

## Research Article

# Effects of Replacement of Fish Meal with Poultry By-Product Meal (PBM) on Growth Performance, Digestive Enzyme, and Immunity of Giant River Prawn (*Macrobrachium rosenbergii*)

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A 56-day feeding trial was performed to determine the effect of replacing fishmeal with 0, 25%, 50%, 75%, and 100% PBM (FM) on growth performance, digestive enzyme, and immunity of juvenile giant river prawn (*Macrobrachium rosenbergii*). Diets were fed in quadruplicate (50 prawns per replicate) to satiation (3–5% of their body weight). The final weight, weight gain, and specific growth rate of prawns were significantly reduced ( $P < 0.05$ ) when 100% PBM was substituted for fish meal, whereas the remaining test diets showed no variation compared to the control ( $P > 0.05$ ). There was no significant difference in survival rates. The moisture, crude protein, and crude fat of the prawn were unchanged ( $P > 0.05$ ). The SOD activity, MDA content, and AKP activity of hemolymph did not change significantly. However, the ACP activity of all replacement groups decreased significantly ( $P < 0.05$ ). The amylase enzyme activity in the intestine of prawn fed 25% of PBM increased significantly compared with the control group, while the trypsin and lipase activities were unchanged. This study showed that PBM up to 75% replacement group does not affect growth performance, and all replacement groups have no adverse effect on intestinal digestive enzyme activity and immunity in juvenile *M. rosenbergii*. Therefore, poultry by-product meal could replace fish meal by up to 75%.

## 1. Introduction

Protein is an essential component of aqua feed [1] for all commercially important aquaculture species, including prawns, which require 25–42% of the total protein by weight. Fish meal (FM) has traditionally been used as a balanced nutritional source to formulate aquaculture feed [2, 3]. Nevertheless, due to a variety of factors, such as climate change, overfishing, and the decline of global fish stocks, developing alternative protein sources is an important research area to enhance aquaculture sustainability [4].

Animal by-products are less expensive, have fewer potentially pathogenic species, and improve palatability and digestibility [5]. Poultry by-product meal (PBM) is prepared

from the processed parts of harvested poultry, such as meat, blood, heads, feet, undeveloped eggs, and viscera. It comprises various elements and suitable nutrient compositions [6, 7]. The PBM is a source of protein with a high biological value comprising an 82% digestibility coefficient and also provides certain amino acids lacking in plant proteins [8]. In addition, PBM contains phosphorus and calcium, which may reduce the need for minerals while requiring a prawn diet formulation [9]. The positive characteristics of PBM have led academia and industry to conduct a plethora of research studies on a wide range of aquatic organisms with different results [6, 10–16]. For example, PBM was effective at the inclusion levels of 21% for Australian snapper, *Chrysophrys auratus* [14], 25% for silver seabream, *Pagrus*

TABLE 1: Chemical composition and amino acid concentration of the PBM and fish meal (%).

Items	PBM	Fishmeal
Crude protein	58.88	67.00
Crude fat	13.40	10.00
Amino acids		
Aspartic acid	4.80	5.78
Threonine	2.28	2.75
Serine	2.25	2.41
Glutamate	8.19	8.19
Glycine	6.06	4.03
Alanine	4.56	4.18
Cystine	0.53	—
Valine	2.45	3.20
Methionine	1.26	1.79
Isoleucine	2.14	2.69
Leucine	4.02	4.72
Tyrosine	1.84	1.95
Phenylalanine	2.12	2.59
Lysine	3.91	5.09
Histidine	1.23	1.96
Arginine	4.10	3.71
Proline	3.85	2.43
17 amino acids	55.59	57.47

*auratus* [12], 14% for red drum, *Sciaenops ocellatus* [13], and 45% for cobia, *Rachycentron canadum* [13]. Freshwater species, including tilapia, *Oreochromis niloticus*, gibel carp, *Carassius gibelio*, and mahseer, *Tor tambroides*, were able to ingest a diet containing up to 100% PBM without showing substantial growth depression [6, 15, 17–19]. However, the complete replacement of FM resulted in a significant reduction in growth performance, intestinal mucosal barrier functions, antioxidant activity, and immunity of barramundi, *Lates calcarifer* [20].

*Macrobrachium rosenbergii* is known as giant freshwater prawn cultivated in several countries for food [21] because of its ability to grow in captive conditions. A number of studies have been conducted testing alternative protein ingredients, but the complete replacement of FM with PBM has not been studied thoroughly. Hence, the present study was conducted to investigate the inclusion of graded levels of PBM on the growth performance, digestive enzymes, and immunity of *M. rosenbergii*.

## 2. Materials and Methods

**2.1. Ethics Statement.** The study was conducted strictly according to the guidance of the care and use of laboratory animals in China. The experimental protocol was approved by the Committee on the Ethics of Animal Experiments at Huzhou University.

**2.2. Formulation and Feed Preparation.** The fishmeal and PBM were provided by New Hope Liuhe Lited Company, Chengdu, Sichuan, China (Table 1). Five experimental diets were formulated including the control (without PBM). The main protein source of the control group was 30% of fish meal, and the fish meal was replaced by 25%, 50%, 75%, and

100% PBM as groups PBM 25, PBM 50, PBM 75, and PBM 100, respectively. Each experimental diet had four replicates and 50 prawns were distributed into each replicate. The crude protein and the crude lipids content of all levels of the experimental diet are shown in Table 2.

All the feed ingredients were homogenized, granulated with a granulator, and strained with 60 holes by a square-meter sieve. All feed ingredients were added accordingly, hand-mixed thoroughly, and mixed with a machine for 20 minutes. Water was added about 30% while continuously mixing to get a good texture for the pellets to be made. The pellets were made with a 1.5 mm diameter mill. The pellets were dried in a hot dried air oven at 50°C for 24 hours. After this, feeds were stored at −4°C until use.

**2.3. Prawn Stocking, Feeding, and Data Collection.** The juvenile prawns (*Macrobrachium rosenbergii*) were provided by Jiangsu Shufeng Aquatic Industry Co., Ltd., China. The feeding trial took place at Huzhou University in Huzhou, Zhejiang Province (China).

Twenty (20) 300-liter white polyethylene tanks were washed and filled with 250 liters of water each; prawns were acclimatized for two weeks and were fed the control diet. Then, fifty (50) randomly selected prawns were distributed, making a total of 1000 prawns for the trial. Diets were fed in quadruplicate (50 prawns per replicate) to satiation (3–5% of their body weight). Prawns were fed twice daily (8:00, 17:00). The dead prawns observed were removed, measured, and registered at any moment. The fecal matter was regularly removed from each tank's bottom, and adjustments were made in 30–50% of the water. Individual tanks were aerated for 24 hours with more than 5 mg/L dissolved oxygen levels, and water was adjusted regularly. A Handy Polaris® meter was used to measure the dissolved oxygen and temperature, and a YSI® pH meter was used to measure pH at 10 cm below the water surface (model pH 100). The water was in a temperature range (26–30°C), with an ammonia nitrogen concentration of less than 0.3 mg/L and a pH of 7.8–8.3.

The prawns were fed for 56 days. Then, the prawns were individually weighed using a scientific balance, and the standard length was determined using a metric ruler. The following growth parameters were calculated after the feeding trial: survival rate (SR), weight gain (WG), and specific growth rate (SGR).

Survival rate (SR, %) =  $100 \times (\text{final fish number} / \text{initial fish number})$ ; weight gain (WG, %) =  $100 \times (\text{final body weight} - \text{initial body weight}) / \text{initial body weight}$ ; specific growth rate (SGR, %/day) =  $((\ln \text{final BW} - \ln \text{initial BW}) / \text{days}) \times 100$ .

**2.4. Digestive Enzyme Assay.** At the end of 8 weeks trial, 12 prawns per treatment were randomly selected. Every prawn's carapace cavity was opened from the dorsal region. Forceps were used to extract the hepatopancreas and the gills. The hepatopancreas and gills were held at 80°C for further research. The telson and uropod were cut and separated from the body (abdomen) by cutting slightly through the sixth segment of the prawn's tail. The intestine

TABLE 2: Formulation and proximate composition of the experimental diets %.

Ingredients	Control	PBM 25%	PBM 50%	PBM 75%	PBM 100%
Fish meal	30	22.5	15	7.5	0
Soy protein concentrate	15.1	15.1	15.1	15.1	15.1
Soybean meal	15.1	15.1	15.1	15.1	15.1
Poultry by-product meal	0	7.5	15	22.5	30
Gelatinized starch	18.12	17.84	17.58	17.32	17.06
Corn gluten meal	6	6	6	6	6
Yeast hydrolysate	3	3	3	3	3
Soy oil	3	3	3	3	3
Soybean phospholipid	2	2	2	2	2
Fish oil	1	1	1	1	1
Monocalcium phosphate	2	2	2	2	2
50% coated methionine	0	0.12	0.22	0.32	0.42
50% coated lysine	0	0.16	0.32	0.48	0.64
Cholesterol	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.5	0.5	0.5	0.5	0.5
Vitamin premix <sup>1)</sup>	0.4	0.4	0.4	0.4	0.4
Mineral premix <sup>2)</sup>	0.5	0.5	0.5	0.5	0.5
Carboxymethyl cellulose	2.78	2.78	2.78	2.78	2.78
Total	100	100	100	100	100
<i>Proximate Chemical Compositions</i>					
Crude protein	45.63	44.61	45.26	44.75	44.93
Crude fat	8.33	8.86	9.49	9.16	10.99
Moisture	4.94	4.61	4.97	5.00	4.86

<sup>1)</sup>The premix gave the following per kg of diets: VA 10 500 IU, VD<sub>3</sub> 7 000 IU, VE 105 mg, VK<sub>3</sub> 8.75 mg, VB<sub>1</sub> 17.5 mg, VB<sub>2</sub> 35 mg, VB<sub>6</sub> 28 mg, VB<sub>12</sub> 0.07 mg, nicotinamide 140 mg, D-calcium pantothenate 87.5 mg, folic acid 8.75 mg, D-biotin 0.28 mg, inositol 350 mg, and VC 1.4 g. <sup>2)</sup>The premix gave the following per kg of diets: FeSO<sub>4</sub>·H<sub>2</sub>O 2.4 g, CuSO<sub>4</sub>·5H<sub>2</sub>O 30 mg, MnSO<sub>4</sub>·H<sub>2</sub>O 90 mg, ZnSO<sub>4</sub>·H<sub>2</sub>O 175 mg, Na<sub>2</sub>SeO<sub>3</sub> 3 mg, Ca(IO<sub>3</sub>)<sub>2</sub> 3.5 mg, CoCl<sub>2</sub>·6H<sub>2</sub>O 6 mg, and zeolite meal 2.29 g.

was removed by carefully pulling the telson and uropod away from the rest of the body. For further research, the intestine was kept at 80°C. Hepatopancreas and intestines were homogenized in separate tubes of 4°C precooled deionized water, left in a 4°C refrigerator overnight, and centrifuged at 4000 r/min for 10 minutes. The crude enzyme solution was removed from the homogenate and deposited at -80°C for testing. Trypsin, lipase, and amylase were examined using Nanjing Jiancheng Bioengineering Co., Ltd., kits.

**2.5. Hemolymph Analysis.** Hemolymph samples were obtained from the third pair of walking legs (pericardial cavity) using 1 mL syringes (needle size 20–25G) and put in a 1.5 ml centrifuge tube before the dissection procedure. The hemolymph was centrifuged at 4°C for 20 minutes at 10000 r/min, and the supernatant was taken and deposited at -80°C. The hemolymph was tested for superoxide dismutase (SOD), malondialdehyde (MDA), alkaline phosphatase, and acid phosphatase using Nanjing Jiancheng Bioengineering Co., Ltd., kits.

**2.6. The Nutritional Components of the Whole Prawn.** Prawns were randomly selected from each experimental group and stored in the refrigerator at -20°C for later use after the feeding trial and a 24-hour fast. Proximate composition, moisture, crude protein, and crude fat were measured. The proportion of crude protein was measured using the Kjeldahl process, the chloroform-methanol

method was used to analyze crude fat, and the combustion method at 550°C was used to determine crude ash.

**2.7. Statistical Analysis.** The data were statistically analyzed using the analysis of variance approach (ANOVA). Prior to performing ANOVA, all data were checked for homogeneity of variance. The impact of various diets on growth parameters (WGR, SGR, and SR), proximate composition of prawns, intestinal digestive enzymes, and immunity were investigated using one-way ANOVA with a significant level ( $P < 0.05$ ).

### 3. Results

**3.1. Effects of Replacement of Fish Meal by the Poultry By-Product Meal on Growth Performance.** Compared with the control group, when the fish meal was 100% replaced by PBM, the final weight, weight gain, and specific growth rate of prawn were significantly reduced ( $P < 0.05$ ), and the difference between 25%, 50%, and 75% groups was not significant ( $P > 0.05$ ). The survival rate of prawns in all feed groups was not different, as shown in Table 3.

**3.2. Effects of Replacement of Fish Meal by the Poultry By-Product Meal on Proximate Composition.** There was no significant difference in the moisture, crude protein, crude fat, and crude ash contents of prawn body composition in different proportions of the PBM instead of fish meal ( $P > 0.05$ ), as shown in Table 4.

TABLE 3: The effect of replacement fish meal by PBM on the growth performance.

Groups	Initial weight (g)	Final weight (g)	SR (%)	WGR (%)	SGR (%/day)
Control	3.12 ± 0.01 <sup>a</sup>	5.36 ± 0.30 <sup>a</sup>	64.00 ± 7.21 <sup>a</sup>	72.07 ± 10.09 <sup>a</sup>	1.08 ± 0.17 <sup>a</sup>
PBM 25	3.12 ± 0.01 <sup>a</sup>	4.77 ± 0.06 <sup>ab</sup>	59.33 ± 0.67 <sup>a</sup>	52.75 ± 1.73 <sup>ab</sup>	0.76 ± 0.02 <sup>ab</sup>
PBM 50	3.12 ± 0.00 <sup>a</sup>	5.24 ± 0.11 <sup>ab</sup>	62.67 ± 2.40 <sup>a</sup>	68.24 ± 3.55 <sup>ab</sup>	0.88 ± 0.09 <sup>ab</sup>
PBM 75	3.13 ± 0.01 <sup>a</sup>	4.98 ± 0.04 <sup>ab</sup>	62.67 ± 2.67 <sup>a</sup>	59.38 ± 0.92 <sup>ab</sup>	0.79 ± 0.04 <sup>ab</sup>
PBM 100	3.14 ± 0.02 <sup>a</sup>	4.66 ± 0.10 <sup>b</sup>	60.00 ± 3.46 <sup>a</sup>	48.87 ± 4.29 <sup>b</sup>	0.63 ± 0.03 <sup>b</sup>

All values are given as the mean ± standard deviation (SD). Means followed by different letters in the same column are significantly different  $t(P < 0.05)$ . <sup>a</sup>WGR: weight gain rate; <sup>b</sup>SGR: specific growth rate; <sup>c</sup>SR: survival rate.

TABLE 4: The effect of replacement fish meal by PBM on proximate composition (%).

Groups	Moisture	Crude protein	Crude fat	Crude ash
Control	72.66 ± 0.57 <sup>a</sup>	18.80 ± 0.83 <sup>a</sup>	1.73 ± 0.75 <sup>a</sup>	4.92 ± 0.10 <sup>a</sup>
PBM 25	72.76 ± 0.72 <sup>a</sup>	18.94 ± 1.11 <sup>a</sup>	1.71 ± 0.30 <sup>a</sup>	5.24 ± 0.20 <sup>a</sup>
PBM 50	72.10 ± 0.79 <sup>a</sup>	18.89 ± 0.80 <sup>a</sup>	1.87 ± 0.60 <sup>a</sup>	5.17 ± 0.54 <sup>a</sup>
PBM 75	71.88 ± 0.65 <sup>a</sup>	19.09 ± 0.21 <sup>a</sup>	1.78 ± 0.05 <sup>a</sup>	5.47 ± 0.18 <sup>a</sup>
PBM 100	71.79 ± 0.54 <sup>a</sup>	18.98 ± 0.55 <sup>a</sup>	2.37 ± 0.71 <sup>a</sup>	5.49 ± 0.33 <sup>a</sup>

All values are given as the mean ± standard deviation (SD). Means followed by different letters in the same column are significantly different ( $P < 0.05$ ).

**3.3. Effects of Replacement of Fish Meal by the Poultry By-Product Meal on Intestinal Digestive Enzymes.** The 25% PBM replacement of the fish meal had the highest enzyme activity. Amylase activity was significantly increased ( $P < 0.05$ ) compared to the other groups. The trypsin and lipase activities of the PBM groups were higher than those of the control group (C), but there was no significant difference ( $P > 0.05$ ), as shown in Table 5.

**3.4. Effects of Replacement of Fish Meal by the Poultry By-Product Meal on Immunity.** In all experimental diets, there was no significant change ( $P > 0.05$ ) in SOD activity, MDA content, and AKP activity in prawn's hemolymph. However, the ACP activity of all the replacement groups decreased significantly ( $P < 0.05$ ), as shown in Table 6.

## 4. Discussion

The evaluation of ingredients is critical for determining their suitability for aquaculture systems. It is also critical to determine an ingredient's energy and nutrient accessibility and its digestibility to an animal. This research revealed that a substantial amount of PBM might be substituted for fish meal in the feed of *M. rosenbergii* without affecting growth performance, feed digestibility, and health. *M. rosenbergii* is a fast-growing species requiring a shorter time to achieve commercial size compared to other aquatic species. This shorter culture window means a reduced feed input demand but a greater maintenance expense. PBM has come out as a viable alternative to fish meal in aquaculture feed formulations for a myriad of purposes, together with its high protein content, low ash content, high palatability, and digestibility, as a great source of cholesterol and phospholipids [22]. In addition, PBM is a less expensive protein source than fish meal and is readily accessible, particularly in

TABLE 5: Effects of replacement fish meal by PBM on intestinal digestive enzymes.

Groups	Amylase (U/dl)	Trypsin (U/mg prot)	Lipase (U/mg prot)
Control	9.05 ± 2.00 <sup>b</sup>	9.85 ± 1.25 <sup>a</sup>	1.76 ± 0.93 <sup>a</sup>
PBM 25	40.23 ± 0.65 <sup>a</sup>	17.82 ± 2.53 <sup>a</sup>	4.12 ± 2.34 <sup>a</sup>
PBM 50	9.78 ± 3.33 <sup>b</sup>	13.03 ± 2.40 <sup>a</sup>	3.01 ± 0.72 <sup>a</sup>
PBM 75	6.31 ± 1.42 <sup>b</sup>	12.74 ± 1.60 <sup>a</sup>	1.94 ± 0.48 <sup>a</sup>
PBM 100	14.98 ± 4.43 <sup>b</sup>	14.00 ± 1.96 <sup>a</sup>	3.25 ± 0.04 <sup>a</sup>

All values are given as the mean ± standard deviation (SD). Means followed by different letters in the same column are significantly different  $t(P < 0.05)$ .

poultry-producing areas [23, 24]. As a result, increasing the amount of PBM in the diets of *M. rosenbergii* can significantly cut feed expenditures. However, to achieve the optimum results, measures for balancing the content of alternative protein sources in the basal feed and promoting *M. rosenbergii* growth must be implemented.

**4.1. Effect of Replacement of Fish Meal by the Poultry By-Product Meal on Growth Performance.** When it comes to growth performance, many researchers focus on the nutritional value of proximate composition, nutritional content, and its effect on prawns' growth [25, 26]. Studies suggested that the total replacement of fish meal could be realized when using a high-quality PBM [6, 27, 28].

The present study showed that the replacement of FM by 25%, 50%, and 75% PBM did not adversely affect prawns' growth parameters. Many research studies support this present study. In diets for Pacific white shrimp *L. vannamei*, a high-quality PBM can substitute up to 80% of the fish meal without the need for amino acid supplements [22] and up to 70% of the fish meal [29] without noticeably impairing growth. Nik Sin et al. [30] also replaced up to 75% of *Macrobrachium rosenbergii*'s diet without negatively affecting the growth performance. Other studies on different PBM inclusion had been studied in crustacean diets without affecting the growth, including 27.7% in *P. monodon* [31], 29.82% in *Macrobrachium nipponense* [32], and 21.2% in *C. quadricarinatus* [33]. Another similarity was also observed in Davis and Arnold [34] and Samocha et al. [35], who also reported that the Pacific white shrimp, *L. vannamei* did not demonstrate any difference in survival or growth when a PBM product was used to replace 80% FM in the diet.

TABLE 6: The effect of replacement fish meal by PBM on immunity.

Groups	<sup>a</sup> SOD (U/mL)	<sup>b</sup> MDA (nmol/L)	<sup>c</sup> AKP (gprot)	<sup>d</sup> ACP (gprot)
Control	1499.62 ± 145.75 <sup>a</sup>	24.39 ± 6.50 <sup>a</sup>	7.25 ± 2.00 <sup>a</sup>	10.55 ± 3.53 <sup>a</sup>
PBM 25	1523.32 ± 120.65 <sup>a</sup>	24.79 ± 2.61 <sup>a</sup>	2.97 ± 0.96 <sup>a</sup>	3.28 ± 0.30 <sup>b</sup>
PBM 50	1490.32 ± 137.39 <sup>a</sup>	19.95 ± 4.22 <sup>a</sup>	1.92 ± 0.44 <sup>a</sup>	3.54 ± 1.36 <sup>b</sup>
PBM 75	1599.72 ± 120.99 <sup>a</sup>	19.32 ± 3.87 <sup>a</sup>	5.44 ± 3.08 <sup>a</sup>	3.20 ± 1.01 <sup>b</sup>
PBM 100	1648.17 ± 135.41 <sup>a</sup>	25.85 ± 3.24 <sup>a</sup>	5.44 ± 1.45 <sup>a</sup>	2.49 ± 0.68 <sup>b</sup>

All values are given as the mean ± standard deviation (SD). Means followed by different letters in the same column are significantly different ( $P < 0.05$ ). <sup>a</sup>SOD: superoxide dismutase. <sup>b</sup>MDA: malondialdehyde. <sup>c</sup>AKP: alkaline phosphatase. <sup>d</sup>ACP: acid phosphatase.

Similarly, the inclusion of 20.1% PBM in the diet of white shrimp produced the best performance [29]. The results of this research are also in line with those found by Cheng et al. [36], Tan et al. [37], and Zhu and Yu [38], with 66%, 80%, and 80% replacement levels, respectively.

The recent study by Nik Sin et al. [30] reported reduced growth performance at 100% PBM replacement in *Macrobrachium rosenbergii*, which agrees with this present study. Also, Karapanagiotidis et al. [28] reported that 100% replacement of FM by PBM resulted in reduced growth performance in gilthead seabream juveniles due to a lower feed intake and low levels of dietary methionine and lysine. In other studies, tilapia (100% PBM [39]), golden pompano *Trachinotus ovatus* (up to 100% PBM [40]), and humpback grouper (100% PBM [15]) also had reduced growth performances. The 100% replacement of fish meal by PBM agrees with this study because growth performance was significantly reduced in the prawns of group PBM100 compared to the control group. PBM protein is characterized by having less methionine and lysine than fish meal, which is thought to be a growth factor in many species, such as hybrid striped bass [41] and Florida pompano *Trachinotus carolinus* [42]. Amino acid deficiency of PBM could be one cause for decreased fish growth at a greater fish meal replacement level (100%). Again, poor digestibility can be another cause for poor growth performance in higher replacement (PBM100). Although the apparent digestibility coefficient of the meals in the current research was not tested, it has been reported that many prawns/fish perform worse in terms of growth because feeds with a lot of poultry by-product meal have a poor digestibility [30, 32, 43, 44]. In experiments on humpback grouper [15] and gilthead seabream [6], it was discovered that diets containing 75% and 100% FM protein substituted with PBM protein resulted in reduced growth performance, which was associated with the reduced digestibility of dietary protein.

**4.2. Effects of Replacement of Fish Meal by the Poultry By-Product Meal on Proximate Composition.** In this present research, replacing fish meal with PBM had no significant effect on the whole-body moisture, crude protein, crude lipid, and crude ash content of prawns. However, the whole-body protein was higher in prawn-fed PBM25 than in the other prawn groups. This observation was in agreement with Yang et al. [44] who reported no significant differences in the whole-body moisture and fat, but a trend of increased

protein and ash contents of gibel carp fed increasing levels of PBM. Again, the whole-body composition of red sea bream *Pagrus major* was not affected by different groups of dietary PBM [18]. Other research studies were on *M. rosenbergii* [30], *L. vannamei* [22], *L. vannamei* [35], *L. vannamei* [29], *M. nipponense* [32], *C. quadricarinatus*, and Pacific white shrimp [34]. Prawns benefit from the fat in PBM because it contains essential nutrients, including phospholipids and cholesterol. Protein and lipid content in the prawn body could be related to changes in their synthesis, deposition rate in muscle, and growth rates [45–47].

In contrast, Chinook salmon *Oncorhynchus tshawytscha* [48] and rainbow trout *Oncorhynchus mykiss* [27, 49] increased whole-body fat of fish-fed PBM diets. Nengas et al. [6] found that increasing dietary PBM in gilthead seabream resulted in significantly decreased carcass lipid, which disagrees with this present study.

**4.3. Effect of Replacement of Fish Meal by the Poultry By-Product Meal on Intestinal Digestive Enzymes.** Trypsin is a protein-digesting enzyme, amylase is a carbohydrate-digesting enzyme, and lipase digests fats and lipids. The hepatopancreas of the giant freshwater prawn is primarily responsible for amylase, trypsin, and lipase production. The enzyme amylase is then secreted into the foregut, breaking down glycosidic bonds [50]. In this study, the increase in all these enzyme activities in the experimental diets indicates an improved capacity for digesting. The preponderance of research validated PBM's excellent digestibility as in giant tiger prawn, *Penaeus monodon* [51], Pacific white prawn [22, 34], Korean rockfish, *Sebastes schlegelii* [19], and rainbow trout [52]. Factors such as nutritional content, palatability, and digestibility of PBM may function as a moderator of the growth effect of PBM supplementation. In this study, PBM-improved digestive enzymes activity is the important factor for good growth performance and immunity in prawns.

**4.4. Effect of Replacement of Fish Meal by the Poultry By-Product Meal on Immunity.** The levels of SOD, MDA, AKP, and ACP can be utilized to assess the prawns' physiological state and immunity response, as is well acknowledged. SOD is the enzyme that converts superoxide anion radicals to hydrogen peroxide and molecular oxygen, and it plays a critical role in regulating cellular reactive oxygen species

(ROS) levels [53]. The type of feed intake is also critical for ROS production. The balance between ROS creation and removal maintains a steady-state ROS level. Malondialdehyde (MDA) is the end product of lipid peroxidation and is widely used as a proxy for oxidative stress [54–56]. The alkaline phosphatase (AKP) level in the hemolymph can be used to determine hepatopancreas-related issues. In summary, elevated SOD and MDA mean the health of the prawns has been compromised. This study showed no significant change in SOD activity, MDA concentration, or AKP activity in prawn hemolymph. The blood parameter values of prawns fed different diets in this study suggested that their condition and health state were normal for prawn-fed PBM meals, which agrees with Rio-Zaragoza et al. [57] and Hernández et al. [58]. The substitution of fish meals with PBM meals had no effect on the body composition of the prawns in this investigation which also showed that prawns were in good health.

## 5. Conclusion

This study showed that PBM up to 75% replacement group has no effect on growth performance, and all replacement groups have no adverse effect on intestinal digestive enzyme activity and immunity of juvenile stage of *M. rosenbergii*. Therefore, poultry by-product meal could replace fish meal by up to 75%.

## Data Availability

The datasets in this study are available from the corresponding author upon reasonable request. All data and materials are available for publication.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

All co-authors have seen and agreed with the contents of the work.

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