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Research Article

Use of mealworm *Eisenia fetida* cultivated in vegetal and animal substrate as a dietary supplement for the whiteleg shrimp *Penaeus vannamei*

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ABSTRACT. Mealworm (MW) from the red wriggler, *Eisenia fetida*, has been used as a dietary substitute or complement in aquaculture diets. However, the substrate in which it is cultivated varies its proximate composition. This study aimed to evaluate the inclusion of MW produced in two substrates-vegetal culture (VC) and animal culture (AC)-in mixed diets for juvenile *Penaeus vannamei*. A 2×3 factorial experimental design was used, consisting of two substrates (VC and AC) and three inclusion levels (5, 10, and 20%), yielding six diets, each with three replicates. Shrimp (2.29 ± 0.13 g) were cultured for 75 days in a recirculating system and were fed twice daily. Growth performance, survival, and digestive enzyme activity were evaluated. Shrimp fed with 10% VC showed a higher final weight (8.07 ± 0.39 g) and weight gain (5.66 ± 0.45 g) ($P < 0.05$) at the end of the experiment. The final weight, weight gain, and specific growth rate were higher in organisms fed with VC, regardless of the inclusion level. Survival was similar (>90%) across all treatments ($P > 0.05$). Except for lysine, the levels of essential and nonessential amino acids increased with the MW substitution level produced using both substrates. Both 10% VC and 10% AC groups showed the highest digestive protease activity (39.83 ± 3.0 and 50.87 ± 4.4 U mg protein^{-1} in VC and AC, respectively). In contrast, the highest chymotrypsin activity was observed in the 5% AC group (6.08 ± 0.43 U mg chymotrypsin^{-1}). The results indicate that incorporating MW from *E. fetida*, produced on either substrate, into a mixed diet alters shrimp growth and enzymatic activity without affecting survival. However, future studies evaluating MW as an ingredient in formulated shrimp feeds should proceed with caution, particularly regarding the characteristics of the worm-culture substrate.

Keywords: alternative protein sources; fish meal replacement; digestive enzyme activity; mixed diets; sustainable aquafeeds

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INTRODUCTION

Due to its biochemical characteristics, fish meal (FM) has been the most important ingredient in the formulation of aquaculture diets. However, the current overexploitation of "feed fish" (e.g. sardines, anchovies) has reduced their availability and increased their cost (Dedeke et al. 2013, Shao et al. 2018), which has prompted an intense search for FM substitutes, including plant- and/or animal-derived ingredients that reduce production costs while being readily available and environmentally friendly, a priority in aquaculture nutrition (Kumlu et al. 2018, Musyoka et al. 2019).

One strategy to reduce FM is to replace it with vegetable meals, either partially or completely. As an important feed strategy adopted by our lab a few years ago, several legumes have been evaluated as FM substitutes in various commercial aquatic species, such as the whiteleg shrimp (*Penaeus vannamei*) and the Nile tilapia (*Oreochromis niloticus*) (Cuevas-Rodríguez et al. 2006, Montoya-Mejía et al. 2016, Valdez-González et al. 2016). For example, Tejeda-Miramontes et al. (2023) studied the inclusion of chickpea flour (*Cicer arietinum*) in the diet of *P. vannamei*, concluding that FM can be substituted up to 60% without affecting performance. On the other hand, González-Félix et al. (2023) demonstrated that the dietary inclusion of a 15% concentrate of the same grain did not compromise the growth of this species in the laboratory or under field conditions and, in addition, yielded a higher profit indicator (1.34) than the control group (1.26). Nonetheless, vegetable ingredients in aquaculture diets typically exhibit inherently low protein content, deficiencies in essential amino acids (EAAs), and antinutritional factors (Dedeke et al. 2013, Chiu et al. 2016, Shao et al. 2018, Musyoka et al. 2019), which regularly hinder organism development during cultivation. From the above, the formulation of mixed diets - containing animal and vegetable protein sources- is presented as a viable option in the production of less costly aquafeed supplies with an adequate energy profile to meet the nutritional requirements of organisms (Chiu et al. 2016).

The mealworm (MW), primarily derived from red wriggler (*Eisenia fetida*), has been studied as an alternative dietary ingredient for commercially important crustaceans and fish. *E. fetida* is the most used worm species due to its easy culture, protein content (50-67%) (Vielma Rondón & Medina 2006), high reproductive rate, and capacity to feed on different organic wastes, which decreases its production costs (Ibáñez et al. 1993, Musyoka et al. 2019). For instance, Dedeke et al. (2013), Chiu et al. (2016), Kumlu et al. (2018), and Musyoka et al. (2019) reported nutritional similarities between MW and FM in experimental diets for the African sharptooth catfish, *Clarias gariepinus*, and the Pacific white shrimp, *P. vannamei*. Although this annelid is rich in proteins, EAA, n-3 PUFA, minerals, and trace elements (Chiu et al. 2016, Kumlu et al. 2018, Musyoka et al. 2019), the concentrations of each compound vary with the substrate they are cultivated in (Kumlu et al. 2018, Musyoka et al. 2019), which would affect their nutritional quality.

The whiteleg shrimp is the most commercially important crustacean worldwide (Choi et al. 2018, Shao et al. 2018, Javahery et al. 2019). *P. vannamei* easily adapts to dietary changes (Gamboa-Delgado et al. 2003) and can digest both animal and vegetal protein (Cuzon et al. 2004); this allows the inclusion of diverse dietary energetic sources in its feed, which is known as a "mixed diet" (Alves & Tavares 2019). Since vegetables, as unique protein source in the diet, are deficient in the quantity and quality of their dietary requirements (Shao et al. 2018), the inclusion of MW in this kind of diets for shrimp could complement the latter profiles, including amino acids and fatty acids (Dedeke et al. 2013), as well as reducing the production cost of the diet.

This study aimed to evaluate the partial substitution of FM with MW, produced from two different substrates and incorporated into a mixed diet based on chickpea flour, on the growth, survival, and enzymatic activity of *P. vannamei* juveniles raised under laboratory conditions. It is hypothesized that dietary inclusion of MW does not compromise shrimp development.

MATERIALS AND METHODS

Experimental diets

One kilogram of live worms (*E. fetida*) was collected from each breeding module (vermicompost beds) from the IPN-CIIDIR-Sinaloa Unit; one enriched with sheep manure (animal culture; AC) and the other with kitchen vegetal waste (vegetal culture; VC). Subsequently, the worms were frozen (-20°C), lyophilized for 48 h under vacuum conditions of 0.220 mBar (FreeZone 2.5 plus LABCONCO), and ground in a Torrey mill (model M-22R, 745 W capacity). Finally, the proximate composition and amino acid profiles of *E. fetida* MW produced on both substrates were obtained (Table 1). Crude protein was determined using the Kjeldahl method (Das et al. 2024), and crude lipids were extrac-

Table 1. Proximate composition of earthworm meal (*E. fetida*) (mean ± standard deviation). NFE: nitrogen free extract. Different superscript letters within the same row indicate significant differences between compost types (vegetal *vs*. animal), according to Tukey's multiple comparison test ($P < 0.05$).

	Vegetal compost	Animal compost
Proximate composition (g kg^{-1})		
Moisture	4.96 ± 0.41^{b}	5.46 ± 0.25^{a}
Crude protein	69.92 ± 0.66^{a}	64.74 ± 0.87^{b}
Lipid	9.83 ± 0.08^{a}	9.60 ± 0.13^{b}
Crude fiber	0.57 ± 0.17^{b}	1.08 ± 0.17^{a}
Ash	5.81 ± 0.06^{b}	8.86 ± 0.08^{a}
NFE	13.87	15.72
Energy (kcal g^{-1})	426.70 ± 2.01^{a}	410.48 ± 1.01^{b}
Essential amino acids		
Lysine	1.69	2.37
Arginine	6.20	6.57
Histidine	8.43	7.92
Isoleucine	2.52	2.19
Leucine	5.71	5.12
Valine	3.46	3.06
Methionine	1.64	1.46
Phenylalanine	3.22	2.62
Threonine	16.27	17.96
Nonessential amino acids		
Aspartate	6.84	6.95
Glutamate	17.17	15.16
Serine	3.81	4.44
Glycine	4.67	4.09
Alanine	4.76	4.60
Tyrosine	7.82	6.63

ted with anhydrous ether using a Soxhlet system (Hewavitharana et al. 2020). Ash was determined using a muffle furnace (AOAC International 2019), crude fiber was obtained using the phenol-sulphuric acid method (Hoang et al. 2025), and nitrogen-free extract was estimated by difference. The amino acid content of MW was determined according to Yu et al. (2023).

Three samples from each diet were pooled and analyzed for proximate composition; the amino acid content of the tested diets (Table 2) was determined according to Yu et al. (2023). Also, the proportion of the total amino acids and total EAA was determined (essential amino acid index, EAAI = EAA content / total EAA content) from each diet (Table 3).

VC and AC mealworms were included at 5, 10, and 20% (Sharifinia et al. 2025) (Table 4) in a chickpea meal-based diet (Tejeda-Miramontes et al. 2023) to formulate and elaborate six experimental diets: 5% VC, 10% VC, 20% VC, 5% AC, 10% AC, and 20% AC.

Growth bioassay

The experiment was conducted in 40 L plastic containers connected to a recirculation system. Six shrimp (2.29 ± 0.13 g) were placed in each container (10 m^{-2}); each experimental diet was replicated three times. The bottoms of the containers were cleaned twice daily by siphoning to remove waste. Shrimp were fed twice daily at 4% of total body biomass. During the 75 culture days, the temperature (31 ± 2°C), salinity (30.5 ± 3), dissolved oxygen (4.7 ± 0.8 mg L^{-1}), ammonium (0.047 ± 0.009 mg L^{-1}), nitrites (0.009 ± 0.004 mg L^{-1}), and nitrates (0.286 ± 0.004 mg L^{-1}), were maintained stable within the culture rate for *P. vannamei* (Nazarudin et al. 2025). Shrimp biometrics (weight and total length) were recorded every week to determine weight gain (WG = final weight - initial weight), mean final weight (MFW = final weight of total organisms / total number of organisms), absolute growth rate (AGR = [final weight - initial weight] / time), specific growth rate (SGR = 100 × [Ln final

Table 2. Formulation of diets used in shrimp *P. vannamei* growth bioassay. VC: vegetal culture, AC: animal culture. Values given in grams. Values expressed in g 100 g^{-1}. Energy expressed in kcal g^{-1}. NFE: nitrogen-free extract; proximate analysis: average of three experimental replicates ± standard deviation.

	Vegetal compost			Animal compost		
	5%	10%	20%	5%	10%	20%
Fish meal	122.4	114.7	92.6	122.4	114.7	92.6
Chickpea flour	516.5	489.2	426.5	516.5	489.2	426.5
Soybean past	190	175	160	190	175	160
Worm meal VC	50	100	200	0	0	0
Worm meal AC	0	0	0	50	100	200
Grenetine	40	40	40	40	40	40
Fish oil	40	40	40	40	40	40
Soybean lecithin	40	40	40	40	40	40
Mix of vitamins	0.1	0.1	0.1	0.1	0.1	0.1
Mix of minerals	1	1	1	1	1	1
Proximal analysis (g kg^{-1})						
Humidity	13.06 ± 0.19	11.94 ± 0.11	13.31 ± 0.07	8.16 ± 0.16	8.64 ± 0.12	9.47 ± 0.09
Proteins	44.03 ± 0.49	43.54 ± 0.79	42.45 ± 0.59	40.69 ± 0.54	43.53 ± 0.11	44.02 ± 0.47
Lipids	10.13 ± 0.09	10.42 ± 0.09	11.00 ± 0.15	10.46 ± 0.07	10.49 ± 0.01	10.69 ± 0.09
Fiber	2.31± 0.09	2.49 ± 0.08	1.46 ± 0.05	1.40 ± 0.05	1.17 ± 0.15	1.20 ± 0.09
Ash	5.15 ± 0.03	5.17 ± 0.05	5.15 ± 0.03	5.66 ± 0.05	5.83 ± 0.01	6.34 ± 0.04
NFE	38.37	38.39	39.95	36.33	38.99	37.75
Energy	418.37 ± 0.46	418.37 ± 0.18	424.30 ± 0.61	420.42 ± 0.59	421.25 ± 0.58	419.78 ± 0.49
Essential amino acids						
Lysine	2.35	2.29	2.15	2.39	2.36	2.29
Arginine	2.74	2.90	3.21	2.76	2.94	3.28
Histidine	1.33	1.70	2.42	1.31	1.64	2.32
Isoleucine	1.37	1.42	1.50	1.35	1.38	1.43
Leucine	2.46	2.61	2.89	2.43	2.55	2.77
Valine	1.55	1.64	1.80	1.53	1.60	1.72
Methionine	0.74	0.78	0.85	0.73	0.76	0.82
Phenylalanine	1.58	1.65	1.79	1.55	1.59	1.67
Threonine	1.96	2.70	4.17	2.04	2.87	4.51
Nonessential amino acids						
Aspartate	3.26	3.42	3.72	3.26	3.43	3.74
Glutamate	5.77	6.32	7.42	5.67	6.12	7.02
Serine	1.47	1.58	1.80	1.50	1.65	1.93
Glycine	1.94	2.07	2.29	1.92	2.01	2.18
Alanine	1.83	1.97	2.22	1.82	1.95	2.19
Tyrosine	1.33	1.66	2.33	1.27	1.55	2.09

weight / Ln initial weight] / time), feed conversion ratio (FCR = feed supplied / WG), and condition factor (K = 100 × [total weight / (total length)^3]). The final survival of each replicate was determined at the end of the bioassay.

Digestive enzyme activities

Two shrimps from each replicate (N = 6 per treatment) were randomly selected on experimental day 75 for enzymatic activity measurements. First, the crustaceans were weighed (wet weight; ww) and kept at -20°C until analysis. Only the hepatopancreas and intestines were analyzed. The anterior section of the head, all appendages, and exoskeleton were discarded during dissection. The remaining tissue was individually weighed and homogenized with cold distilled water (4°C) in a v/w proportion of 4 mL water g^{-1} fresh organ tissue. Raw extracts were separated by centrifugation at 14,000 rpm for 10 min at 4°C, and the clarified crude extract was kept at -20°C until analyzed for soluble proteins and enzyme activity. Analytical measurements were performed in quadruplicate for all enzymatic anal-

Table 3. Essential amino acid index (EAAI) of the experimental diets and the whole body of *P. vannamei.*

Amino acid	Diets						
	Vegetal compost			Animal compost			*P. vannamei* juveniles
	5%	10%	20%	5%	10%	20%	(2 g)
Lysine	14.64	12.99	10.36	14.85	13.36	11.00	14.36
Arginine	17.05	16.40	15.44	17.15	16.59	15.78	13.7
Histidine	8.29	9.59	11.63	8.12	9.30	11.13	3.8
Isoleucine	8.50	8.01	7.22	8.40	7.82	6.89	10.78
Leucine	15.28	14.74	13.89	15.09	14.40	13.31	16.29
Valine	9.64	9.26	8.65	9.52	9.02	8.25	10.58
Methionine	4.60	4.40	4.11	4.54	4.30	3.93	1.44
Phenylalanine	9.82	9.35	8.62	9.63	9.00	8.04	11.26
Threonine	12.17	15.27	20.07	12.69	16.21	21.67	9.06

Table 4. Biological evaluation of *P. vannamei* after 75 days of laboratory culture. *Significant difference ($P < 0.05$); n.s.: no significant difference ($P > 0.05$). SGR: specific growth rate. FCR: feed conversion rate. Different superscript letters within the same column indicate significant differences among treatments, according to Tukey's multiple comparison test ($P < 0.05$). C×I indicate the interaction effect between compost type (C) and inclusion level (I) evaluated by two-way ANOVA.

	% Inclusion	Initial weight (g)	Final weight (g)	Weight gain (g)	SGR (% d^{-1})	FCR	Survival (%)
Vegetal compost	5	2.19 ± 0.22	7.01 ± 0.42b	4.81 ± 0.26ab	1.55 ± 0.07	2.34 ± 0.03	95.24 ± 8.2
	10	2.40 ± 0.07	8.07 ± 0.39a	5.66 ± 0.45a	1.61 ± 0.10	2.04 ± 0.22	90.47 ± 4.8
	20	2.26 ± 0.07	7.42 ± 0.22ab	5.16 ± 0.14ab	1.58 ± 0.01	2.06 ± 0.08	90.47 ± 4.8
Animal compost	5	2.34 ± 0.18	7.42 ± 0.58ab	5.07 ± 0.67ab	1.53 ± 0.17	2.16 ± 0.36	95.23 ± 8.2
	10	2.25 ± 0.12	6.64 ± 0.55b	4.38 ± 0.67b	1.43 ± 0.18	2.25 ± 0.29	90.47 ± 4.8
	20	2.33 ± 0.14	6.62 ± 0.12b	4.28 ± 0.04b	1.39 ± 0.05	2.53 ± 0.20	90.47 ± 4.8
Two-way ANOVA							
Compost		n.s.	*	*	*	n.s.	n.s.
Inclusion		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C×I		n.s.	*	*	n.s.	n.s.	n.s.

yses. A control sample (blank) was also included, and the enzyme reagent was added after the reaction had ceased.

Protein concentrations in the enzyme raw extracts were measured using the Bradford (1976) method. The process involved combining 8 µL of crude extract, 792 µL of distilled water, and 200 µL of Bradford reagent in glass tubes, then gently vortexing. Absorbance was read at 595 nm. Bovine serum albumin (05470, Sigma-Aldrich, St. Louis, MO) was used as the protein standard.

Alkaline digestive protease activity was quantified according to the method described by Arreola et al. (2023), using 1% azocasein as a substrate. Lipase activity was determined using β-naphthyl caprylate as the substrate (Nolasco-Soria 2023). Lipase activity was expressed as lipase units mg $protein^{-1}$ (one lipase unit was the number of enzymes required to increase 0.01 absorbance units at 540 nm min^{-1}). Amylase activity was measured as described by Arreola et al. (2023), using 1% starch in 50 mM Tris-HCl at pH 7.5 as the substrate; its activity was expressed as amylase units mg $protein^{-1}$ (one amylase unit was defined as the quantity of enzyme that increased the absorbance units by 0.01 at 550 nm min^{-1}). Trypsin activity was determined using BAPNA as the substrate (Nuntapong et al. 2019). Samples were adapted to a 96-well microplate by adding 10 µL of crude extract, 160 µL 60 mM Tris-HCl at pH 8.0, 10 µL 192 mM $CaCl_2$ at pH 8.0, and 10 µL 9.6 mM BAPNA dissolved in DMSO, in each well to start the reaction. Chymotrypsin activity was deter-

mined (Satjarak et al. 2024) by adding 9.6 mM SAPNA dissolved in dimethyl sulfoxide (DMSO). For both enzymes, the absorbance at 414 nm was recorded every 15 s for 30 min. At the end of the assay, the linear coefficient was calculated to determine the increase in absorbance per second. The enzyme activity was calculated using the molar extinction coefficient of p-nitroaniline (8800).

Statistical analysis

Growth and enzyme activity values were tested for normality (Lilliefors) and variance homogeneity (Bartlett's test; Ge et al. 2025). A bifactorial ANOVA and Tukey's multiple range test were used to compare the mean digestibility of the diet ingredients. The factors and levels analyzed corresponded to worm substrates and their dietary inclusion percentages. Statistica 7.0 software (StatSoft, Tulsa, OK, USA) was used for the analysis, with significance set at $P < 0.05$.

RESULTS

Proximate composition of mealworm and experimental diets

The VC group presented a higher content of crude protein, lipids, and energy ($P < 0.05$) and a higher amount of histidine, isoleucine, leucine, valine, methionine, phenylalanine, glutamate, glycine, alanine, and tyrosine. Meanwhile, the AC treatments showed higher humidity content, crude fiber, ash, nitrogen-free extract ($P < 0.05$), and a higher quantity of lysine, arginine, threonine, aspartate, and serine (Table 1).

Table 3 shows the EAAI percentages of the experimental diets. Except for histidine and threonine, the EAAI of the remaining amino acids decreased as the MW inclusion level increased. The VC diets showed lower concentrations of isoleucine, leucine, valine, and phenylalanine than the AC diet. The percentages of arginine, histidine, methionine, and threonine in the experimental diets were higher than those in the whole shrimp body.

Growth bioassay

Shrimp fed with 10% VC obtained the highest final weight (8.07 ± 0.39 g) and WG (5.66 ± 0.45 g) ($P < 0.05$; Table 4). The final weight, WG, and SGR were higher in shrimps fed with the VC diets, regardless of the inclusion level ($P < 0.05$). The FCR and survival rates did not differ significantly among the treatments ($P > 0.05$).

Enzymatic activity

The highest digestive protease activity was observed in the AC group (50.87 ± 4.4 U mg protein^{-1}), whereas chymotrypsin activity was highest in the 5% AC group (6.08 ± 0.43 U mg protein^{-1}) ($P < 0.05$). The inclusion of 10% of MW produced in both substrates showed the highest protease activity (39.83 ± 3.0 and 50.87 ± 4.4 U mg protein^{-1} in VC and AC, respectively; $P < 0.05$). The source of the substrate did not affect the activity of any enzyme studied. Only protease and chymotrypsin activities showed significant differences ($P < 0.05$) due to the joint effect of substrate and dietary inclusion level (Table 5).

DISCUSSION

Previous studies have reported that MW from red wriggler (*E. fetida*) contains nutritional properties (proteins and amino acids) comparable to those of FM (Mohanta et al. 2016, Vodounnou et al. 2016, Musyoka et al. 2019). It has been documented that the nutritional profile of *E. fetida* is determined by the quality of the substrate and processing techniques (handling, harvest, killing, drying, intestinal evacuation, and testing procedures; Musyoka et al. 2019). Gunya & Masika (2022) found that the protein and lipid content of *E. fetida* mealworms oscillates between 50.1-66.2 and 5-20% in wet and dry weight, respectively. These values coincide with the concentrations obtained for the two substrates from the present experiment (VC and AC) but differ from those reported by Zhenjun (2010), Zakaria et al. (2013), and Mohanta et al. (2016) (54.6, 76.5, and 52%, respectively). Such differences may be explained by factors such as the substrate used for worm cultivation and the evacuation of intestinal content performed in those studies, which was not done in the present study. Zhenjun (2010) concluded that the intestinal content of the worm reduces crude protein and increases the fiber and ash content in MW. In the present study, the intestinal content of *E. fetida* was not discarded due to practical limitations, aiming to facilitate its potential application by producers, who would likely find the additional technology required for intestinal evacuation difficult to implement (Musyoka et al. 2019). This decision may also explain the high protein content (> 64.74 ± 0.87 g kg^{-1}) obtained in MW from both substrates, which supports its inclusion in shrimp diet.

Methionine and lysine are the main limiting amino acids when FM is substituted and/or when protein from vegetal sources is included (Djissou et al. 2016). All amino acids, except lysine, increased with the inclusion levels of VC and VA (El-Saidy & Gaber 2002).

Table 5. Digestive enzymes of *P. vannamei* after 75 days of laboratory culture period. *Significant difference ($P < 0.05$); n.s.: no significant difference ($P > 0.05$). Different superscript letters within the same column indicate significant differences among treatments, according to Tukey's multiple comparison test ($P < 0.05$). C×I indicate the interaction effect between compost type (C) and inclusion level (I) evaluated by two-way ANOVA.

	% Inclusion	Proteases (U mg protein^{-1})	Trypsin (U mg protein^{-1})	Chymotrypsin (U mg protein^{-1})	Lipases (U mg protein^{-1})	Amylases (U mg protein^{-1})
Vegetable compost	5	37.99 ± 3.9^{b}	2.50 ± 0.55	5.51 ± 0.68^{ab}	1.34 ± 0.23	0.0108 ± 0.0010
	10	39.83 ± 3.0^{b}	2.31 ± 0.47	5.68 ± 0.19^{ab}	1.60 ± 0.04	0.0101 ± 0.0010
	20	35.07 ± 2.7^{b}	2.01 ± 0.33	5.91 ± 0.44^{a}	1.18 ± 0.23	0.0120 ± 0.0014
Animal compost	5	35.64 ± 1.4^{b}	2.17 ± 0.46	6.08 ± 0.43^{a}	1.14 ± 0.18	0.0122 ± 0.0010
	10	50.87 ± 4.4^{a}	1.75 ± 0.27	5.82 ± 0.25^{a}	1.12 ± 0.28	0.0112 ± 0.0011
	20	34.69 ± 2.2^{b}	2.01 ± 0.42	5.19 ± 0.35^{a}	1.39 ± 0.21	0.0123 ± 0.0006
Two-way ANOVA						
Compost		n.s	n.s	n.s	n.s	n.s
Inclusion		*	n.s	n.s	n.s	n.s
C×I		*	n.s	*	n.s	n.s

However, the concentration of each amino acid was within the necessary range to fulfill all requirements in shrimp diets (Nunes et al. 2014), which supports the suitability of the inclusion levels tested in this study.

The EAAI is widely used to screen potential protein sources (Moreno-Arias et al. 2017). Several studies have reported changes in EAAI proportions when fish meal is replaced with vegetable sources (Bunda et al. 2015, Moreno-Arias et al. 2017), with the aim of searching for low-cost protein sources. In the present study, histidine and threonine increased as the inclusion level of MW produced in both substrates also increased, which could be explained by the fact that the level of these enzymes in VC and AC is higher than those reported for FM (Musyoka et al. 2019). The percentages of isoleucine, leucine, valine, and phenylalanine in the experimental diets were lower than those in the shrimp body. The latter suggests that VC and AC are deficient in EAA; therefore, it is essential to include them in the diet of *P. vannamei* to complement its nutritional requirements. Some of these EAA can be found in different sources. Valine is present in soybeans, cheese, peanuts, mushrooms, whole grains, and vegetables; isoleucine is abundant in meat, fish, poultry, eggs, cheese, lentils, nuts, and seeds; leucine is contained in dairy, soybeans, beans, and legumes; and phenylalanine is found in dairy, meat, poultry, soy, fish, beans, and nuts (Khadka 2021), which could enrich the mealworm with VC and AC.

The results indicate that the growth of *P. vannamei* varied according to the worm culture substrate. The average SGR for all treatments (1.52% d^{-1}) was similar to that reported by Rodríguez-González et al. (2018; 1.3 to 1.6% d^{-1}) for the same crustacean, using FM as a protein source. However, shrimp fed with 10% VC grew 1.558% d^{-1}, indicating that the vegetal substrate derived from domestic waste presented a proximate composition that favors the development of *P. vannamei* juveniles (Fox et al. 2010, Zhenjun 2010, Musyoka et al. 2019). The FCR presented an interval of 2.04 for 10% VC to 2.53 for 20% AC ($P > 0.05$). Nunes et al. (2024) reported that the FCR for juvenile *P. vannamei* should range from 1.45 to 1.56. Although the 10% CV diet resulted in the lowest FCR, it seems that more dietary adjustments are required to reduce it to optimal production levels. The survival rate obtained in the present study (90.47-95.24%) was like that reported by Chiu et al. (2016; 88.8 to 96.6%) for shrimp fed with MW.

Liu et al. (2009) and Musyoka et al. (2019) mention that adding animal or vegetal protein is related to protease activity, which has an important function in shrimp growth. A 10% inclusion of MW from vegetal sources improved shrimp growth and promoted the generation of bacteria from the genus *Bacillus* sp. in the digestive system (Chiu et al. 2016). In the AC group, the inclusion of 5-10% MW in the mixed diet significantly increased ($P < 0.05$) the enzymatic activity of chymotrypsin. Some authors have argued that MW from vegetal compost should produce more enzymatic activity in the shrimp digestive system (Musyoka et al. 2019, Omont et al. 2019), improving survival, growth, intestinal microbiota, and immune response (Zhou et al. 2020). Nolasco-Soria (2023) documented that the digestive enzyme activity of whiteleg shrimp increases when the protein quantity in their diet increases, which favors protein synthesis and survival. Conversely, methionine plays an essential role

in the growth of *P. vannamei* and contributes to physiological and metabolic processes. Methionine is considered the most limiting EAA in commercial shrimp diets (Fox et al. 2010). In the present study, methionine quantities were above 2.4 ± 0.02%, which is the minimum required for this species (Chen et al. 2024). However, Martínez-Córdova et al. (2018) and Sharifinia et al. (2023) concluded that the weight of whiteleg shrimp decreases when fed high amounts of fiber and live insect byproducts.

CONCLUSION

Based on the findings of this study, we concluded that including 10% dietary level of *E. fetida* MW produced in vegetal compost does not compromise the growth performance of *P. vannamei* juveniles. Vermicomposting of agro-industrial and urban waste is a simple biotechnology that can be used to produce MW as an alternative ingredient for shrimp farming, as it provides important dietary elements such as proteins, amino acids, and digestive enzymes. From a cost-benefit perspective, MW offers advantages such as production from low-cost organic substrates and the potential to reduce dependence on expensive marine protein sources. However, processing steps such as freezing and lyophilization may increase costs, limiting feasibility in areas without adequate infrastructure. Overall, moderate inclusion levels (10%) appear to be nutritionally adequate, economically viable, and consistent with the sustainable circular use of organic residues.

Credit the author's contribution

G. Rodríguez Quiroz: conceptualization, validation, methodology, formal analysis, writing-, original draft, project administration, supervision, review and editing; J.P. Tejeda Miramontes: conceptualization, validation, methodology, formal analysis, writing and original draft; H. Nolasco-Soria: methodology, validation, supervision, review and editing; M. García-Ulloa: review and editing; M. Muñoz-Peñuela: review and editing; C. Hernández: review and editing; G.B. Mendoza Maldonado: review and editing; H. Rodríguez González: funding acquisition, project administration, supervision, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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