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Research Article

Effect of dietary inclusion of earthworm (*Eisenia fetida*) meal on the growth performance, body composition, and survival rate of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931) postlarvae

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Abstract

The increasing demand and cost of fishmeal, together with seeking more sustainable feed ingredients in aquaculture have pushed the investigation of alternative protein sources, such as earthworm (EW; *Eisenia fetida*). A 50-day feeding trial was conducted to evaluate the effects of dietary replacement of fishmeal by EW meal on the growth performance, body composition, and survival rate of Pacific white shrimp (*Litopenaeus vannamei*) postlarvae. Triplicate groups of shrimps (mean weight 0.05 g) were fed with 5 isonitrogenous and isolipidic experimental diets including 0% (EW0; control), 25% (EW25), 50% (EW50, 75% (EW75), and 100% (EW100) EW meal replacing fishmeal. The final body weight, weight gain, and specific growth rate revealed significant differences among the groups, with the lowest values observed in the EW75 and EW100 groups compared to the control group (P<0.05). However, the incorporation of the 25% and 50% EW meal in the shrimp feed had the highest survival rates and also increased their capacity to convert feed into shrimp biomass. The proximate analysis indicated that 25% and 50% of fishmeal could be replaced with EW meal without any negative effects on the protein and lipid content of the shrimp carcass. Obtained results indicate that optimum replacing up to 20% of fishmeal with *E. fetida* meal in the diet of Pacific white shrimp (*L. vannamei*) does not negatively impact growth performance, survival, or body composition.

Introduction

The fast-growing aquaculture sector, together with high demand and prices for traditional protein sources (fishmeal/soy) is pushing new research into the exploration of alternative protein sources for aquafeed (Alfiko *et al*., 2022). In the last two decades, plant protein-rich feeds like oilseed meals, as protein sources in fish diets, mostly substituting fishmeal (Gatlin III *et al.,* 2007). However, it has been demonstrated that plant protein-based diets or complete replacement of fishmeal with vegetables could have adverse effects on fish growth, health, and welfare (Minghetti *et al*., 2017; Novriadi *et al.,* 2018). The amino acid profile of plant-derived ingredients does not completely match the essential amino acid requirement of fish (Jobling, 2016), and also the presence of the anti-nutritional factors in such ingredients can tiger some adverse side-effects on fish health and interfere with nutrient digestion and absorption (Hardy, 2010; Bandara, 2018). Among the alternatives, terrestrial animal by-products, especially the poultry by-product meal, are the most promising cost-effective ingredients for aquafeeds (Bandara, 2018). Terrestrial animal by-products contain a good balance of essential amino acids and good digestibility (Hatlen *et al*., 2015; Barreto-Curiel *et al*., 2016), despite these advantages, the aquafeed inclusion of terrestrial animal by-products is still limited by consumer acceptance constraints and a strict regulation on their use due to the risk of developing human diseases.

Today, research for cost-effective and sustainable alternative protein sources, such as some non-conventional animals (Sogbesan and Ugwumba, 2008), has arisen. Earthworms are generally rich in proteins (~65%) and lipids (~11%), and serve as a valuable source of bioavailable minerals, making them a suitable nutritional ingredient for farmed aquatic animals (Ng *et al.,* 2001; Dynes, 2003). *Eisenia fetida* (EW) is one of the earthworm species used in aquafeed due to its high protein content (Stafford and Tacon, 1985) and essential amino acids profile (Vielma-Rondón *et al*., 2003; Fadaee, 2012), making it a candidate as a potential feedstuff for manufacturing aquafeeds (Chiu *et al.,* 2016; Musyoka *et al*., 2019). Previous studies have used it as a substitute for fishmeal in the aquafeeds. Velasquez *et al*. (1991) reported that replacing 25% and 50% fishmeal with *E. fetida* meal (EW) in rainbow trout (*Oncorhynchus mykiss*) diet improved growth rate, feed conversion efficiency, and protein efficiency ratio compared to the control diet with 0 % replacement. Moreover, replacing up to 75% of fishmeal with earthworm meal can significantly enhance growth rates and feed conversion ratios in fish species like butter catfish, *Ompok pabda* (Chakraborty *et al*., 2021), and tilapia, *Oreochromis niloticus* (Russio *et al*., 2022).

Pacific white shrimp (*Litopenaeus vannamei*) is the most commercially important shrimp species in global aquaculture due to its rapid growth rate, high survival, and adaptability to varying environmental conditions. The early developmental stages- particularly the postlarval phase- is critical for survival and growth performance, with optimal nutrition and immune competence serving as key determinants of success in shrimp hatchery systems. Compared with other species, *L.*

vannamei requires a lower protein (and hence cheaper) diet (20-35%) during culture than *P. monodon*, *P. chinensis*, or *P. stylirostris* (36-42%) (Briggs *et al*., 2004). This study aims to evaluate the potential of *E. fetida* meal to partially or completely replace fishmeal in the shrimp diet. Moreover, this study has employed statistical methods such as variance analysis and broken line model (linear and quadratic) to determine the optimum percentage of fishmeal replacement with EW in the *L. vannamei* diet based on both growth performance parameters and survival rate.

Materials and methods

Shrimp husbandry

This study was conducted at the Hormozgan Fisheries Organization farm, Bandar Abbas, Iran. Pacific white shrimp (*L. vannamei*) postlarvae were randomly selected from a private shrimp farming site (Bandar-e-Jask, Hormozgan, Iran). Seven hundred and fifty postlarvae of *L. vannamei* were randomly distributed into 15 round polyethylene tanks, which were filled with 250 seawater, and each tank stocked with 150 shrimp (mean initial body weight about 0.05 g). Shrimp were acclimated to the experimental condition before the start of the dietary trial. Water in the holding tanks was changed daily (approximately 50%) and its salinity and temperature were 40.0±1.5‰, and 29.0±0.5°C, respectively during the experimental period. The mean dissolved oxygen content and pH of water was 5.8±0.2 mg l^{-1} and 7.9±0.2, respectively. Oxygen was supplied by supplying air continuously through an air stone from an air-blower. Water quality parameters were monitored daily to ensure the fish welfare.

Preparation of experimental diets

The *Eisenia fetida* meal (EW) was provided by a private company (Pishgaman Zist, Iran). The mealworm meal was composed of approximately 55% crude protein, 7.3% crude fat, 16% ash, 14. 7% fiber, and 70% water. To prepare the experimental diets, five isonitrogenous (crude protein, CP: ~33.26 % dry matter), and isolipidic (crude fat- 6.79 % dry matter) were formulated (Table 1).

Table 1: The ingredients of experimental dies with different replacement percentages (0, 25, 50, 75, and 100) of fishmeal by earthworm (*Eisenia fetida*) meal (EW) in Pacific white shrimp (*Litopenaeus vannamei*) diet.

Ingredients (%)	Experimental diets				
	EW0	EW25	EW50	EW75	EW100
Herring meal	35.00	26.25	17.50	8.75	0.00
Earthworm	0.00	8.75	17.50	26.25	35.00
Soybean meal	21	21	21	21	21
Cottonseed meal	15	15	15	15	15
Wheat	8.5	8.5	8.5	8.5	8.5
Barley	9	9	9	9	9
Glucose	7.5	7.5	7.5	7.5	7.5
Molasses	2	2	2	2	2
Di-calcium phosphate	1.4	1.4	1.4	1.4	1.4
†Vitamin	0.6	0.6	0.6	0.6	0.6
††Proximate composition (% dry matter)					
Moisture	3.5 ± 0.15	3.6 ± 0.08	3.5 ± 0.10	3.7 ± 0.04	3.6 ± 0.07
Crude protein	33.35 ± 1.25	33.35 ± 1.20	33.17 ± 1.52	33.30 ± 1.12	33.16 ± 1.57
Crude lipid	7.02 ± 0.09	6.99 ± 0.57	6.98 ± 0.33	6.65 ± 0.56	6.32 ± 0.32
Ash	11.49 ± 0.58	11.50 ± 1.53	11.83 ± 0.99	11.88 ± 0.75	11.83 ± 1.00

† Each 1000-gram vitamin supplement contains 3600000 international units of vitamin A, 800000 international units of vitamin D3,14400 mg of vitamin E, 800 mg of vitamin K, mg of vitamin B1, 2640 mg of vitamin B2, 1176 mg of vitamin B6, 11880 mg of niacin,40 mg biotin, 100000 mg Choline chloride.

†† Data (mean ± SE) with different letters among the treatments are significantly difference (p< 0.05).

The diets formulated, as fed basis to include increasing levels of EW as a partial or complete replacement for fishmeal, corresponding to 0% (EW0), 25% (EW25), 50% (EW50), 75% (EW75), and 100% (EW100) replacement levels, respectively. Diets were tested in triplicate; shrimp were fed by hand at 5 % of the body weight four times per day (6, 12, 18, and 24 h) for 50 days. Feces and uneaten food were removed from the bottom of the tank by siphoning every night.

Shrimp growth and sampling

At the end of the trial, shrimp will be fasted for 24 h before being anesthetized and individually weighed (BWf). Two fish from each replicate will be sacrificed with an overdose of the same anesthetic to sample. All morphometric indices were calculated as follows (Niu *et al.*, 2009; Chiu *et al.*, 2016):

WG = BWf (g) – BWi (g);

SGR (% day^{-1}) = [(ln BWf – ln BWi)/t] ×100/days;

FCR = FI (g) / WG (g);

FCE = WG (g)/ FI (g);

Survival (%) = final number of shrimps/initial number of shrimps×100

Where, WG = Weight Gain, BWf = final Body Weight, BWi = initial Body Weight, SGR = Specific Growth Rate, FCR = Feed Conversion Ratio, FI = Feed Intake, and PCE = Feed Conversion Efficiency.

Proximate composition of the experimental diets and shrimp carcass

The experimental diets and shrimp carcasses were analyzed for proximate composition. Crude protein content was determined by the Kjeldahl method (Kjeltec2100 Distillation Unit, Foss Tecator, Hoganas, Sweden); lipid content was determined by ether-extraction method; moisture content was determined by oven-drying samples at 105◦C for 24 h; and ash content was determined by burning samples in a muffle furnace at 550◦C for 5 h and all methods were according to standard AOAC (1995).

Statistical analysis

Data was analyzed using SPSS ver.16.0 (Chicago, Illinois, USA). All data are presented as mean±standard error of the mean. One-way ANOVA was performed at a significance level of 0.05 following confirmation of normality and homogeneity of the variance. Duncan's procedure was used for multiple comparisons when statistical differences were found among groups by the one-way ANOVA. The optimal percentage of fishmeal replacement with EW in the *L. vannamei* diet was determined based on both growth performance parameters and survival rate simultaneously, using a desirability function model analysis by using the R desirability package’s linear and quadratic model (Bates *et al*. 2015; Mani and Ebrahimi, 2022).

Results

Effect of experimental diets on shrimp growth performance

Partial or complete fishmeal replacement with earthworm meal had different effects on growth performance indices, including final body weight (BWf), final length (Lf), weight gain (WG), specific growth rate (SGR), and survival rate (SR) in shrimp (Table 2).

Table 2: The growth performance parameters and survival of Pacific white shrimp (*L. vannamei*) that were fed different experimental diets including EW0: basal diet, EW25: 25% fishmeal replacement by earthworm (*Eisenia fetida*), EW25: 25% fishmeal replacement by earthworm (*Eisenia fetida*), EW50: 50% fishmeal replacement by earthworm (*Eisenia fetida*), EW75: 75% fishmeal replacement by earthworm (*E. fetida*), EW100: 100% fishmeal replacement by earthworm (*E. fetida*).

Parameters	EW0	EW25	EW50	EW75	EW100
BWf (g)	4.69 ± 0.38^a	4.76 ± 0.71^a	4.72 ± 0.48^a	3.12 ± 0.36^b	3.04 ± 0.17^b
Lf (cm)	6.67 ± 0.06^a	6.6 ± 0.10^a	6.57 ± 0.20^a	6.13 ± 0.06^b	6.23 ± 0.04^b
WG (g)	4.64 ± 0.38^a	4.72 ± 0.70^a	4.67 ± 0.48^a	3.07 ± 0.36^b	2.99 ± 0.17^b
SGR (%)	9.22 ± 0.52^a	9.55 ± 0.29^a	9.37 ± 0.14^a	8.39 ± 0.18^b	8.21 ± 0.19^b
FCR	1.65 ± 0.06	1.54 ± 0.10	1.53 ± 0.14	1.81 ± 0.10	1.78 ± 0.15
FCE	0.62 ± 0.02	0.64 ± 0.05	0.68 ± 0.06	0.59 ± 0.03	0.60 ± 0.02
SR (%)	60.22 ± 3.66^a	65.34 ± 5.70^a	67.56 ± 3.86^a	43.33 ± 2.04^b	42.89 ± 1.90^b

Abbreviation: BWf= final body weight, Lf= final length, WG= weight gain, SGR= specific growth rate, FCR= feed conversion ratio, FCE= feed conversion efficiency, and SR= survival rate. Data (mean ± SE) with different letters among the treatments are significantly difference ($p<0.05$).

The results indicated that replacing 75% and 100% of fishmeal with earthworm meal in the shrimp diet significantly reduced growth performance and survival compared to the control diet with 0% EW ($p<0.05$). However, feed conversion ratio (FCR) and feed conversion efficiency (FCE) showed no significant differences between control and experimental diets ($p>0.05$). The highest WG, SGR, and SR were recorded in shrimp fed with EW25 and EW50 diets, along with the control group. Shrimp fed with EW0, EW25, and EW50 diets exhibited significantly higher WG and survival compared to those fed with EW75 and EW100 diets. Given that shrimp fed with EW25 and EW50 diets had no significant differences in growth performance and survival compared to the control, it can be claimed that earthworm meal can partially substitute up to 50% of fishmeal without negative effects on shrimp growth and survival. Furthermore, based on the desirability function model (quadratic regression analysis) using growth performance indices and survival, shrimp showed to have the best growth performance when replacing approximately 20% of fishmeal in the diet with EW (Fig. 1).

Effect of experimental diets on shrimp carcass composition

Proximate analysis of shrimp carcass composition was evaluated including moisture and crude among the experimental diets and the control group (Table 3). The crude protein content was significantly different among the experimental group compared to the control ($p<0.05$). The results indicated that 75% and 100% levels of fishmeal replacement with EW significantly reduced the crude protein content in shrimp carcasses compared to the 25%, 50%, and control groups ($p<0.05$).

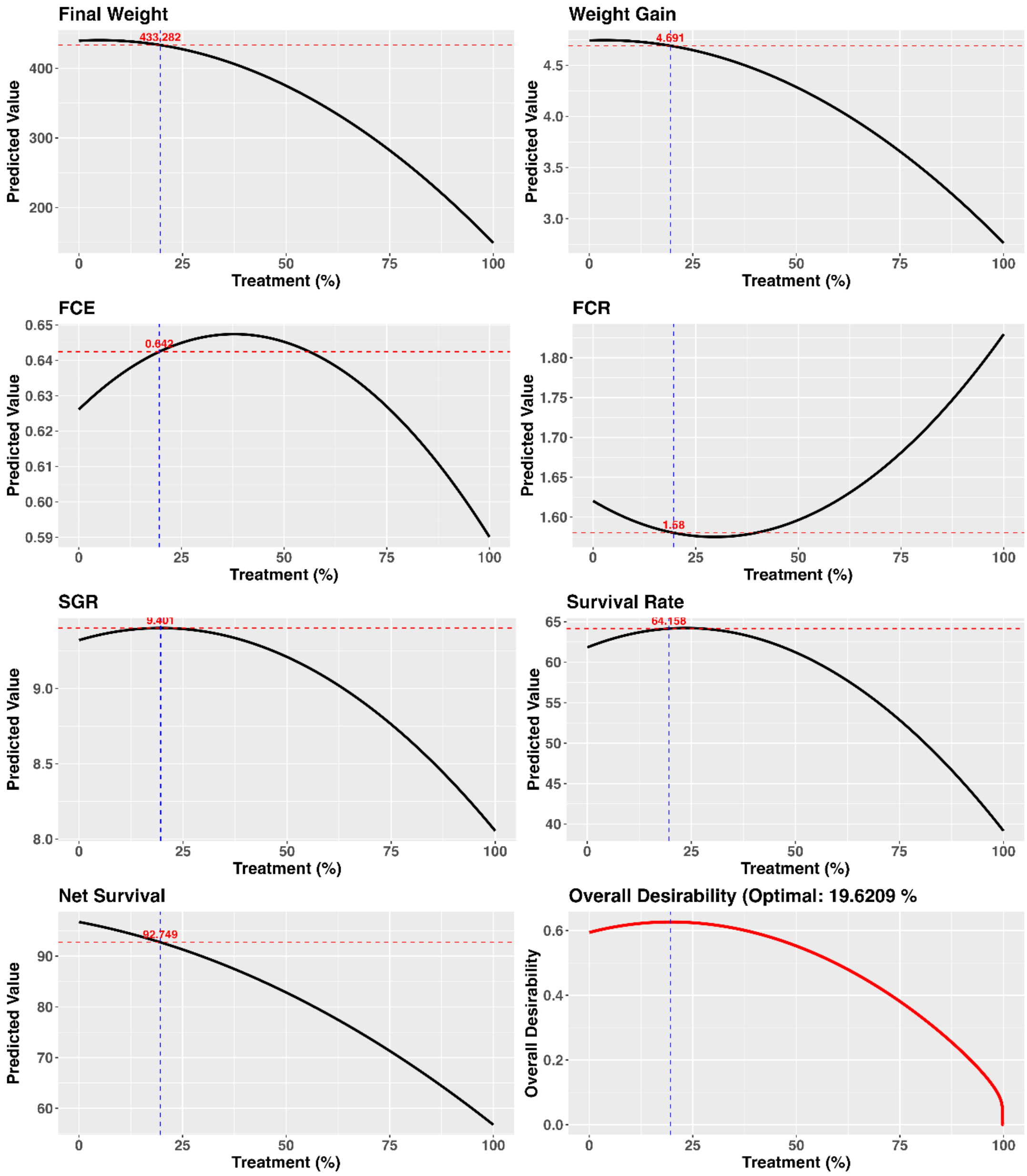

Figure 1: Desirability function model output of growth performance indices and survival rate of Shrimp against various levels of fishmeal replacement with EW (0%, 25%, 50%, 75%, and 100% fishmeal replacement with earthworm). Values are presented as mean from triplicate groups (n=3) of fish.

Table 3: Body composition (% in wet weight) of Pacific white shrimp (*Litopenaeus vannamei*) that were fed different experimental diets including EW0: basal diet, EW25: 25% fishmeal replacement by earthworm (*Eisenia fetida*), EW25: 25% fishmeal replacement by earthworm (*E. fetida*), EW50: 50% fishmeal replacement by earthworm (*E. fetida*), EW75: 75% fishmeal replacement by earthworm (*E. fetida*), EW100: 100% fishmeal replacement by earthworm (*E. fetida*).

Diets	Body composition (% in wet weight)			
	Moisture	Crude protein	Crude fat	Ash
EW0	75.93 ± 0.11	19.35 ± 0.18 [a]	0.65 ± 0.07	1.86 ± 0.04
EW25	75.64 ± 0.21	19.35 ± 0.17 [a]	0.66 ± 0.05	1.87 ± 0.03
EW50	75.93 ± 0.15	19.17 ± 0.21 [a]	0.62 ± 0.01	1.87 ± 0.02
EW75	75.75 ± 0.10	18.30 ± 0.11 [b]	0.59 ± 0.06	1.88 ± 0.05
EW100	75.62 ± 0.25	18.16 ± 0.08 [b]	0.58 ± 0.06	1.87 ± 0.03

Data (n= 9; mean ± SE) with different letters among the treatments are significantly difference (p<0.05).

Discussion

Earthworm (*E. fetida*) meal has been considered a potential substitute for fishmeal in aquafeed (Chiu *et al.*, 2016; Musyoka *et al.*, 2019). Many studies have been conducted on the nutritional values of the formulated diets with EW, and their possible influences on growth performance and proximate body composition of the fish (Chaves *et al.*, 2015; Beg *et al.*, 2016). However, research on the effects of EW on growth performance indices in seawater shrimp remains comparatively limited and has not yet been thoroughly investigated. Therefore, the present study investigated the potential of *E. fetida* meal to partially or completely replace fishmeal in the Pacific white shrimp (*L. vannamei*) diet, with an emphasis on shrimp growth and welfare.

The result of the current study revealed that a partial fishmeal replacement level of EW up to 50% does not negatively affect the growth indices and survival of *L. vannamei*. Since the *L. vannamei* fed with EW25 and EW50 diets showed no significant difference in weight gain compared to the control group (EW0), it can be claimed that EW can be used as a partial substitute for fishmeal up to 50% in the shrimp diet. In line with these findings, Valasquez *et al.* (1991) reported that replacing 25% and 50% of fishmeal with *E. fetida* in the diet of rainbow trout (*Oncorhynchus mykiss*) resulted in growth performance comparable to that of the control group (0% replacement). The best growth performance was observed at 50% fishmeal replacement with EW in the diet of Indian carps, including ruhu (*Labeo rohita*), mrigal (*Cirrhinus mrigala*), and catla (*Catla catla*) (Beg *et al.*, 2016).

However, a decrease in shrimp growth performance was observed when fishmeal was replaced with over 50% EW, the survival rate was also reduced at 75% and 100% fishmeal replacement with EW. These results are consistent with the findings of Chakraborty *et al*. (2021), who reported that complete replacement of fishmeal with earthworm (*Perionyx excavatus*) meal hindered growth and feed utilization parameters in juvenile butter catfish (*Ompok pabda*). In another study, the depression of growth performance was observed when *P. excavatus* replaced 40% of the fishmeal in the diet of Nile tilapia (Das *et al.*, 2024). In contrast, it has been reported that replacing fishmeal with *E.*

fetida meal at a 75% level in the diet of Nile tilapia, *Oreochromis niloticus* (Ahmed *et al.*, 2020).

In this study, the decreasing growth performance parameters in the EW75 and Ew100 groups may be because of the chitin (an insoluble polymer of glucosamine) content in the hard cuticle of the earthworm (Beg *et al.*, 2016). It has been reported that high chitin content in aquafeeds depressed growth in numerous fish species (Maulu *et al.*, 2022). A decrease in growth and feed utilization in African catfish (*Clarias gariepinus*) fed with high levels of mealworm (*Tenebrio molitor*) occurred due to the high chitin levels (Ng *et al.*, 2001). Moreover, the depression in the growth performance of the fish at higher fishmeal replacement levels may also attributed to the low methionine level present in earthworms compared to fishmeal (El-Ouny *et al.,* 2023). Inadequate methionine dietary has been reported to hinder growth in fish species (Zhou *et al.*, 2006; Tang *et al.*, 2009; Wu *et al.*, 2017). In the present study, the reduction in shrimp survival rate fed with EW75 and EW100 compared to control group may has been caused by high level of coelomic fluid of the earthworm, which is known to be toxic to fish (Kobayashi *et al.,* 2001; Ngoc *et al.*, 2016; Musyoka *et al*., 2019), which likely contributed to the poor growth performance of fish (Das *et al*., 2024).

This study has used statistical analysis such as variance analysis and broken line model to estimate the optimum replacement fishmeal level with EW. The desirability function is an accepted technique for determining the optimal measure of an independent variable (or more) which can determine optimal levels for one or more responses (Mani and Ebrahimi, 2022). Based on the desirability function model between growth performance parameters and inclusion of EW levels in the present study, *L. vannamei* showed to have the best growth performance and survival rate when replacing 20% of fishmeal in the diet with EW. In agreement with our findings, it has been demonstrated that the quadratic regression analysis between final body weight and inclusion of EW level, Nile tilapia showed to have the best growth performance when replacing 17.5% of fishmeal in the diet with EW (Das *et al*., 2024). Similarly, replacing fishmeal with EW meal up to 20% in the dietary trial of Nile tilapia did not negatively affect the growth and physiological parameters of fish (El-Ouny *et al.*, 2023).

In the current study, a significant decrease in the body crude protein in *L. vannamei* fed diets with 75% or 100% fishmeal replacement with EW exhibits inappropriate levels of EW inclusion in the diets, which could hinder the growth and welfare of this species. In line with our findings, Musyoka *et al*. (2020) reported that the body crude protein content of Nile tilapia was reduced only in the fish fed a diet in which 100% fishmeal was replaced with *P. excavates*. This reduction may be attributed to the quality and composition of the amino acid profile in earthworm proteins, particularly the deficiency of essential amino acids such as lysine, methionine, and cysteine (Das *et al.,* 2024).

Conclusion

Obtained results indicate that optimum replacing up to 20% of fishmeal with *E.*

fetida meal in the diet of Pacific white shrimp (*L. vannamei*) does not negatively impact growth performance and survival rate. However, higher replacement levels (above 50%) had adverse effects on growth indices and survival. Therefore, *E. fetida* can be considered an applicable alternative protein source for partial replacement of fishmeal in the diet of *L. vannamei*.

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Confilicts of interest
The authors have no cofilicts of interest.

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