA) was used to determine significant differences ($p < 0.05$) among dietary treatments. Where ANOVA revealed significant effects, Duncan's Multiple Range Test was applied to compare treatment means. All statistical analyses were performed using GraphPad Prism version [*Insert Version Number*]. Water quality data were compared against recommended ranges for the species.

2.6. Chemical Analysis: The proximate composition of the feed ingredients and experimental diets was determined following the standard methods of AOAC (2005). Moisture was determined by drying samples in an oven at 100 °C for 24 hours. Ash content was determined by combusting samples in a muffle furnace at 500 °C for 10 hours. Lipid content was analyzed by Soxhlet extraction with petroleum ether. Crude protein was estimated by the micro-Kjeldahl method (N × 6.25). Nitrogen-Free Extract (NFE) was calculated by difference: NFE = 100% - (%Moisture + %Crude Protein + %Lipid + %Crude Fiber + %Ash).

The essential amino acid (EAA) profiles of the shrimp muscle and the experimental diets were analyzed using an amino acid analyzer. Approximately 1.0 mg of sample was hydrolyzed in 6 N HCl at 110 °C for 24 hours under vacuum. The hydrolysate was filtered (0.2 µm) and injected into the analyzer equipped with an ion-exchange resin column maintained at 53 °C. Amino acids were separated using a sodium citrate buffer gradient (pH 3.3, 4.3, and 4.9) at a flow rate of 0.225 mL/min and post-column derivatized with ninhydrin. The derivatives were detected at 570 nm and 440 nm (for proline) (Ju et al., 2008).

Table 2: Formulation and proximate composition of the experimental diets (g/100 g diet)

Ingredients	Diet A	Diet B	Diet C	Diet D	Diet E
Fish Meal	60	0	0	0	0
Earthworm Meal	0	30	20	15	12
Maggot Meal	0	30	40	45	48
Soybean Meal	15	15	15	15	15
Corn Meal	9	9	9	9	9
Wheat Flour	3	3	3	3	3
Fish Oil	2.2	2.2	2.2	2.2	2.2
Palm Oil	2	2	2	2	2
Vitamin+Minerals	6	6	6	6	6
CMC	2.3	2.3	2.3	2.3	2.3
Cr_2O_3	0.5	0.5	0.5	0.5	0.5
Total	**100**	**100**	**100**	**100**	**100**
Proximate Analysis					
Protein (%)	37.78	38.64	39.49	39.92	40.17
NFE (%)	8.48	12.09	10.51	9.71	9.24
Lipids (%)	12.72	11.91	12.36	12.58	12.72
Energy (kcal/100g)	329.83	340.46	335.32	332.75	331.21

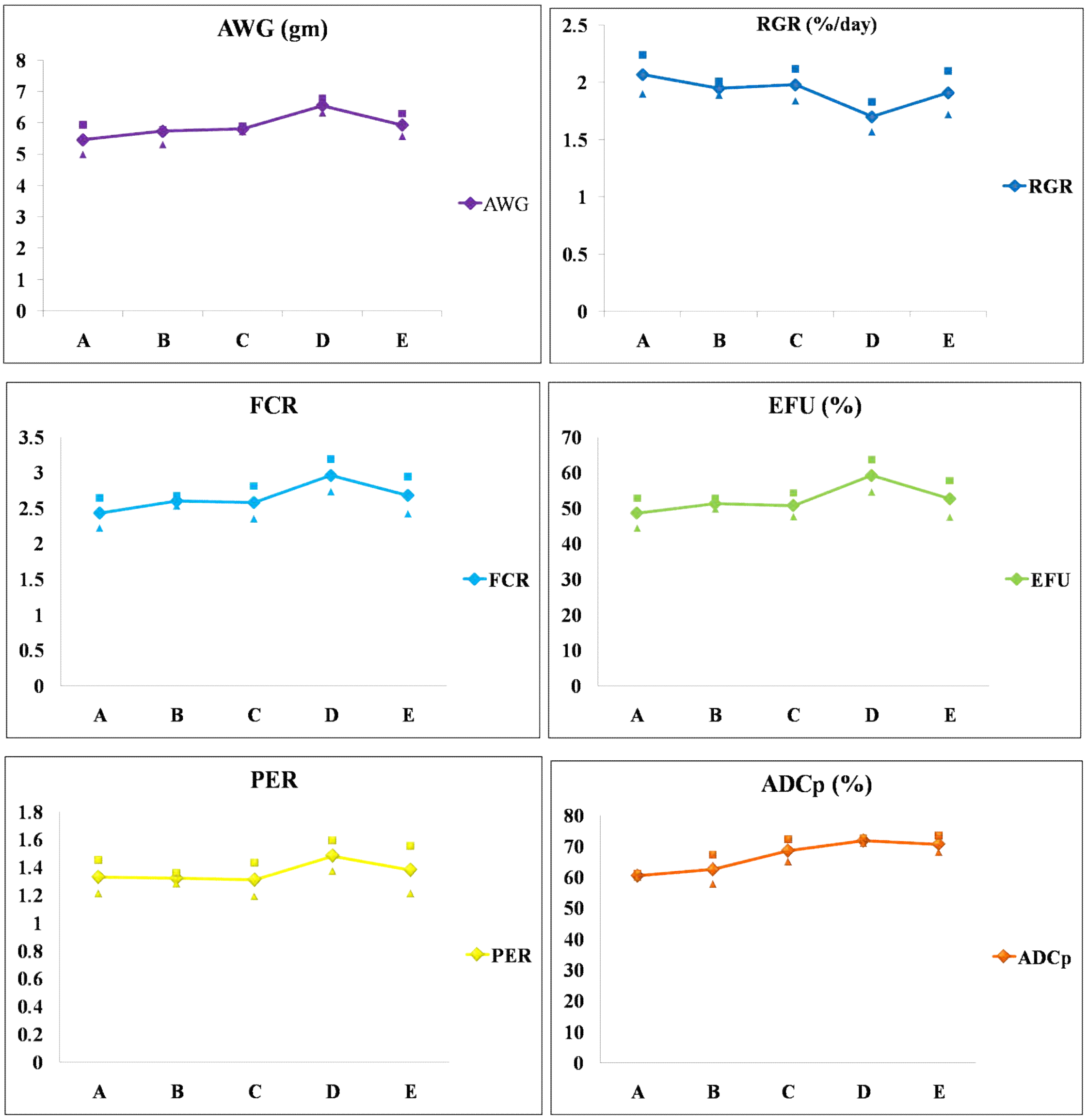
3. Results

The growth performance, feed utilization, and nutritional composition of *Litopenaeus vannamei* fed diets with different earthworm and maggot meal ratios are summarized in Tables 3, 4, and 5.

Table 3. Growth performance and feed utilization of Litopenaeus vannamei fed experimental diets (Mean ± SD)

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E
AWG (g)	5.46 ± 0.48	5.75 ± 0.03	5.80 ± 0.08	6.55 ± 0.23	5.93 ± 0.36
RGR (%/day)	2.07 ± 0.17	1.95 ± 0.06	1.98 ± 0.14	1.70 ± 0.13	1.91 ± 0.19
FCR	2.43 ± 0.21	2.60 ± 0.07	2.58 ± 0.23	2.96 ± 0.23	2.68 ± 0.26
EFU (%)	48.56 ± 4.18	51.19 ± 1.43	50.72 ± 3.51	59.14 ± 4.56	52.59 ± 5.14
PER	1.33 ± 0.12	1.32 ± 0.04	1.31 ± 0.12	1.48 ± 0.11	1.38 ± 0.17
ADCp (%)	60.38 ± 0.60	62.37 ± 4.69	68.49 ± 3.59	71.71 ± 0.82	70.59 ± 2.73
SR (%)	93.33	95.00	93.33	96.67	96.67

Replacement of fish meal with earthworm and maggot meal significantly influenced shrimp growth and feed utilization (Table 3). Diet D (earthworm:maggot, 1:3) yielded the most pronounced results, showing the highest absolute weight gain (AWG) and feed efficiency (EFU). The apparent protein digestibility (ADCp) was also highest in Diet D, with Diets C and E also showing substantially improved ADCp over the control. While the protein efficiency ratio (PER) was numerically highest for Diet D, the difference was not statistically significant. Survival rates were high (>93%) across all dietary treatments.



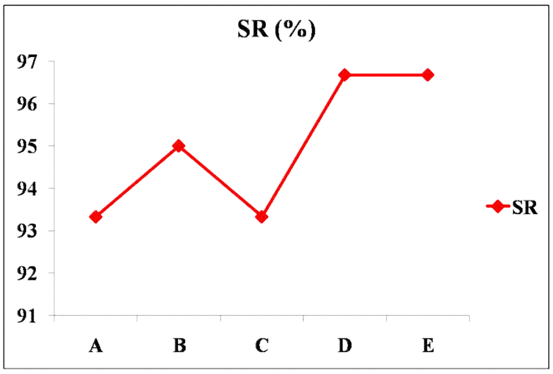


Figure 1. Growth performance and feed utilization parameters of *Litopenaeus vannamei* fed experimental diets. (A) Absolute Weight Gain (AWG), (B) Relative Growth Rate (RGR), (C) Feed Conversion Ratio (FCR), (D) Feed Efficiency (EFU), (E) Protein Efficiency Ratio (PER), (F) Apparent Protein Digestibility (ADCp). Data are presented as mean ± SD (n=3). Different letters above bars indicate significant differences ($p < 0.05$, one-way ANOVA, Duncan's test).

Table 4. Proximate composition (% wet weight) of *L. vannamei* muscle at the start (Initial) and end of the experiment, fed different experimental diets (Mean ± SD).

Sample	Protein	Lipids	Carbohydrates	Ash
Initial	59.62 ± 2.44	5.68 ± 0.26	31.37 ± 0.87	3.33 ± 0.12
Diet A	62.33 ± 1.56	4.31 ± 0.19	29.14 ± 3.72	4.22 ± 0.11
Diet B	63.27 ± 1.39	4.37 ± 0.17	27.13 ± 2.30	4.23 ± 0.14
Diet C	62.78 ± 1.78	4.56 ± 0.41	28.05 ± 1.03	4.61 ± 0.33
Diet D	68.32 ± 3.09	4.24 ± 0.17	23.76 ± 3.59	3.68 ± 0.11
Diet E	65.95 ± 2.57	4.00 ± 0.14	26.09 ± 1.73	3.96 ± 0.09

The experimental diets also affected the final body composition of the shrimp (Table 4). Shrimp fed Diets D and E exhibited significantly higher body protein content compared to the control and other diets, with Diet D showing the highest accumulation. This increase in protein was accompanied by a corresponding decrease in body lipid and carbohydrate content.

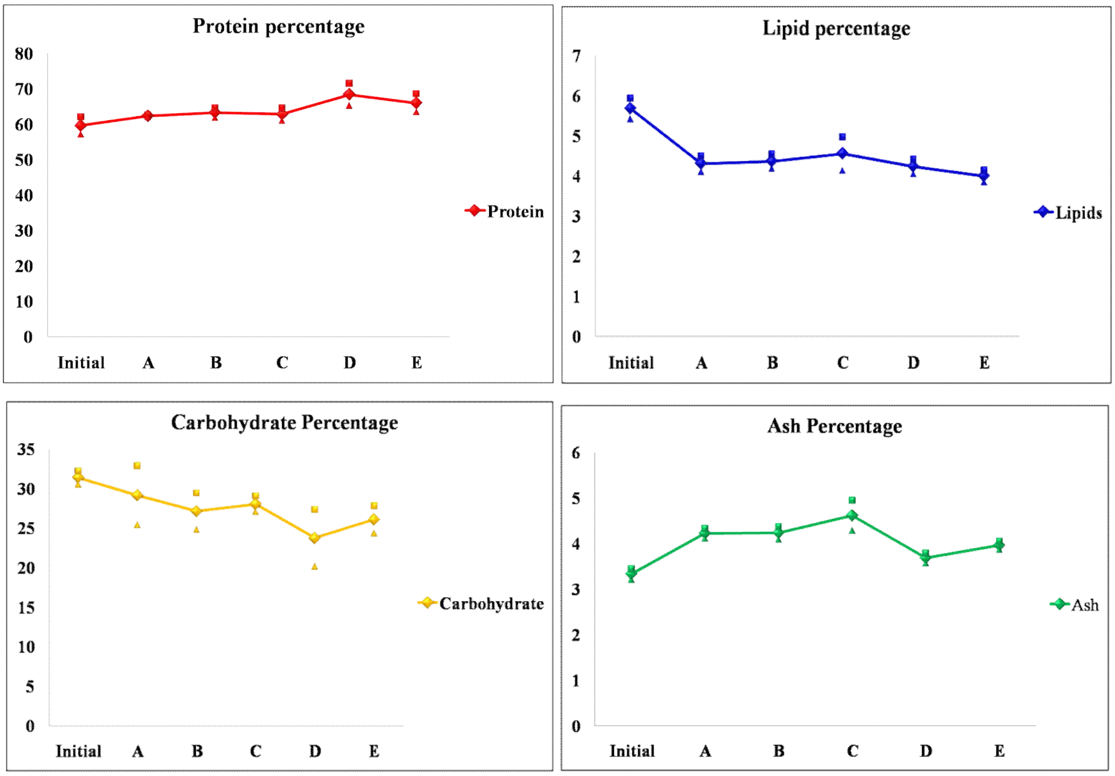


Figure 2. Proximate composition of *Litopenaeus vannamei* muscle after feeding with experimental diets. (A) Crude Protein, (B) Crude Lipid, (C) Carbohydrate, (D) Ash. Data are presented as mean ± SD. Different letters above bars indicate significant differences ($p < 0.05$).

Table 5. Essential amino acid (EAA) profile (g/kg dry matter) of the experimental diets compared to the optimum requirement for *L. vannamei*.

EAA	Optimum*	Diet A	Diet B	Diet C	Diet D	Diet E
Arginine	61.36	55.87	54.51	60.88	56.64	68.61
Histidine	18.88	25.48	20.66	18.64	19.95	19.95
Isoleucine	38.70	67.95	45.90	41.86	43.88	35.90
Leucine	65.14	42.47	53.55	64.69	71.40	52.65
Lysine	58.06	54.55	66.75	57.08	58.24	65.82
Methionine	25.49	35.86	36.34	22.30	27.92	29.52
Phenylalanine	40.59	40.77	22.19	41.86	43.08	25.93
Threonine	34.46	36.43	37.49	34.21	43.88	51.06
Valine	40.59	18.12	45.14	39.00	33.91	49.46

The essential amino acid (EAA) profiles of the experimental diets were largely conformant with the known requirements of *L. vannamei* (Table 5). While some divergences were observed, particularly in Diets A and E, most EAA levels fell within the expected range. Diet D was characterized by higher levels of leucine, phenylalanine, and threonine relative to the optimum.

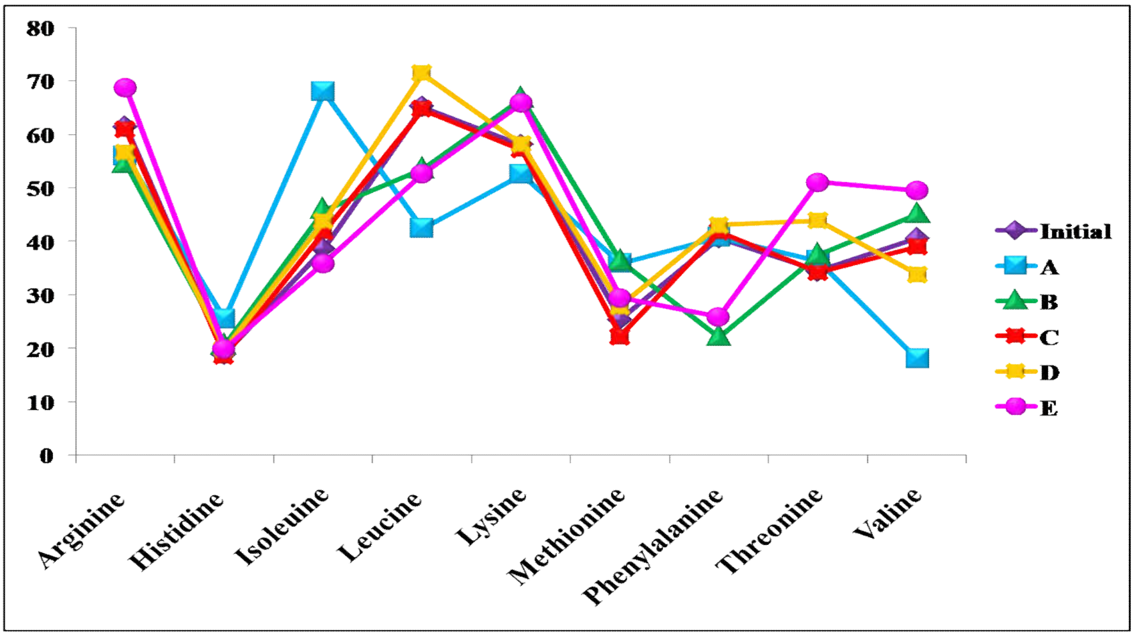


Figure 3. Essential amino acid (EAA) profile of the experimental diets. The purple line represents the optimum EAA requirement for *Litopenaeus vannamei*.

4. Discussion

The development of alternative feeds holds significant potential to reduce costs, increase yield, and mitigate the environmental impact of the aquaculture sector. While fish meal is an optimal ingredient for shrimp feed, its depletion and rising cost have necessitated the search for sustainable alternatives. Animal-based protein sources have generally demonstrated better efficacy than plant-based meals in shrimp nutrition (Kureshy, 2002). Non-conventional sources like earthworm and maggot meal are particularly promising due to their high protein content, lower production costs, and shorter cultivation cycles (Djissou et al., 2016), making them ideal candidates for fish meal replacement.

In the present study, the complete replacement of fish meal with various combinations of earthworm and maggot meal yielded positive results. Shrimp fed the experimental diets (B-E) showed improved growth performance and feed utilization compared to the control (Diet A). Specifically, Diet D (earthworm: maggot, 1:3) consistently produced the most favorable outcomes, with significantly higher absolute weight gain (AWG), feed efficiency (EFU), and apparent protein digestibility (ADC_p), alongside a lower feed conversion ratio (FCR). The enhanced growth can be directly attributed to improved nutrient digestibility, as a positive correlation between high ADC_p, improved PER, and reduced FCR has been well-established (Jabir et al., 2012). The superior performance of Diet D suggests that a higher inclusion level of maggot meal is particularly beneficial for nutrient absorption and growth in *L. vannamei*. Furthermore, the high survival rates (>90%) across all treatments indicate that none of the experimental diets induced negative health effects or degraded water quality, a finding consistent with previous studies using similar alternative protein sources (Djissou et al., 2016; Rachmawati & Nurhayati, 2022).

Proximate analysis revealed that the experimental diets had higher crude protein levels than the fish meal-based control. More importantly, the body composition of the shrimp was significantly influenced by the dietary treatments. Shrimp fed Diets D and E exhibited a significantly higher final body protein content compared to the control, with Diet D again yielding the highest value. This increase in protein retention is likely a direct consequence of the higher ADC_p and lower FCR observed in these groups, leading to more efficient utilization of dietary protein for growth.

The growth performance of aquatic animals is profoundly influenced by both the protein level and the essential amino acid (EAA) profile of the diet (Peres & Oliva-Teles, 2009). Diets that closely mirror the EAA requirements of the species promote optimal growth and minimize nitrogen waste (Akiyama et al., 1992; Adesina, 2012). In this study, the EAA profile of Diet D was found to most closely align with the known optimum requirements for *L. vannamei* (NRC, 2011). Notably, Diet D contained elevated levels of leucine and threonine, which may have contributed to its enhanced performance, though this warrants further investigation. The suboptimal growth in other dietary groups can be reasonably attributed to greater deviations in their EAA profiles from the ideal. The absence of water quality degradation throughout the experiment suggests that any EAA imbalances in Diets A-E were within a manageable range and did not lead to significant nitrogen catabolism (Médale et al., 2009).

In conclusion, this study demonstrates that a 1:3 ratio of earthworm to maggot meal (Diet D) can effectively replace 100% of the fish meal in diets for *L. vannamei*, resulting in superior growth, enhanced feed efficiency, and improved protein retention. These findings align with a growing body of evidence supporting the use of earthworm and maggot meals as viable, unconventional protein sources in aquaculture (Aniebo et al., 2009; Jabir et al., 2012; Pucher et al., 2014; Ezewudo et al., 2015; Rachmawati & Nurhayati, 2022). Future research should focus on optimizing the earthworm-to-maggot ratio for different shrimp life stages and investigating the efficacy of different species within these genera.

5. Conclusion

This study demonstrates that fish meal can be successfully replaced with a combination of earthworm and maggot meal in diets for Pacific white shrimp, *Litopenaeus vannamei*. Among the tested ratios, Diet D, with an earthworm to maggot ratio of 1:3, was the most effective, resulting in significantly improved growth performance and feed utilization. This was evidenced by the highest values for absolute weight gain, feed efficiency (EFU: 59.14%), protein efficiency ratio (PER: 1.48), and apparent protein digestibility (ADC_p: 71.71%), coupled with a lower feed conversion ratio (FCR: 1.70). The high survival rate (96.67%) confirms that this dietary replacement has no adverse effects on shrimp health. Therefore, the 1:3 earthworm-maggot meal combination presents a viable and sustainable alternative to fish meal in commercial feeds for *L. vannamei*.

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