



Evaluation of Copepods as an Alternative Feeding Strategy in Semi-Intensive Culture of Pacific White Shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Indonesia is the fourth-largest global shrimp exporter but faces higher production costs than Ecuador. Using copepods in semi-intensive farming offers a potential solution, as their high content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can enhance shrimp growth and efficiency. The present study aimed to evaluate the effects of copepods as a live-feed supplement on growth and feed efficiency of vannamei shrimp (*Litopenaeus vannamei*) in the semi-intensive culture. A completely randomized design was established with three treatment groups and three replications. A total of 315 post-larvae *Litopenaeus vannamei* were kept at a density of 35 shrimp per tank, with each tank measuring $1.3 \times 1.3 \times 0.73 \text{ m}^3$ during a 50-day cultivation period. The treatment groups included a commercial feed-only control group (P1), a commercial feed with copepods inclusion (P2), and a copepods-only group (P3). The growth (absolute daily growth, absolute body weight, absolute weight/length), feed efficiency, hepatopancreas histopathology, proximate composition, and water quality (temperature, salinity, pH, dissolved oxygen, total dissolved solids, alkalinity, total ammonia nitrogen, total organic matter, total bacteria count, total vibrio count) were assessed. The current results indicated that P2 achieved the highest growth performance among the treatment groups. Furthermore, P2 provided higher nutritional value for protein and fat than P1 and P3, showing the healthiest tissue structure, mild atrophy, and sloughing, with the lowest hemocyte infiltration, supporting improved epithelial health. Water quality remained within or approximately within the recommended ranges. Therefore, combining copepods with commercial feed improved growth, survival, and feed efficiency compared with using either copepods or commercial feed alone.

Keywords: Copepod, Shrimp growth, Vanname shrimp, Water quality

INTRODUCTION

Ecuador is currently leading the global shrimp supply, with export volumes in 2023 rising significantly to 1.07 million tons, a 20.6% increase from 2022. Indonesia ranks fourth among shrimp-producing countries, with a 3.28% decline in export volume in 2023 compared to the previous year, totaling 240,400 tons (FAO, 2023a). Shrimp export prices in Indonesia are higher than in Ecuador, a leading global market influencer that substantially affects worldwide prices. Nevertheless, the Food and Agriculture Organization indicated that Ecuadorian shrimp sizes 31-40 and 61-70 were priced at 8.14 USD and 6.47 USD, respectively, compared with Indonesian products, which were priced at 8.65 USD and 7.25 USD (FAO, 2023b). The significant 0.78 USD price difference for the 61-70 size indicated higher operating costs.

Operational cost efficiency can be improved by reducing production input, particularly feed, which accounts for 60-70% of the total costs (Ulum et al., 2018; Macusi et al., 2023; Corne et al., 2024). Due to the high feed cost, there is a need for alternatives, such as copepods, zooplankton commonly found in estuaries and mangroves with adequate fatty acid profiles (Dayras et al., 2021) and available at different sizes, from nauplii to adults (Martino et al., 2024). Nauplii stage copepods (N1-N6), measuring 100-230 μm , are substantially smaller than rotifers, which are 145-150 μm , making them easy for vannamei shrimp larvae to consume (Ismi et al., 2021). Compared to rotifers with an Eicosapentaenoic acid (EPA) of 8.26% and *Artemia salina*, copepods provide a greater EPA of 9.25% and Docosahexaenoic acid (DHA) of 24.41 (Samat et al., 2020; Wiradana et al., 2020). The omega-3 HUFADHA (22:6n-3) and EPA (20:5n-3), along with the n-6 series highly unsaturated fatty acids (HUFA) arachidonic acid (ARA; 20:4n-6), play important roles in fish larval development. Therefore, deficiency of HUFA can impair fish growth, reproduction, and survival, causing pale or swollen liver, myocarditis, intestinal steatosis, lordosis, fin erosion, and shock syndrome (Samat et al., 2020). Compared to other

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species, shrimp have a limited ability to elongate and desaturate linolenic acid into EPA and DHA (Hartik et al., 2017). This limitation can be overcome by feeding copepods that contain several enzymes, such as delta-5, delta-6 desaturase, and elongase, which are useful for converting short-chain essential fatty acids (LNA 18:3n3) into long-chain forms, including DHA (22:6n3), EPA (20:5n3), and other omega-3 highly unsaturated fatty acids (Novianto and Efendy, 2020). The high HUFA content enhances feed palatability, promoting larval growth and feed consumption rate (Herawati et al., 2023). Fish and shrimp consume different species of copepod nauplii (Suminto et al., 2020). Consequently, incorporating copepods into initial maintenance can reduce feed production input costs and improve competitiveness in the global market (Burbano et al., 2020). Therefore, the present study aimed to evaluate the effects of copepods as a dietary supplement on *Litopenaeus vannamei* cultured in a semi-intensive system by examining key production parameters, including growth performance, survival rate (SR), and feed conversion ratio (FCR).

MATERIALS AND METHODS

Ethical approval

The current study was conducted in accordance with the guidelines of the Faculty of Aquaculture Technology, Jakarta Technical University of Fisheries, Jakarta, Indonesia. All stages of study procedures were carried out according to standards in both laboratory and field testing. Analysis of the test biota was carried out by physical observation every day, with food provided according to the treatment group.

Experimental design

The current study used a completely randomized design with three treatment groups, each having three replications. The treatments included the control group that received only the commercial feed (P1), the second group that received the commercial feed with copepods (P2), and the third group that received only copepods (P3). *Litopenaeus vannamei* were used at the post-larva stage, with a density of 35 shrimp per tank, in tanks with dimensions of $p \times l \times t = 1.3 \times 1.3 \times 0.73 \text{ m}^3$. These shrimps were obtained from the Cultivation Hatchery at Politeknik AUP in Serang, Banten, Indonesia, for the 50-day maintenance. During this period, *Litopenaeus vannamei* were given P1, P2, and P3 three times a day (Table 1). The commercial feed (Gold Coin Marine Shrimp Feed, Vietnam) features a particle size between 0.5 and 2.2 mm, with a nutritional composition including 36% crude protein, 5% crude fat, 4% crude fiber, 15% ash, 12% moisture, and a metabolic energy content of 3.200 kcal/kg. Copepods from the Brackishwater Aquaculture Development Center in Jepara, Indonesia, were cultured in 15-liter jars. The seawater salinity was about 20-23 g/l, and the temperature ranged from 28 to 32°C. The copepod life cycle, from egg to adult, was found to be seven days. During the 50-day maintenance of *Litopenaeus vannamei*, copepods were initially supplied at a quantity of 1,000,000 individuals. Subsequently, 45,000 copepods were administered weekly for each treatment (Martínez-Córdova et al., 2011).

Making fermented rice bran

Fermented rice bran (FRB), locally sourced from operated rice mills in Serang, Banten, Indonesia, was used as copepods feed. A previous comparative study reported that probiotic fermentation decreased phytic acid and trypsin inhibitor in rice bran (Wang et al., 2024). For preparation, 500 g of dried and ground rice bran was placed in a 1000 ml beaker and autoclaved at 121°C for 20 minutes (Ihtifazhuddin et al., 2017). Fermented rice bran was prepared using 1 kg of rice bran, 1 L of fresh water, and 10 g of yeast (NKL Indonesian brand), then incubated for 24 hours at 28-30°C under static conditions (loosely covered, stirred 2-3 times), until the slurry reached an end-pH of 4.5-5.5. Subsequently, the culture was fermented for 24-48 hours at 28-30°C (Rengpipat et al., 1998) without aeration, using 8 L of seawater, 1 L of molasses, 200 mL of EM4, and 50 g of NaHCO₃ mixed into the FRB.

Application of fermented rice bran

The FRB was applied before and after shrimp stocking until the water culture reached approximately 30-35 cm in transparency. Before shrimp stocking, the culture water was sown three times a day (at 8:00 a.m., 2:00 p.m., and 6:00 p.m.) with FRB and Dolomite lime at doses of 100-200 mgL⁻¹ and 5 mgL⁻¹. (Saparuddin and Haryani, 2015). The time and frequency of FRB implementation were the same before and after stocking, with the dose at 1-5 mgL⁻¹. The dose was decreased when water transparency fell below 20-30 cm and increased when water transparency exceeded 30-50 cm.

Feeding

Feed requirements were estimated utilizing the feeding rate (%) method (Antunes et al., 2018; Inayah et al., 2023), which was derived from the average body weight (ABW) and calculated based on the biomass of *Litopenaeus vannamei*. The feed management followed the SNI 7772:2013 guidelines for semi-intensive *Litopenaeus vannamei* cultivation in brackish-water ponds (Table 1).

Table 1. Feeding schedule using commercial feed for *Litopenaeus vannamei* over a 50-day culture

Average body weight (g.Ind ⁻¹)	Feeding rate (%)	Feeding	
		Frequency (times a day)	Time (Local time)
0.05-1.0	25		
1.1-2.5	15		
2.6-5.0	10		
5.1-8.0	7	3	09.00, 14.00, dan 19.00
8.1-11	5		
11.1-18.0	3		
18.1-22.0	2		

Shrimp growth and survival rate

Several growth parameters were analyzed, including average daily growth (ADG), ABW, absolute growth (weight/length), and SR. The ADG was the average daily weight gain of shrimp over a given period and can be used to determine the growth rate. The ABW was the average weight of shrimp based on sampling results. In the present study, Average daily growth and ABW were calculated using the following formula (Mahendra et al., 2023).

$$\text{ADG (g.d}^{-1}\text{)} = \frac{\text{ABW sampling II (g)} - \text{ABW sampling I (g)}}{\text{Sampling period (day)}} \quad (\text{Formula 1})$$

$$\text{ABW (g.ind}^{-1}\text{)} = \frac{\text{Shrimp sampel weight (g)}}{\text{Number of shrimp sampel (ind)}} \quad (\text{Formula 2})$$

Absolute weight growth was calculated using Formula 3 (Susilowati et al., 2014).

$$\text{W (g)} = \text{Wt (g)} - \text{W}_0\text{ (g)} \quad (\text{Formula 3})$$

In this equation, W is absolute weight gain (g), Wt is final weight (g), and W₀ indicates initial weight (g).

Absolute length growth was calculated using Formula 4 (Kurniaji et al., 2021).

$$\text{L (cm)} = \text{Lt (cm)} - \text{L}_0\text{ (cm)} \quad (\text{Formula 4})$$

In this equation, L is absolute length growth (cm), Lt indicates final length (cm), and L₀ is initial length (cm).

Survival rate was calculated based on Formula 5 (Mahendra et al., 2023).

$$\text{SR (\%)} = \frac{\text{Number of shrimp sampel (ind)}}{\text{Number of stocking shrimp (ind)}} \times 100 \quad (\text{Formula 5})$$

Feed conversion ratio was calculated by Formula 6 (Witoko et al., 2018).

$$\text{FCR} = \frac{\text{F}}{\text{Wt} - \text{W}_0} \quad (\text{Formula 6})$$

In the equation, F is the amount of feed given during the rearing period (g), Wt indicates final biomass (g), and W₀ is initial biomass (g).

Histological analysis

The method of Saputra et al. (2025) was used to perform histopathological examination of hepatopancreas in *Litopenaeus vannamei*. Shrimp health was assessed by visually inspecting the color and structure of the hepatopancreas. After determining the health condition, hepatopancreas were collected and stored in Davidson's fixative (Sigma-Aldrich, St. Louis, Missouri, USA) for 24 hours (Saputra et al., 2025). For observation and differentiation of hepatopancreas and intestinal cells, staining was performed using hematoxylin and eosin (H&E; Sigma-Aldrich, St. Louis, Missouri, USA).

Proximate of *Litopenaeus vannamei*

The biochemical or proximate method was used to determine the nutritional content of *Litopenaeus vannamei* in the chemistry laboratory of the Fisheries Business Expert Polytechnic, Jakarta, Indonesia. The tests were carried out in accordance with the Indonesian National Standards using the Official Methods Chapter 4. Protein content was determined using the Kjeldahl method, ash content was analyzed through an ash furnace at 500°C for 4 hours, and fat content was evaluated with the Soxhlet fat extraction method (AOAC, 2005).

Water quality

The physical water parameters observed were temperature with a thermometer, salinity with a refractometer, pH with a pH meter, and dissolved oxygen (DO) with a DO meter. Light intensity was measured at the time of the initial preparation of the maintenance using a Lux meter. The chemical parameters measured were total DO, alkalinity, total

ammonia nitrogen, and total organic matter. Furthermore, the measured biological parameters included total *Vibrio* and total bacterial counts (Asni et al., 2023).

Data analysis

The effect of each treatment on predetermined parameters was tested using single-factor Analysis of Variance (ANOVA) at the 95% confidence level ($p < 0.05$). Subsequently, an LSD test was conducted to determine the best treatment using IBM SPSS version 22.0.

RESULTS

Average daily growth

The highest ADG was obtained in P2 at 0.36 ± 0.051 g/d, followed by P1 and P3 at 0.26 ± 0.011 g/d and 0.06 ± 0.009 g/d, respectively (Figure 1). The current results indicated that ADG varied significantly among the treatment groups ($p < 0.05$). Group P2 exhibited a significantly higher ADG compared to groups P1 and P3 ($p < 0.05$), with Group P1 surpassing Group P3 ($p < 0.05$).

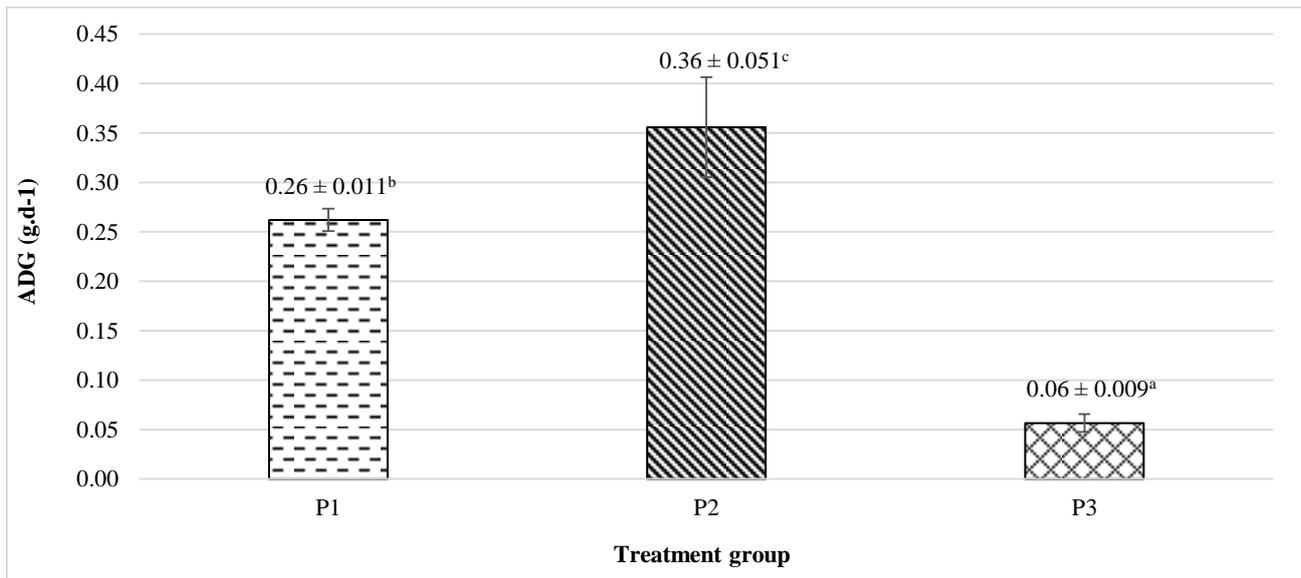


Figure 1. Average daily growth of Pacific white shrimp (*Litopenaeus vannamei*) over a 50-day culture period with different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. Values were represented as mean \pm standard deviation. ^{a, b, c} Different superscript letters indicated significant differences among treatments ($p < 0.05$).

Average body weight

In the present study, the initial weight of shrimp was 0.8 g ind^{-1} . Differences among the treatment groups were detected at the third sampling, with P2 being higher than P1 and P3. Other sampling indicated differences among the treatment groups. The increase of ABW during the culture period is presented in Figure 2.

In the present study, the highest ABW of approximately $18.60 \pm 2.525 \text{ g ind}^{-1}$ was observed in P2, followed by P1 and P3, which recorded $13.91 \pm 0.570 \text{ g ind}^{-1}$ and $3.63 \pm 0.448 \text{ g ind}^{-1}$ (Figure 3). The current results indicated that ABW varied significantly among treatment groups ($p < 0.05$). Fisher's LSD revealed that P2 had a significantly higher ABW than P1 and P3, with P1 exceeding P3 ($p < 0.05$). Biologically, the current results indicated that combining commercial feed with copepods promoted greater somatic growth than feeding only the commercial feed. The administration of copepods was also insufficient to match mixed or commercial feed. This suggested that shrimp nutritional needs were met by both commercial feed and live copepod feed.

Absolute weight growth

In the present study, the highest AWG was obtained in P2 of $17.80 \pm 2.525 \text{ g}$, followed by $13.11 \pm 0.570 \text{ g}$ and $2.83 \pm 0.448 \text{ g}$ in P1 and P3, respectively (Figure 4). The present findings indicated that AWG differed significantly among treatment groups ($p < 0.05$). Fisher's LSD test indicated that P2 had significantly higher AWG than P1 and P3 ($p < 0.05$), and P1 was significantly greater than P3 ($p < 0.05$).

Absolute length growth

In the present study, the highest absolute length growth was in P2 at $8.4 \pm 0.80 \text{ cm}$, followed by P1 and P3 at $7.1 \pm 0.24 \text{ cm}$ and $2.6 \pm 0.06 \text{ cm}$, respectively. The current findings indicated a significant treatment effect on absolute length growth ($p < 0.05$). Fisher's LSD indicated that P2 had significantly greater absolute length growth than P1 and P3 ($p < 0.05$), with P1 significantly exceeding P3 ($p < 0.05$; Figure 5).

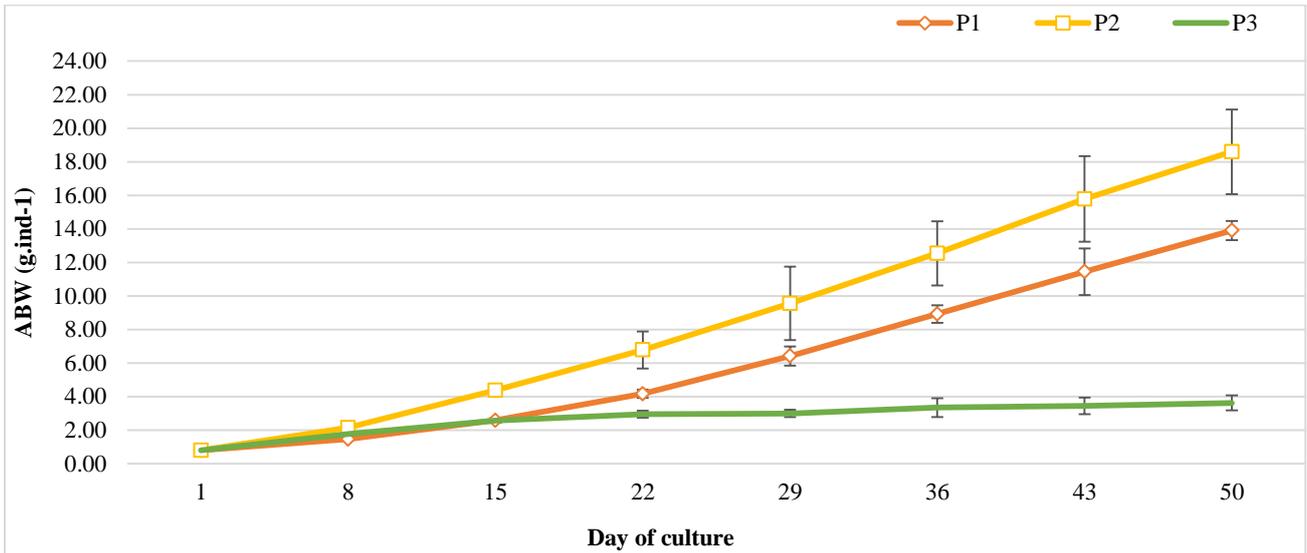


Figure 2. Temporal change in average body weight of Pacific white shrimp (*Litopenaeus vannamei*) over a 50-day culture period fed different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only.

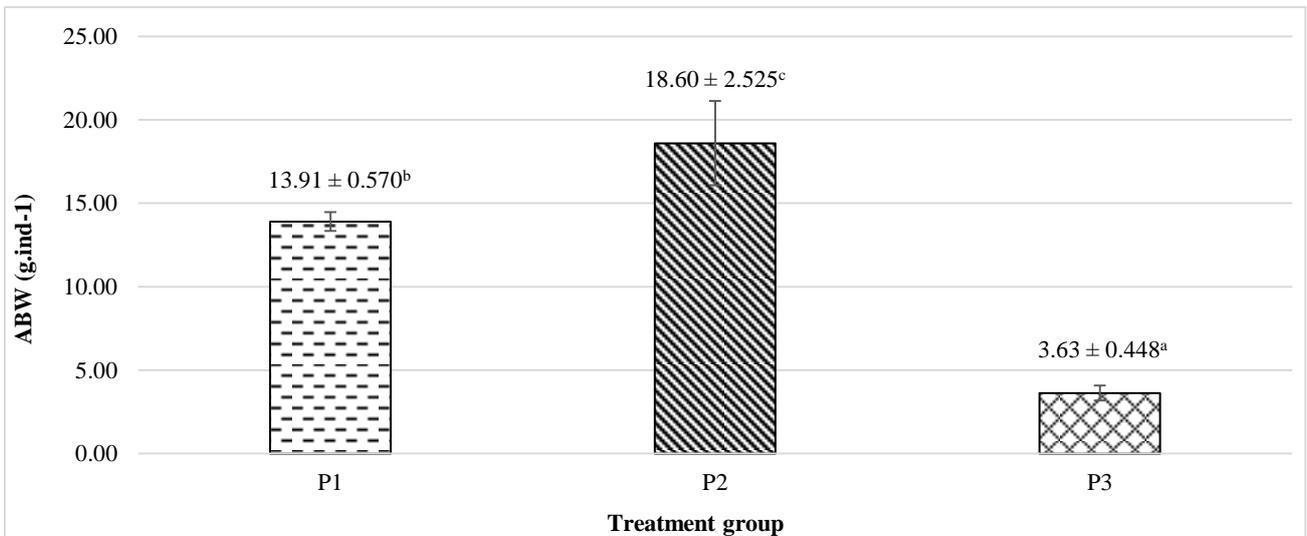


Figure 3. Average body weight of Pacific white shrimp (*Litopenaeus vannamei*) over a 50-day culture period fed different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. Values were represented as mean ± standard deviation. ^{a, b, c} Different superscript letters indicated significant differences among treatments ($p < 0.05$).

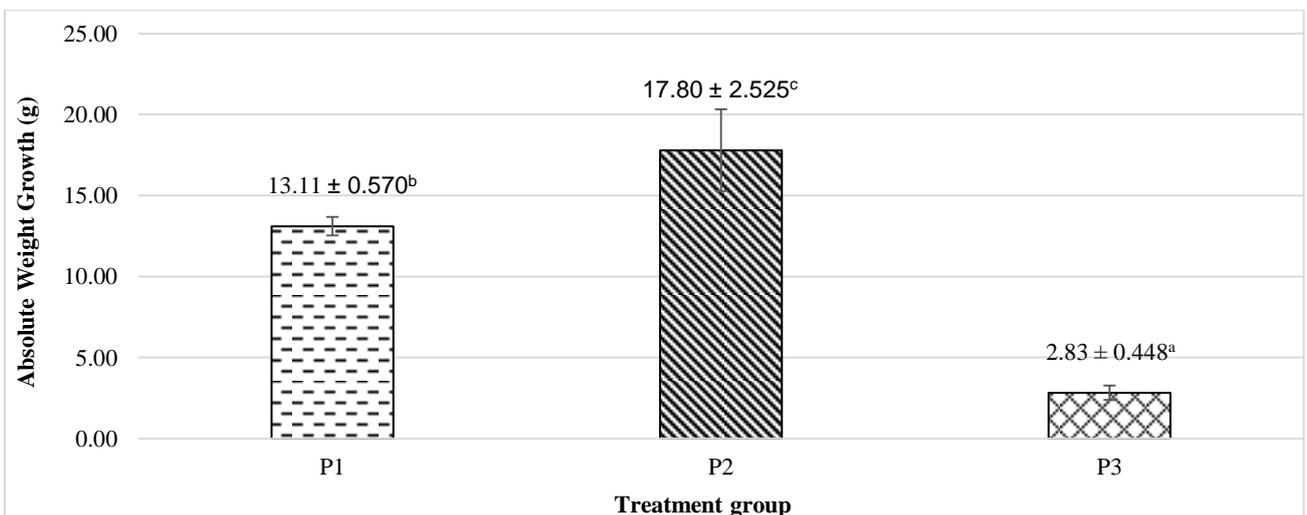


Figure 4. Absolute weight growth of *Litopenaeus vannamei* over a 50-day culture period fed different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. Values were represented as mean ± standard deviation. ^{a, b, c} Different superscript letters indicated significant differences among treatments ($p < 0.05$).

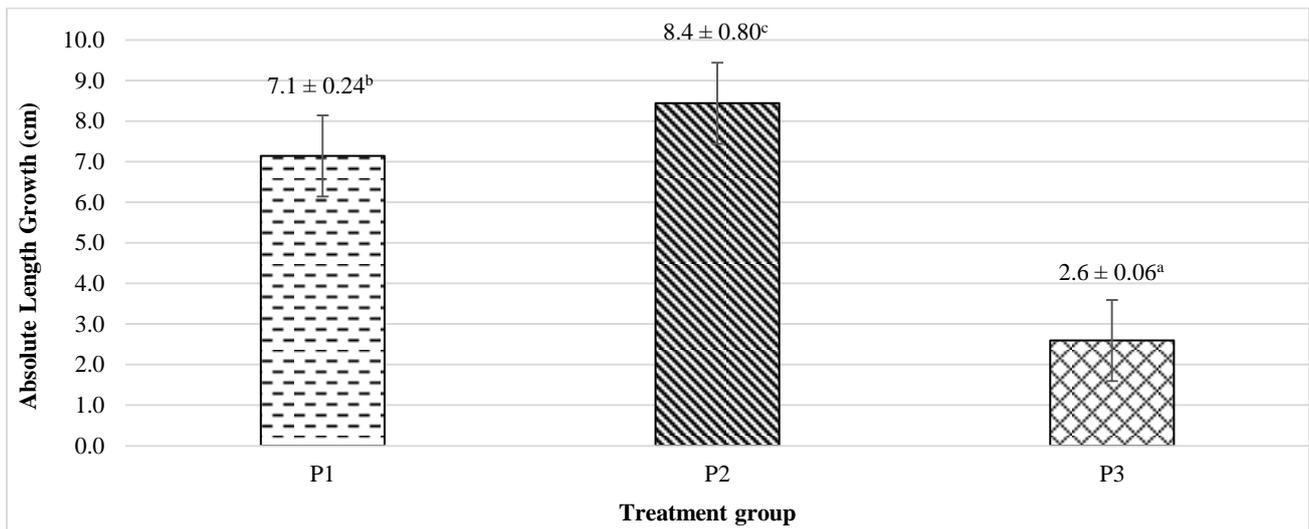


Figure 5. Absolute length growth of Pacific white shrimp (*Litopenaeus vannamei*) over a 50-day culture period fed different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. Values were represented as mean ± standard deviation. ^{a, b, c} Different superscript letters indicated significant differences among treatments ($p < 0.05$).

Survival rate

In the present study, the SR was $99.02 \pm 1.698\%$, 89.22 ± 6.123 , and $79.41 \pm 8.824\%$ in P2, P1, and P3, respectively (Figure 6). The current findings indicated that SR in *Litopenaeus vannamei* differed significantly among treatment groups ($p < 0.05$). The SR in P2 was significantly higher than in P3 ($p < 0.05$). However, the difference between P2 and P1 was not significant ($p > 0.05$).

Feed conversion ratio

In the present study, the best FCR of 1.07 ± 0.116 was achieved in P2, followed by P1 (1.41 ± 0.172) and P3 (0; Figure 7). The current findings demonstrated that FCR differed significantly between treatment groups ($p < 0.05$). Group P2 had a significantly lower FCR than P1 ($p < 0.05$). Since P3 received no formulated feed, FCR was not applicable and was excluded from the analysis. Shrimp growth in P3 was supported by live copepods and FRB-derived productivity; the total FCR (including live-feed inputs) was greater than zero and could not be directly compared without quantifying live-feed biomass. A lower FCR value indicates better feed quality, suggesting higher feed digestibility (Zainuddin et al., 2014).

Scheme implementation

The color of cooked harvested shrimp across treatments, including P1, P2, and P3, differed (Figure 8a). These differences were attributed to variation in the feeding schemes used to apply copepods. The raw coloration of shrimp was due to crustacyanin, a carotenoprotein in which astaxanthin was bound to protein. The complex appeared green-blue to purplish and showed an absorption maximum of approximately 580 nm.

Histopathology of hepatopancreas tissue

The results of histological analysis demonstrated aggregated transformed microvilli (ATM), hepatopancreas tubule atrophy, and sloughing, as well as hemocyte infiltration (Figure 9). Differences in hepatopancreas organ test results are presented in Table 2. In P2, ATM and sloughing cells were found in hepatopancreas lumen (Figures 10a and 10b), hemocyte infiltration in intertubular space (Figure 10c), and atrophy in hepatopancreas tubules (Figure 10d). Hemocyte infiltration in P2 was the lowest among the treatment groups. The combination of commercial feed and copepods in P2 indicated a more normal tissue condition than the other treatment groups. Therefore, shrimp's nutritional requirements can be fulfilled using a combination of commercial feed and copepods. Atrophy and inflammation were found in hepatopancreas tubules (Figure 11a), hemocyte infiltration in the intratubular space (Figure 11b), sloughing in tubules (Figure 11d), and ATM in the hepatopancreas lumen. Furthermore, B cells were dominant in P3, whereas R cells were rarely observed in hepatopancreatic tubules (Figure 11c).

Proximate of *Litopenaeus vannamei*

Proximate analysis of *Litopenaeus vannamei* (protein, carbohydrate, fat, fibre, ash, and moisture) was conducted at the beginning day of the culture (DOC-0) and the end of the culture period (DOC-50) to assess nutrient changes under different feeding diets (Table 3). The lowest protein content, at $9.50 \pm 1.087\%$, was observed in P3. Meanwhile, the highest value, $17.52 \pm 0.904\%$, was obtained in P2.

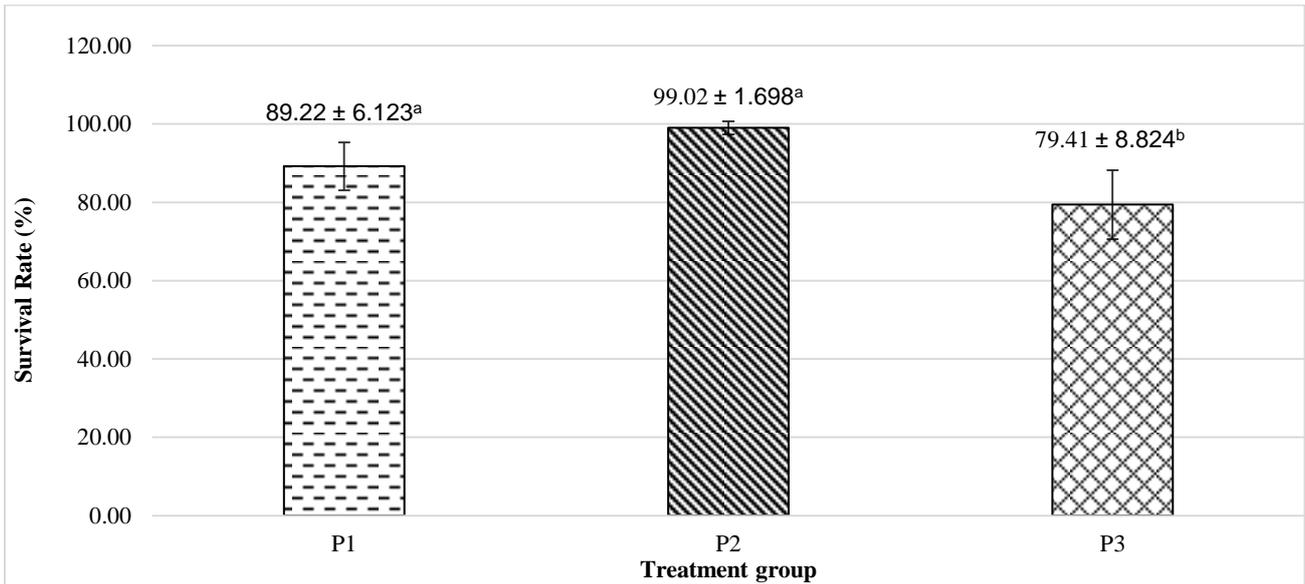


Figure 6. Survival rate of Pacific white shrimp (*Litopenaeus vannamei*) over a 50-day culture period with different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. Values were represented as mean ± standard deviation. ^{a,b} Different superscript letters indicated significant differences among treatments ($p < 0.05$).

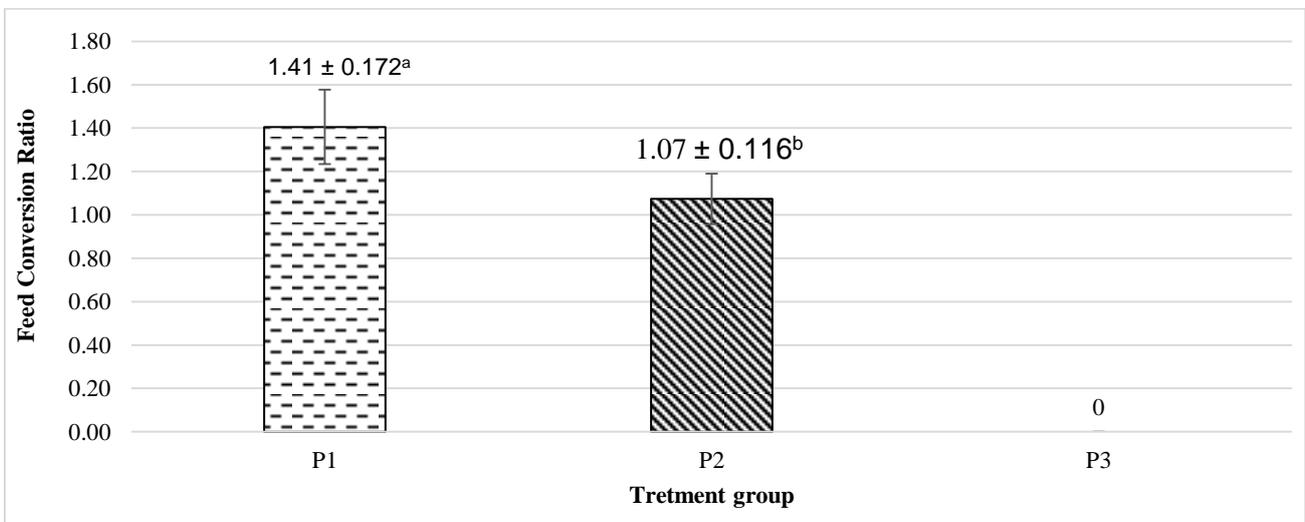


Figure 7. Feed conversion ratio of *Litopenaeus vannamei* over a 50-day culture period fed different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. Values were represented as mean ± standard deviation. ^{a,b} Different superscript letters indicated significant differences among treatments ($p < 0.05$).

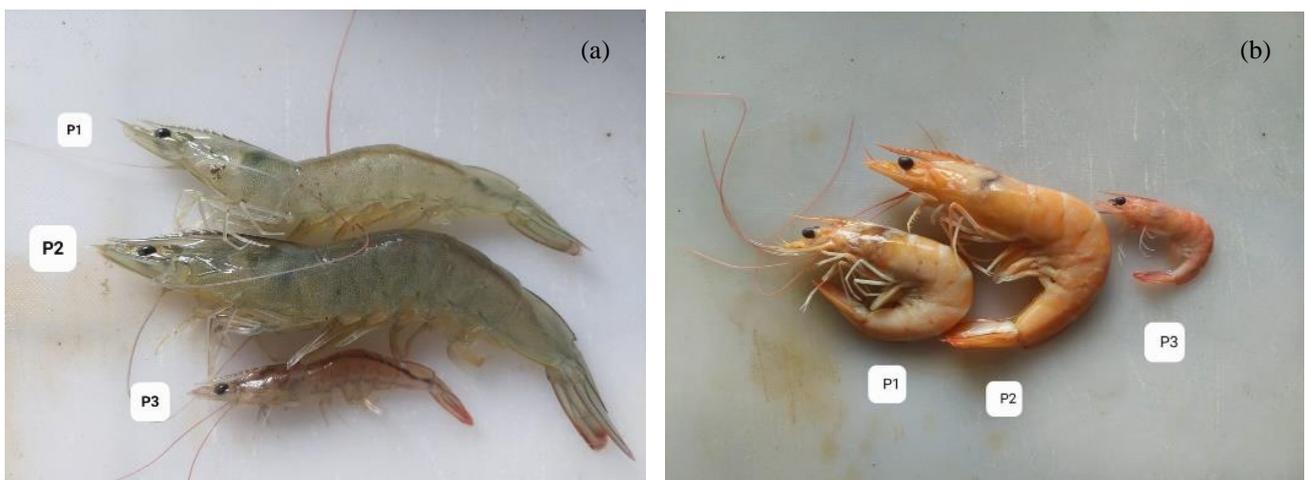


Figure 8. Harvest appearance and post-cooking color of *Litopenaeus vannamei* at the end of the 50-day culture fed different diets. P1: Commercial feed only, P2: Commercial feed + live copepods, and P3: Live copepods only. **a:** Harvested (raw) shrimp, **b:** Cooked shrimp.

Table 2. Differences in histopathology test results of the hepatopancreas organ of *Litopenaeus vannamei* after 50-day culture

Parameter	Treatment	P1	P2	P3
Aggregated Transformed Microvilli (ATM)		+	+	+
Atrophy in the hepatopancreas tubules		+	+	+++
Hemocyste infiltration		+++	++	+
Sloughing		+++	++	+
Blister-like cell		++	+++	+++
Resorptive cell		+++	+++	+

Note: (+) indicates the number of cells. In P1, aggregated transformed microvilli were found in the hepatopancreas lumen, while atrophy and sloughing were in hepatopancreas tubules, and hemocyte infiltration in the intertubular space. P1: Commercial feed only (control), P2: Commercial feed + live copepods, P3: Live copepods only

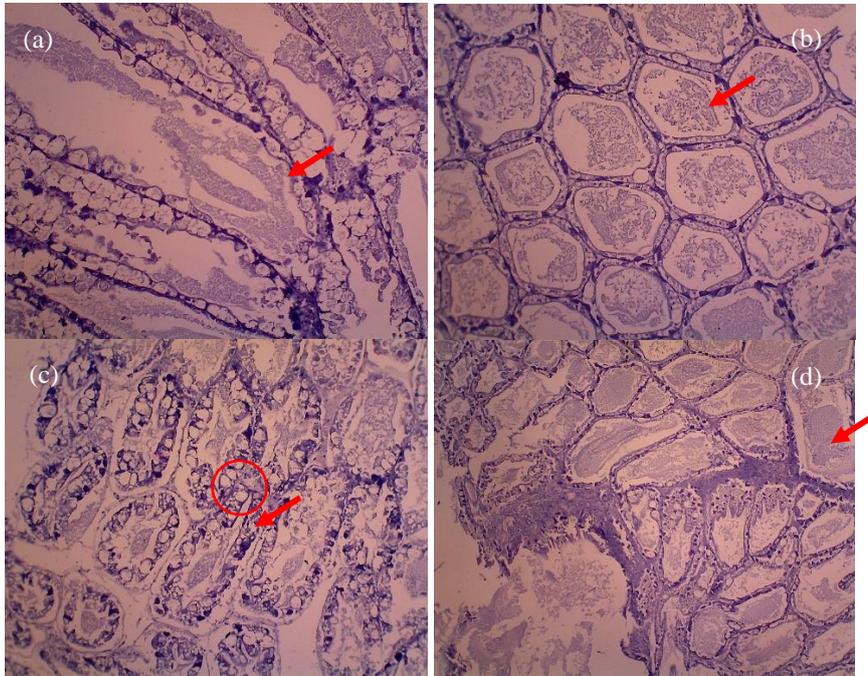


Figure 9. Hepatopancreas of *Litopenaeus vannamei* fed only commercial feed after 50 days of culture. **a:** Aggregated transformed microvilli (Arrow, 100x), **b:** Hepatopancreas atrophy (Arrow, 100x), **c:** Hepatopancreas sloughing (Arrow and circle, 100x), **d:** Hepatopancreas infiltrating cells (Arrow, 40x).

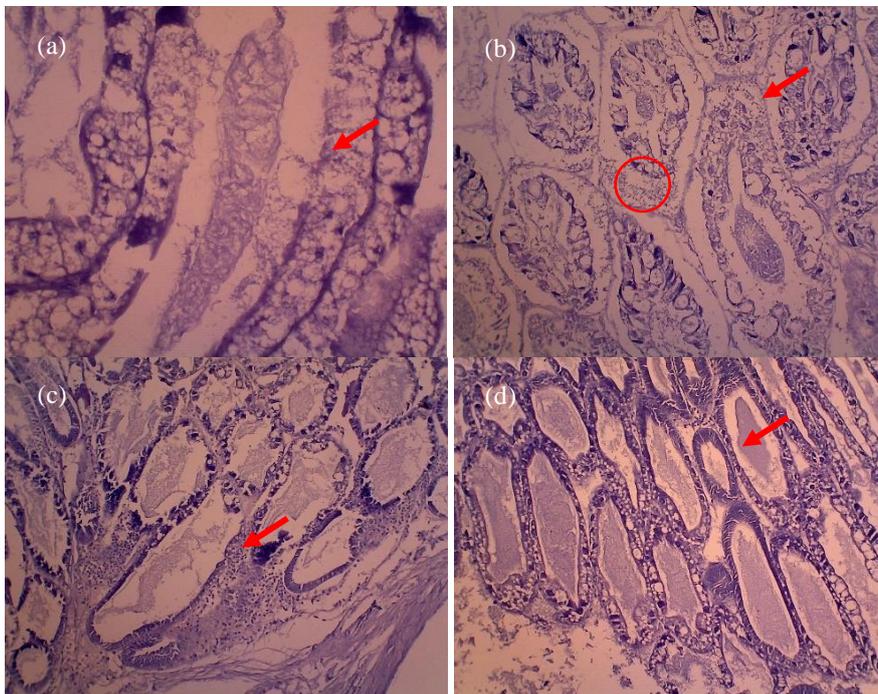


Figure 10. Hepatopancreas of *Litopenaeus vannamei* fed commercial feed supplemented with live copepods after 50 days of culture. **a:** Hepatopancreas Aggregated transformed microvilli (Arrow, 400x), **b:** Hepatopancreas sloughing (Arrow and circle, 100x), **c:** Hepatopancreas infiltrating cells (Arrow, 100x), **d:** Hepatopancreas atrophy (Arrow; 100x).

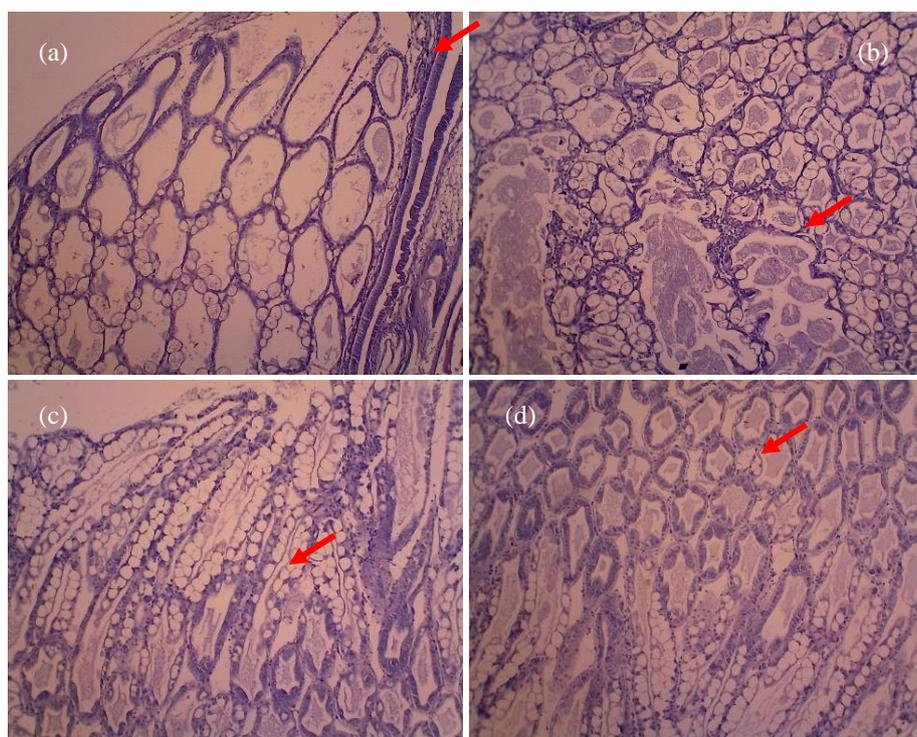


Figure 11. Hepatopancreas of *Litopenaeus vannamei* fed only live copepods after 50 days of culture. **a:** Hepatopancreas atrophy and inflammation (Arrow, 100x), **b:** Hepatopancreas hemocyte infiltration (Arrow, 40x), **c:** Hepatopancreas B cell proliferation (Arrow, 100x), **d:** Hepatopancreas sloughing (Arrow, 100x).

Table 3. The average proximate tests of Pacific white shrimp (*Litopenaeus vannamei*) at the end of the 50-day culture

Treatment	Nutritional Content (%)											
	Protein		Carbohydrate		Fat		Fibre		Ash		Moisture	
	Day of culture DOC-0	Day of culture DOC-50	Day of culture DOC-0	Day of culture DOC-50	Day of culture DOC0	Day of culture DOC-50	Day of culture DOC-0	Day of culture DOC-50	Day of culture DOC-0	Day of culture DOC50	Day of culture DOC-0	Day of culture DOC-50
P1	0.56	17.19	0.56	1.07	1.12	0.40	1.22	1.10	2.84	3.34	80.14	76.91
P2	0.56	17.52	0.56	1.30	1.12	0.79	1.22	1.25	2.84	3.35	80.14	75.79
P3	0.56	9.50	0.56	0.87	1.12	0.38	1.22	1.09	2.84	3.96	80.14	84.21

DOC denotes the number of days elapsed since the culture began, typically starting on the day the animals (shrimp post-larvae) were first stocked into the cultivation tanks (Pilli, 2022). P1: Commercial feed only (control), P2: Commercial feed + live copepods, P3: Live copepods only.

Water quality

Water temperature

The temperature results for each treatment did not comply with SNI 7772:2013 for *Litopenaeus vannamei* in semi-intensive pond culture, which was approximately 28-31.5°C. This was due to weather conditions (cloudy, rainy, blocking sunlight) during the culture period and fluctuating water temperature, which was unsuitable for shrimp.

Salinity

Salinity is an important parameter that significantly affects osmoregulation. This process is the efforts of aquatic organisms to control the balance of water and ions between the body and the environment (Jayanti et al., 2022). The results of salinity measurement in *Litopenaeus vannamei* maintenance media ranged from 23.2 to 25.9 g.l⁻¹.

pH

The pH of the culture water ranged from 8.02 to 8.5. These values were in the tolerance limit for shrimp growth, requiring approximately 6.5-9.0 (Suwarsih et al., 2016). The pH results for each treatment remained within the SNI 7772:2013 range of 7.5-8.5.

Dissolved oxygen

The results of DO measurements in *Litopenaeus vannamei* culture water ranged from 4.10 to 4.99 mg.L⁻¹. These values were in accordance with SNI 7772:2013, namely a minimum of > 3.5 mg.L⁻¹. Therefore, the DO suitability range for *Litopenaeus vannamei* culture ponds is >3 mg.L⁻¹ (Novriadi et al., 2021).

Total dissolved solids

Total dissolved solids (TDS) measurement is important to determine the stability of culture water. The results of TDS measurements in the culture media were in normal conditions, ranging from 17.2 to 21.5 mg.L⁻¹, which was below the recommended value of < 40 mg.L⁻¹ (Mustofa, 2017).

Alkalinity

Alkalinity measurement was important to determine the total concentration of basic elements in water, such as Calcium Carbonate (CaCO₃). Alkalinity of the culture media ranged from 106.2 to 300.9 mg.L⁻¹, meeting the SNI 7772:2013 requirement of 100-150 mg.L⁻¹.

Total ammonia nitrogen

Ammonia in water exists in two forms, including free (un-ionized form/NH₃) and ionized (NH₄⁺), which are collectively called total ammonia nitrogen. Specifically, total ammonia nitrogen measurement is important for determining the accumulation of ammonia produced from uneaten feed residue, shrimp metabolism, and the decomposition of dead organisms. Based on the measurement results, total ammonia nitrogen concentration in the culture media ranged from 0 to 0.294 mg.L⁻¹ (Figure 12).

Total organic matter

Total organic matter concentration in *Litopenaeus vannamei* culture media was in the range of 41.06-173.3 mg.L⁻¹ (Figure 13). The highest total organic matter concentration was found in P2, followed by P3 and P1. Specifically, P2 indicated a decreasing trend in total organic matter concentration, whereas the remaining two treatment groups indicated an increasing trend.

Total bacteria count

In P1 and P3, the number of bacterial colonies increased from the beginning of maintenance to the end, whereas in P2 it decreased (Table 4). The decrease observed in P2 was due to the provision of commercial feed and FRB, which formed bio-colloids fed by shrimp. The FRB was made from a combination of probiotics and prebiotics.

Total vibrio count

In P1, the number of *Vibrio* bacterial colonies increased from the beginning to the end of the culture period, but decreased in P2. At DOC 29, a significant increase in P3 was observed, with a reduction at the end of rearing (p < 0.05; Table 5). According to SNI 8008:2014 on intensive *Litopenaeus vannamei* production in lined ponds, the maximum allowable total vibrio count is 1×10³.

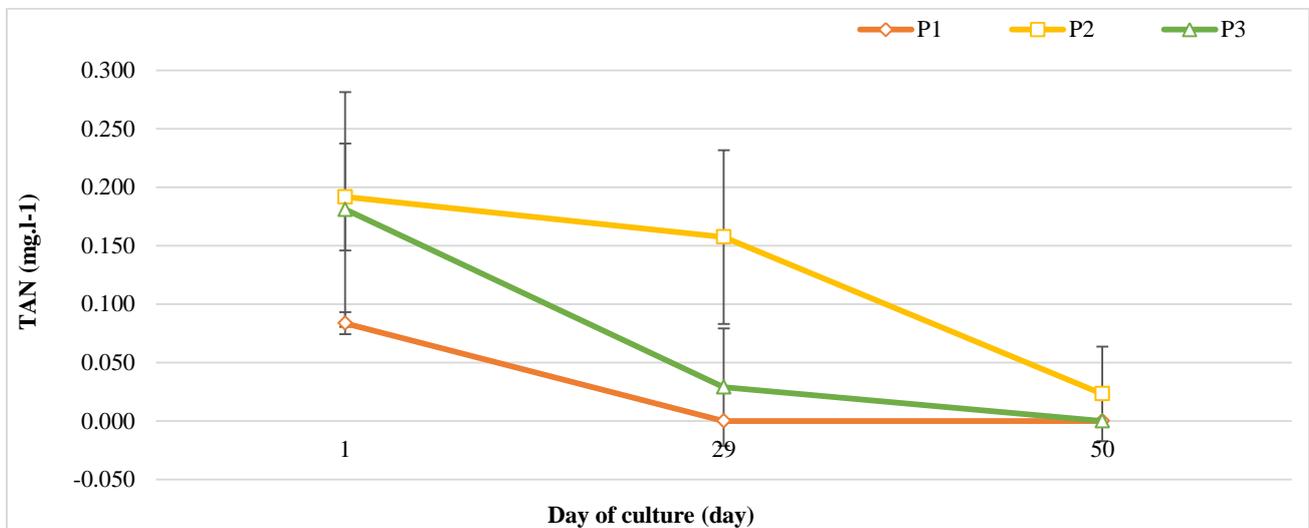


Figure 12. Total ammonia nitrogen in the culture of the *Litopenaeus vannamei* during the rearing period fed different diets. P1: Commercial feed only (control), P2: Commercial feed + live copepods, P3: Live copepods only.

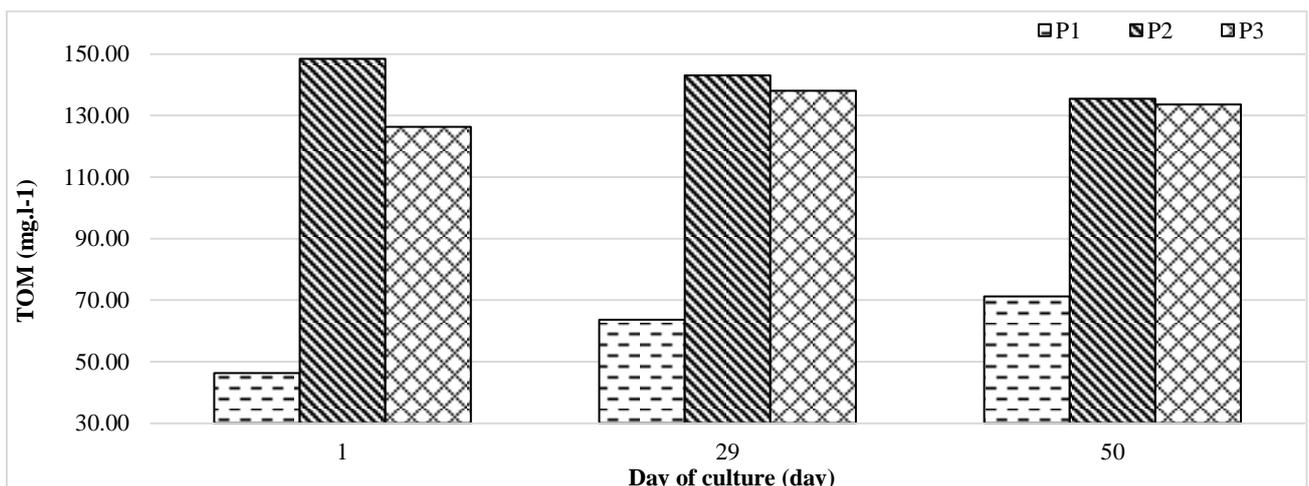


Figure 13. Total organic matter in the culture of the *Litopenaeus vannamei* during the rearing period fed different diets. P1: Commercial feed only (control), P2: Commercial feed + live copepods, P3: Live copepods only.

Table 4. Total heterotrophic bacteria from the culture of *Litopenaeus vannamei* tanks fed different diets at days 1, 29, and 50

Treatment	Total bacteria (CFU.ml ⁻¹)		
	DOC 1	DOC 29	DOC 50
P1	6.4×10^2	5.3×10^4	5.5×10^4
P2	2.2×10^5	3.1×10^5	9.5×10^4
P3	4.6×10^4	8.2×10^5	2×10^5

DOC: Day of culture. P1: Commercial feed only (control), P2: Commercial feed + live copepods, P3: Live copepods only.

Table 5. Total *Vibrio* count from the culture of *Litopenaeus vannamei* at days 1, 29, and 50 fed different feeding diets

Treatment	Total bacteria (CFU.ml ⁻¹)		
	DOC 1	DOC 29	DOC 50
P1	5×10^1	1.6×10^3	1.1×10^3
P2	8.6×10^3	7.3×10^3	1.3×10^3
P3	3×10^1	1.2×10^3	2.1×10^2

DOC: Day of culture. P1: Commercial feed only (control), P2: Commercial feed + live copepods, P3: Live copepods only.

DISCUSSION

Average daily growth

The current results indicated that the highest ADG was observed in P2 because nutrient needs were optimally met with commercial feed and copepods. The use of copepods meets shrimp's essential fatty acid needs, indicating that the combination provides a higher protein content than commercial feed. The high protein content was due to the addition of copepods, which had a protein content of 52.4%-57.6% on a dry-weight basis (Khanjani et al., 2022). Protein is a molecule composed of amino acids as a tissue-building substance, which can maintain, form, and replace damaged tissue (Sultana et al., 2022). In the present study, shrimps given only commercial feed had a lower ADG than P2, by 0.1 g.d⁻¹ (27.78%). Since the ideal ADG is 0.14 g.d⁻¹ (Bahri et al., 2020), commercial feed did not meet shrimp's nutritional needs, underscoring the importance of supplementing the diet with amino and fatty acids. Specifically, amino acids act as chemoattractant that increase the feeding rate of whiteleg shrimp.

Fatty acids such as linoleic, linolenic, stearic, and EPA are required by *Litopenaeus vannamei* (Rohmanawati et al., 2022). These nutrients can be supplied through live feed, which provides protein, lipids, carbohydrates, vitamins, minerals, and amino acids (Santhanam et al., 2020). In copepods, EPA, DHA, and ARA are found in the polar lipid fraction (phospholipids), with additional stores in triacylglycerols (Dhont et al., 2013), and PUFA content is higher in phospholipids (Prasetyo et al., 2020). Phospholipids can increase survival, growth, lipid metabolism, and stress resistance (Parisenti et al., 2011a), while EPA and DHA help maintain healthy cell function (Prasetyo et al., 2020; Wijaya et al., 2021). When EPA is provided alongside DHA, growth is improved (Kim and Lee, 2004; Imanisa et al., 2019) because DHA plays a role in the development of the nervous and vision system of marine fish. Meanwhile, EPA plays a role in physiological processes (Hu et al., 2016). In fish, ARA (20:4n-6) serves as a major precursor for prostaglandins (eicosanoids) in fish, with dietary requirements at lower proportions than DHA (Xu et al., 2024).

DHA and EPA in polar lipids (PL) are more effective for the development of fish larvae than non-polar lipids (NL). In copepods, the ratio of DHA/EPA in polar lipids is 3.69%, while ARA content is 1.79% 1,79% (Matsui et al., 2021). This is in line with previous studies where the DHA/EPA ratio of polar lipids from hepatopancreas and muscle increases with dietary DHA/EPA (Hu et al., 2016). For fish larvae, feed provides optimal nutrients when DHA exceeds EPA (DHA/EPA > 1) and ARA is supplied (Muller-Feuga et al., 2007). Therefore, the combination of commercial feed and copepods as additional nutrients provides the best growth of vaname shrimp.

In this study, P3 produced the lowest ADG because copepods were unable to replace commercial feed as a source of nutrition. The consumption rate of *Litopenaeus vannamei* was likely to be higher than the growth rate of copepods. The life cycle of copepods is relatively long (7-14 days) and goes through 6 nauplii and 5 copepodite phases, with a sexual reproduction system (Santhosh et al., 2018). Furthermore, adult copepods have egg sacs containing 8-22 eggs, which hatch after 24-36 hours into nauplii (Lestari et al., 2018).

The high protein content demonstrated that shrimp are a high-quality protein source (Khan et al., 2014). Based on the present findings, the use of copepods as live feed was able to increase the protein content of *Litopenaeus vannamei*. Carbohydrate, fat, fiber, ash, and water content values were caused by feed nutrition and culture environment. The nutritional composition of *Litopenaeus vannamei* was influenced by DOC, feed, and feeding frequency (Syed et al., 2017).

Average body weight

Based on SNI 7311:2009 concerning the production of *Litopenaeus vannamei*, the stage PL 10 requires a feed particle size of approximately 200-300 μm . This size range enables the consumption of copepods nauplii, measuring 60-225 μm , and the copepodite of 225-600 μm (Karlsen *et al.*, 2015). A previous study had reported that adult copepods were 300 μm long (Lestari *et al.*, 2018). For comparison, the ideal copepod size ranges from 60 to 100 μm (nauplii) for small pelagic marine fish larvae (Barroso *et al.*, 2013). Therefore, the administration of commercial feed and copepods could have met the nutritional needs of shrimp. This commercial feed has a minimum protein content of 36% which was in accordance with the nutritional needs of shrimp. In penaeid shrimp, the optimum protein requirement for growth ranges from 28-60% (Jusadi *et al.*, 2015), while *Litopenaeus vannamei* needs 30% (Rosyida, 2007). Based on the present study, additional nutrients obtained in P2 were supplied from copepods, which served as a supplemental feed for shrimp, supporting beneficial bacterial communities in the aqua mimicry system. This helps stabilize water quality and accelerate growth (Manan *et al.*, 2023).

Copepods contain protein, carotenoids, free amino acids (taurine), peptides, vitamins, and minerals (selenium, iodine, copper, and manganese), which are essential for shrimp growth (Karlsen *et al.*, 2015). Protein and astaxanthin contents are higher than in rotifers and *Artemia* (Dhont *et al.*, 2013). Astaxanthin acts as an antioxidant or as a provitamin compound (Karlsen *et al.*, 2015). Taurine is needed as a nutrient that plays a role in metabolic and physiological functions in shrimp (Yue *et al.*, 2012). Furthermore, trace elements such as zinc (Zn), copper (Cu), selenium (Se), iron (Fe), manganese (Mn), iodine (I), cobalt (Co; via vitamin B₁₂), chromium (Cr), and molybdenum (Mo) play an important role in immune function and disease prevention (Lall and Kaushik, 2021). In addition to these elements, copepods also have nutritional advantages (high ω -3 HUFA; Pan *et al.*, 2022), containing long-chain unsaturated fatty acids/highly HUFA, including EPA (20:5n-3), DHA (22:6n-3), and ARA (20:4n-6). These compounds are very important for larval survival, growth, and metamorphosis (Khanjani *et al.*, 2022). The HUFA is an integral component of cell membranes and eicosanoid precursors required for moulting (Hu *et al.*, 2016). Increasing HUFA content in live feed can notably increase the survival and growth in marine fish (Muller-Feuga *et al.*, 2007).

Absolute weight growth

Feeding shrimp with only commercial feed was not able to meet the nutritional needs. This was because Shrimp require essential fatty acids and nutrients for mechanical energy (locomotion), reproduction (biosynthesis of ovarian/testicular tissue), growth (moulting and muscle tissue synthesis), and osmotic regulation (Ceccaldi, 1989). Generally, fish feed is distinguished by high lipid content, which includes a significant proportion of unsaturated fatty acids. In aquatic feed, the presence of an abundance of redox-active metal ions and reactive oxygen species (ROS) leads to the rapid oxidation of unsaturated fatty acids (Xu *et al.*, 2023). The oxidation-prone nature of unsaturated fatty acids indicates that processing, storage, and transportation can change fatty-acid profiles and reduce quality in feed (Prasetyo *et al.*, 2020). Therefore, *Litopenaeus vannamei* must obtain EPA and DHA from ingested marine microorganisms.

The use of copepods is needed as a feed supplement for *Litopenaeus vannamei* to meet the nutritional needs of shrimp. Commercial feed provides protein or energy well, but can be short on some forms of lipids and micronutrients that shrimp use most efficiently (Chen *et al.*, 2023). This is due to the high levels of LC-PUFA, polar lipids including phospholipids, DHA/EPA ratio, digestive enzymes, vitamins, and carotenoids, which cause better growth performance compared to artemia and rotifers (Barroso *et al.*, 2013). Copepods' production is positively related to the DHA/EPA ratio in feed for cultured biota (Muller-Feuga *et al.*, 2007). The use of copepods at 0.2 individuals mL⁻¹ in biofloc systems has been shown to improve the growth of vanname shrimp (Abbaszadeh *et al.*, 2022).

Copepods cultured using FRB by adding yeast contain higher EPA, DHA, and ARA values than microalgae, *Isochrysis galbana*, *Dunaliella*, and *Rhodomonas tisbury* (De Lima *et al.*, 2013). The consumption of yeast by copepods would produce fatty acids by converting simple fats found into omega-3 fatty acids (Vakati *et al.*, 2023). As a type of copepods, *Tigriopus* sp. contains substantial n-3 HUFA 12% DHA and 7% EPA even under exclusive baker's-yeast diets. Furthermore, yeast-fed copepods have higher EPA (20:5n-3) and DHA (22:6n-3) percentages than conspecifics fed *C. calcitrans*, *D. tertiolecta*, or *I. galbana* (Monroig *et al.*, 2013).

The provision of FRB to the culture media causes an increase in the formation of colloids made from rice bran particles and different organisms, including bacteria, algae, protozoa, and copepods (Tailly, 2019). The FRB can increase the population of *Lactobacillus* sp. after 24 and 48 hours of fermentation (Sapwarobol *et al.*, 2021). Rice bran particles function as a substrate for heterotrophic bacteria that dominate biocolloids in the aquamimicry system (FAO, 2018). In this system, FRB serves as additional nutrition for shrimp that are stocked (Chakravarty *et al.*, 2018), providing zooplankton as a direct food source (Chakravarty *et al.*, 2018) and improving water quality (Romano and Kumar, 2017). In aquamimicry, the growth of phytoplankton and zooplankton is generated from carbon sources, and probiotics function as additional nutrition for cultured fish and shrimp (Deepak *et al.*, 2020).

Absolute length growth

The provision of copepods combined with commercial led to high ALG. Specifically, copepods cultured with FRB contained 16.79% protein, 14.92% fat, 17.36% ash, and 50.94% carbohydrate (Khanjani et al., 2022). In the aquamimicry system, FRB is used as an additional feed source, providing prebiotic substrates that help maintain the survival and growth of beneficial bacteria in shrimp gut (Manan et al., 2023). Bound phenolics released from rice bran dietary fiber show prebiotic potential in vitro by stimulating beneficial microbes and inhibiting pathogenic bacteria (Zhang et al., 2019). Microorganisms or enzymes can be used to increase the solubility of carbon sources in water and accelerate the breakdown of nutrients, and increase growth (Satyantini et al., 2020). Copepods also contain taurine, which plays a role in physiological functions in osmoregulation in fish and other animals (Yue et al., 2012). Taurine is essential for osmoregulation and bile acid conjugation. However, deficiency of taurine promotes lipid accumulation and mitochondrial dysfunction, leading to oxidative stress, neurologic abnormalities, and heart failure (Karlsen et al., 2015).

Survival rate

In this study, SR was classified into three groups, namely high, medium, and low, with values of >70%, 50-60%, and < 50%, respectively (Saepudin et al., 2022). The combination of commercial feed and copepods had produced the highest SR value. The content of DHA (22:6n-3) in copepods is important for the survival and growth of fish larvae. Deficiency of this fatty acid compromises larval health, which reduces growth and feed efficiency, thereby causing anemia and a high mortality rate (Olivotto et al., 2010). Among essential fatty acids, DHA outperforms EPA in promoting larval health, stress resilience, growth, and survival. The EPA heavy diet compared to DHA can disturb phospholipid balance, which is associated with low growth and survival in marine fish larvae (Rainuzzo et al., 1997; Amin et al., 2022). Optimal DHA or EPA should be more than one mol for all marine fish larvae (Santhosh et al., 2018). This suggests that DHA:EPA ratio significantly influences marine fish larval performance, including survival, with higher DHA/EPA targets in live-feed enrichments related to improved growth and survival (Pan et al., 2022). The negative effects of excessive n-3 HUFA administration can be caused by disruption of polar membrane lipids due to excessive accumulation of EPA and DHA in the tissue (Kim and Lee, 2004). Copepods supply astaxanthin, which acts as an antioxidant to increase stress resistance, support hepatopancreas function, and protect EPA/DHA and other PUFA in membranes as well as storage lipids from lipid peroxidation (Parisenti et al., 2011a).

Food conversion ratio

Combined commercial feed and live feed copepods can meet the complete nutritional needs of shrimp. This is because HUFA content in copepods acts as an attractant capable of increasing the palatability (Herawati et al., 2023), feed efficiency, and shrimp immune (Khanjani et al., 2022). The DHA and EPA are also essential for the growth and survival of marine fish larvae (Dhont et al., 2013). However, the occurrence of copepods improves growth performance, the immune system, and feed conversion efficiency of *Litopenaeus vannamei* (Abbaszadeh et al., 2022). *Lactobacillus* sp. found in FRB can increase feed digestibility due to the simplification of complex proteins into simpler protein, thereby enabling feed absorption (Syadillah et al., 2020).

Scheme implementation

Deficiency of essential fatty acids can compromise fish health by reducing fecundity and embryogenesis, causing larval mortality, growth abnormalities, mispigmentation, visual defects, impaired feeding under low light, abnormal behavior, and compromised membrane function at low temperatures (Pangkey, 2011). The application of copepods as live feed provides benefits in the aquaculture industry by increasing larval survival, growth rates, pigmentation, and intestinal development, serving as a source of exogenous enzymes (Rasdi and Qin et al., 2014). Compared to the present study, the prominent dark color of raw shrimp was caused by the use of higher carotenoid concentrations and a longer period of time (Parisenti et al., 2011b).

The color of shrimp changes after cooking because the complex compound dissociates and free astaxanthin appears normal, ranging between yellow, orange, and red, with a wavelength of 470-472 nm (Rhys et al., 2011). Therefore, a greater amount of astaxanthin in shrimp contributes to a darker color (Parisenti et al., 2011a). Applying copepods as live feed changes the color of shrimp after boiling to a deep orange due to a high content of carotenoids, particularly astaxanthin, that acts as an antioxidant or a provitamin A compound (Karlsen et al., 2015). Astaxanthin is a carotenoid compound and xanthophyll derivative that provides red pigment to products with significant biological activity (Maharani et al., 2023). The product produced from the aquamimicry concept is red shrimp (astaxanthin, amino acids, and PUFA), which increases the selling value as organic shrimp (Deepak et al., 2020). Generally, shrimp with bright red color have a greater price and are accepted by consumers (Hasan et al., 2022).

Histopathology of hepatopancreas tissue

In P1, there was a hemocyte infiltration caused by the entry of bacteria, as excessive production led to abnormal tissue. Generally, shrimp lack the ability to develop antigens, making their immune system non-specific and primarily mediated by hemocytes and plasma proteins. Hemocytes remove foreign particles through phagocytosis, encapsulation, and nodular aggregation in the shrimp immune system (Prastiti *et al.*, 2023). Compared to others, P2 showed the most normal tissue architecture and the lowest hemocyte infiltration, indicating better shrimp health. Hemocytes serve as a defense system that can be used to assess health (Ismawati *et al.*, 2019). The results show that meeting shrimp nutritional needs through a combination of commercial feed and copepods supports proper immune function and tissue integrity (Ismawati *et al.*, 2019; Deepti *et al.*, 2024).

Atrophy in P3 caused widening of the hepatopancreas lumen. This showed that the hepatopancreas lumen was empty because of low feed intake (Anjaini *et al.*, 2018). In the decapod hepatopancreas, B-cells (blister cells) mediate intracellular digestion and the packaging/transport (apocrine release) of digestion products. Meanwhile, F-cells are the primary site of digestive-enzyme synthesis, and R-cells store lipids and glycogen (Al-Mohanna and Nott, 2009). In this study, histopathological tests were carried out on the hepatopancreas organ to determine the condition of *Litopenaeus vannamei* tissue with the application of a feeding scheme. In crustaceans, the hepatopancreas is the main organ for metabolic processes, lipid storage (triglycerides and phospholipids; Liu *et al.*, 2021). R-cells (Restzellen) are multi-vacuolated absorptive cells, storing absorbed nutrients as glycogen and lipid (Vogt, 2019). Furthermore, R-cells commonly sequester mineral deposits, including calcium, magnesium, phosphorus, and sulfur (Patrachotpakinkul *et al.*, 2022). Hepatopancreas is the main organ of acid metabolism, which is very sensitive to nutritional conditions (Hu *et al.*, 2016). This suggests that severe damage to hepatopancreas structure will cause stress in shrimp, thereby increasing the risk of high sensitivity to viral and bacterial infections (Dharmawan *et al.*, 2020). Histopathological changes can also provide information on the level of stress, susceptibility, and adaptation of the organism's ability to deal with stress (Zhahrah *et al.*, 2016).

ATM is generally derived from the peeling of shrimp hepatopancreatic tubule epithelial cells. These thin epithelial cells negatively affect shrimp growth and survival. Peeled epithelial cells accumulate at the hepatopancreas-midgut junction and are excreted as feces through the midgut. Severe cases can cause a phenomenon called White Feces Syndrome (WFS; Sriurairatana *et al.*, 2014). Atrophy is a reduction in the size of cells or tissues that occurs due to less usage, thereby causing loss of substances. This phenomenon reduces epithelial height or number in the shrimp hepatopancreas, which allows the widening of the tubule lumen. Epithelial atrophy is a common lesion reported in shrimp hepatopancreas pathology (Bonaldo and Sandri, 2013), caused by a lack of feed intake for several days (Suryana *et al.*, 2023). Sloughing hepatopancreas provides a growth matrix for bacteria, leading to significant secondary *Vibrio* infection. At the terminal phase, during extensive hepatopancreatic destruction, infected sites show hemocyte infiltration (Widanarni *et al.*, 2016).

Water quality

Water culture temperature measurement is important because shrimp are cold-blooded organisms (Poikilothermic). In this study, the temperature of culture media ranged from 27.3 to 28.9°C, showing a fluctuation of approximately 1.6°C. Sudden temperature fluctuations of $\pm 2^\circ\text{C}$ can cause stress in *Litopenaeus vannamei* (Suwarsih *et al.*, 2016), as the optimal range is 26-33°C (Novriadi *et al.*, 2021). The temperature parameter generally affects the appetite and eating habits of shrimp, as a lower value decreases feed intake, causing weakness, inactivity in swimming, and appearance of black spots (Nanga *et al.*, 2023). When the temperature increases, body's metabolic system will run faster, as a larger amount of energy is required to adjust to higher temperature conditions (Zufadhillah *et al.*, 2018).

Salinity value is influenced by the levels of ions such as chloride, bicarbonate, carbonate, magnesium, sodium, and calcium, which are dissolved in water. This is because water evaporation occurs daily, which is approximately 0.2 cm.d^{-1} (Krishna, 2021). Measurement results in each treatment are in accordance with SNI 7772:2013 concerning *Litopenaeus vannamei* semi-intensive culture system in tanks. Salinity affects moulting by influencing osmoregulation process and the osmotic pressure of water. Low salinity inhibits moulting due to the low availability of minerals. Meanwhile, high salinity contains elevated concentrations of electrolytes, which increase osmotic pressure and inhibit moulting process (Jayanti *et al.*, 2022). Shrimp cultured at water salinity of 35-40 g.l^{-1} grow slower than 15-25 g.l^{-1} , 5-10 g.l^{-1} has faster growth but becomes more susceptible to disease (Asrin *et al.*, 2020).

Compared to other parameters, pH shows the degree of acidity or alkalinity of water culture, which closely relates to physical, chemical, and biological factors (Supriatna *et al.*, 2020). The optimal pH range for *Litopenaeus vannamei* culture is 7.5-8.5 (Novriadi *et al.*, 2021). This value is often influenced by the concentration of CO_2 . During the day, photosynthesis occurs, decreasing CO_2 concentration and increasing pH, which raises ammonia level. At night, all

organisms in the water release CO₂ from respiration, causing a decrease in water pH and reducing shrimp blood pH (acidosis), impairing oxygen transport (Supriatna et al., 2020).

DO is important because it affects the amount of hemocyanin in crustacean blood, essential for nutrient oxidation and energy production. The energy obtained from this catabolic process is used for digestion and assimilation of feed, consumption, and activation in anabolic activities (Wahyuni et al., 2022). DO, nutrients, and blood cells distribute oxygen and metabolites in the shrimp's body, enabling energy production for growth and activity. Increasing biomass during the rearing period can cause a rise in DO, particularly at night, which is used for the respiration process (Wahyuni et al., 2022). Low DO concentrations do not supply oxygen to the bottom of the pond, creating anaerobic sediment conditions. The optimum alkalinity level for shrimp cultivation is 150-200 mg.l⁻¹ because it can maintain pH stability and supports bacterial nitrification processes (Manullang et al., 2023). Alkalinity functions as pH buffer, which is important in maintaining the sensitivity of cell membranes in nerve and muscle tissues. Low alkalinity values cause shrimp to moult frequently and increase susceptibility to disease. Meanwhile, high alkalinity values provide strong pH buffering capacity and supply calcium for cellular osmotic regulation (Listriyana et al., 2023).

High TDS values can affect growth, respiration, and make biota more easily stressed (Satrio et al., 2022). In this study, the increase in TDS was due to the implementation of FRB in the culture media. The TAN concentration in all treatment groups was in the normal range but tended to decrease with culture period. The optimal range of TAN for shrimp cultivation is <1.0 mg.l⁻¹ (Novriadi et al., 2021), with ammonia (NH₃) being more toxic than NH₄⁺ due to the ability to penetrate cell membranes more easily (Wahyuningsih et al., 2020). High ammonia concentration reduces oxygen transport in the blood and hinders the growth of *Litopenaeus vannamei* (Farabi and Latuconsina, 2023). The high TOM was caused by the elevated amount of organic material accumulation. TOM has a negative correlation with the water transparency, indicating that denser pond water causes a higher concentration of organic matter (Ariadi et al., 2021).

Total bacteria and vibrio count

EM4 probiotics contain a mixed culture of fermented microorganisms, lactic acid bacteria (*Lactobacillus casei*), and yeast (*Saccharomyces cerevisiae*; Telaumbanua et al., 2023). A previous study reported that FRB could increase the population of *Lactobacillus* sp. after 24 and 48 hours (Sapwarobol et al., 2021). As a feed source, FRB provides a high surface area for bacteria to colonize (Biofixation; FAO, 2018). In this study, the probiotic used was *Lactobacillus casei*, with antimicrobial activity against pathogenic bacteria causing vibriosis in shrimp through *Vibrio harveyi*. *L. casei* is capable of producing antibacterial substances, particularly bacteriocins (Jati et al., 2017). Bacteriocins produced by *L. casei* can damage the molecular structure of bacterial cells, causing leakage and death. Furthermore, *Lactobacillus* sp. can produce lactic acid (H₂O₂) using the lactase enzyme to eliminate bad bacteria that are negative for *Litopenaeus vannamei* or their competitors in the intestine (Syadillah et al., 2020).

CONCLUSION

Litopenaeus vannamei given commercial feed supplemented with copepods indicated significantly higher performance over a 50-day culture period. Among treatment groups, P2 improved major production parameters, including growth performance, feed efficiency, and SR. Additionally, P2 improved shrimp physiological and quality characteristics, as evidenced by higher crude protein levels, enhanced hepatopancreatic health, and a bright orange coloration after cooking. Water quality was also within or approximately the recommended ranges. Future studies are recommended to investigate copepods as a feed supplement at appropriate dosages for fish or other marine aquatic species.

DECLARATIONS

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Availability of data and materials

All data generated during the study are relevant and included in this published article. The data to support the present study's findings are available upon reasonable request to the corresponding author.

Authors' contributions

Moch Nurhudah, Tubagus Haeru Rahayu, and Luh Krisna Weda Yanti conducted the research, administration, and conceptualization. Sinung Rahardjo and Reza Shah Pahlevi contributed to the data analysis for the Histological and proximate analysis. Yenni Nuraini, Erni Marlina, Margono, and Umidayati conducted Research execution and Interpretation of data. Arie Kiswanto, Mohsan Abrori, Ren Fitriadi, and Muhammad Ikhwan Ihtifazhuddin wrote the draft and prepared the article. All authors checked and approved the last edition of the manuscript for processing and publication in this journal.

Competing interests

The authors affirmed that there are no competing interests.

Ethical considerations

The authors declared that the manuscript is original, has not been published elsewhere, and confirmed the latest version before publication. The authors declared that no AI tools were used for writing and preparing the article.

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