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FEED MANAGEMENT INNOVATIONS FOR ENHANCING THE GROWTH PERFORMANCE OF VANNAMEI SHRIMP LARVAE (LITOPENAEUS VANNAMEI)

Inovasi Manajemen Pakan Untuk Meningkatkan Performa Pertumbuhan Larva Udang Vannamei (*Litopenaeus vannamei*)

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ABSTRACT

Feed management is a crucial component in the cultivation of vannamei shrimp, particularly during the larval stage which requires complex and varied nutritional needs. This study aims to explore innovative feed management strategies by combining natural and artificial feeds in an industrial-scale setting at Hatchery PT Suri Tani Pemuka, Bali. Natural feeds used included *Thalassiosira sp.* and *Artemia salina*, along with six types of artificial feeds administered throughout the larval rearing period from Nauplius to Post Larva (PL) 11 stages. Growth parameters, larval health, and water quality were regularly monitored. The results showed that this feed combination provided high nutritional content (45–60% protein, 16–23% fat) and favorable larval survival rates, ranging from 63.62% to 76.15% across the three rearing tanks. There were no significant differences in larval length among tanks (P>0.05), and water quality parameters remained within optimal thresholds. Microscopic observations supported visual assessments, indicating good physiological development in the larvae. This innovative feeding strategy demonstrates strong potential to enhance the sustainability and efficiency of vannamei shrimp larval aquaculture.

Key words: Artificial Feed, Feed Innovation, Larval Growth, Natural Feed, Vannamei Shrimp

ABSTRAK

Manajemen pakan merupakan komponen krusial dalam budidaya udang vannamei, khususnya pada fase larva yang memiliki kebutuhan nutrisi kompleks dan beragam. Penelitian ini bertujuan mengeksplorasi inovasi manajemen pakan melalui kombinasi pakan alami dan buatan dalam skala industri di Hatchery PT Suri Tani Pemuka, Bali. Pakan alami berupa *Thalassiosira* sp. dan *Artemia salina*, serta enam jenis pakan buatan digunakan selama masa pemeliharaan larva dari stadia Nauplius hingga Post Larva (PL) 11. Parameter pertumbuhan, kesehatan larva, dan kualitas air diamati secara berkala. Hasil penelitian menunjukkan bahwa

kombinasi pakan tersebut mampu menghasilkan kandungan nutrisi tinggi (protein 45–60%, lemak 16–23%) dan tingkat kelulushidupan larva yang baik, yaitu 63,62–76,15% pada tiga bak pemeliharaan. Tidak terdapat perbedaan signifikan dalam pertumbuhan panjang antar bak (P>0,05), dan parameter kualitas air masih berada dalam ambang optimal. Pengamatan mikroskopis mendukung temuan visual, menunjukkan perkembangan fisiologis larva yang baik. Inovasi dalam strategi pemberian pakan ini berpotensi meningkatkan efisiensi budidaya larva udang vannamei secara berkelanjutan.

Kata Kunci: Inovasi pakan, Larva udang vannamei, Pakan alami, Pakan buatan, Pertumbuhan larva

INTRODUCTION

Feed management is critical in aquaculture practices (Imron & Samara, 2022). In vannamei shrimp (*Litopenaues vannamei*) farming, larvae exhibit varying feeding behaviors depending on their size, age, and environmental conditions, making it challenging to determine the appropriate amount of feed to provide (Joshua *et al.*, 2022). Moreover, competition among larvae for feed can affect individual consumption levels, potentially resulting in unequal nutrient intake (Dewi *et al.*, 2019). Despite the crucial role of feed management, limited research has focused on improving feeding techniques in shrimp production.

Vannamei shrimp larvae require feed rich in protein, lipids, and vitamins to support optimal development (Rohmanawati *et al.*, 2022). Therefore, a balanced and high-quality feed formulation is essential to ensure proper growth, target size achievement, and disease resistance. Rotifers (*Brachionus* sp.), artemia, moina, copepods, and bloodworms are the most commonly used and discussed live feeds in vannamei shrimp larviculture (He *et al.*, 2022; Joshua *et al.*, 2022; Li *et al.*, 2021). The live feed provides rich nutrients and stimulates the larvae's natural feeding behavior, contributing to optimal growth and health (Kandathil *et al.*, 2020). However, live feed production carries a potential risk of disease transmission, which can negatively impact larval health and farming productivity. These risks arise because live feeds, such as *Artemia salina* and rotifers, may be contaminated by pathogens present in the culture environment. Further research on live feed cultivation and management techniques will provide innovative solutions to meet this need.

Combining natural and artificial feeds can enhance the growth efficiency and disease resistance of vannamei shrimp larvae, thereby improving overall farming productivity (Haris *et al.*, 2024). Artificial feed is formulated with specific nutritional content tailored to the larvae's developmental stages. Additionally, artificial feed usage can reduce the disease transmission risks often associated with natural feed. For example, snakehead fish (*Channa striata*) fed with a combination of 50% *Tubifex* worms and 50% commercial feed showed a specific growth rate of 5.49% per day, compared to 3.89% with 100% commercial feed) (Syamsunarno & Sunarno, 2022). Furthermore, vannamei shrimp larvae fed with 100% artificial feed demonstrated a higher absolute growth in length (2.34 cm) than those fed exclusively on natural feed (1.08 cm) or a 50:50 feed combination (1.61 cm) (Darsiani *et al.*, 2024).

Innovations in feed management are crucial steps toward improving the growth performance of vannamei shrimp larvae. By integrating natural and artificial feeds and applying more effective feeding techniques, the challenges in shrimp farming can be addressed. This study aims to explore and develop innovative feed management strategies that enhance larval growth efficiency and strengthen disease resistance. Through an evidence-based, industrial-scale approach, the outcomes of this research are expected to contribute significantly to more sustainable and productive vannamei shrimp farming practices.

RESEARCH METHODE

This research was conducted at an industrial-scale shrimp hatchery supplying both domestic and international markets, namely "Hatchery PT Suri Tani Pemuka" located in Negara, Bali. This research was conducted from July 2024 to January 2025. The study was carried out on an industrial scale, meaning all experiments and observations were performed in a real, operational aquaculture environment. In this context, no experimental replications were conducted; instead, replication was represented by the use of multiple tanks, with identical treatment procedures applied. Data collection was conducted directly at the hatchery site, where vannamei shrimp larvae were reared using both natural and artificial feeds according to standard industry procedures.

Natural Feed Culture

Two types of natural feed were used in this study: phytoplankton from the microalgae species *Thalassiossira sp.* and zooplankton in the form of *Artemia salina* cysts Supreme Plus® (produced by Golden Wes Artemia) and *Artemia salina* instar E and instar 1 (made by PT. IANDV BIO Indonesia). 360 grams of *Artemia salina* cysts Supreme Plus® were cultured in 500 liters of water under 18-watt lighting and continuous aeration for 24 hours until hatching. To assess hatching results, samples were taken from five different points using a 1000 μ L micropipette. The harvested *Artemia* was rinsed (dipped) with fresh water and treated with 5 mL of iodine for 1 minute, followed by a freshwater rinse. The *Artemia salina* instar E and one were stored in a freezer at -40°C until use.

Starter cultures of *Thalassiossira sp.* phytoplankton were obtained from the Gondol Center for Marine Aquaculture Research and Fisheries Extension. The Thalassiossira sp. was cultured in pure form using Difco[™] agar medium at a temperature of 23–25°C under 2000 lux light intensity. A volume of 150 µL of the Thalassiossira inoculum was placed into petri dishes and evenly spread using a spider tool. The cultures were incubated for 5-7 days. Grown colonies were then selected and transferred to Erlenmeyer Agar (EA) medium. The selection process was based on several criteria: high cell density, clear and bright pigmentation, uniform surface growth with no empty areas, consistent cell shape, uniform size, and absence of contamination (Jessica, Ardiansyah, & Muhammad Yasir, 2024). Selected cultures were then transferred to 250 mL of EA medium and incubated for another 5–7 days. After incubation, 50 mL of Thalassiossira from the EA culture was poured into 450 mL of EPC (Erlenmeyer Pure Control) medium and incubated for 48 hours at room temperature under 2000 lux lighting. The culture process in the EPC medium was carried out for 3 days before continuing with the Plastic Inoculation (IP) stage. This phase used plastic bags suspended on a culture rack, with additional lighting to aid the growth and development of the culture. Before the IP culture, 10 liters of seawater were settled in plastic bags for 24 hours. Then, 500 ppm of chlorine was added with complete aeration. After 1 hour, 500 ppm of thiosulfate was added to neutralize the chlorine, followed by a 5-minute settling period. Afterward, 7.5 mL each of vitamin solution, NP, FeCl, and silicate fertilizer were added. Once the water medium was ready, the EPC-cultured starter was transferred into the IP medium, incubated for 72 hours, and then transitioned to mass culture.

The mass-scale culture was conducted in concrete tanks containing 7 tons of seawater. Before culture, the water in the tanks was treated with 3000 ppm chlorine and aerated thoroughly. After 1 hour, thiosulfate was added to neutralize the chlorine, followed by the addition of fertilizers in the following doses: 60 mL of nitrate, 5 grams of EDTA, 4 mL of sodium phosphate, 13 mL of silicate, and 5 mL of AGP. The starter culture used for the mass culture originated from plastic bag cultures on Day 3 (DOC 3), with 12 bags of culture added per tank. The culture was maintained for 2 days. The percentage of Artemia hatching rate were calculated using the following formula according to (Perdana *et al.*, 2021):

 $HR = \frac{Average number of hatched cysts (units)}{Sample cyste} \ge 100\%$

Fertilizer Preparation

The fertilizers used in the pure culture of *Thalassiossira* sp. were intended to support and accelerate algal growth. Four fertilizers were utilized: vitamins, NP (nitrate-phosphate), FeCl (iron chelate), and silicate. The preparation of each type is described as follows:

- A vitamin solution was prepared by dissolving 25 grams of thiamine, 0.1 grams of biotin, and 0.1 grams of vitamin B12 in 1 liter of water, then autoclaving for 5 minutes at 121°C.
- **NP solution** was made by dissolving 150 grams of NaNO₃ and 10 grams of NaH₂PO₄ into 1 liter of water, followed by autoclaving for 5 minutes at 121°C.
- **FeCl solution** was prepared by mixing 6.3 grams of FeCl and 8.7 grams of EDTA (Triplex III) into 1 liter of water and autoclaving for 5 minutes at 121°C. After cooling, trace metals were added, consisting of 4.4 grams of ZnSO₄, 1.2 grams of NaMoO₄, 2 grams of Cobalt III, 36 grams of MnCl₂, and 1.9 grams of CuSO₄.
- A silicate solution was prepared by autoclaving 1 liter of water for 5 minutes at 121°C, and then, after cooling, 50 mL of sodium silicate solution was added.

Artificial Feed Preparation

Six types of artificial feed, Royal Caviar, Elevia 1, Elevia 2, Elevia 3, Epibal, and Frippak, were used in this study. Prophylaxis supplements were also applied as complementary feed. The type of prophylaxis used was adjusted according to the shrimp's specific growth stages (Table 1).

Stadia	Types of Propylaxis
N – PL.10	Vitamin C, Epicin D + sugar
N, Zoea 1 – 3, Mysis 3	EDTA
Zoea 3, Mysis 3, Post larvae 1 – 10	Virkon
Zoea 2, Mysis 2	Sodium Bicarbonat
Naupli	Agp

Table 1. Types of Propylaxis According to Developmental Stages

Feed Dosage and Feeding Frequency

The total dosage and frequency of feed administration are presented in Tables 2 and 3 below.

Preparation of Vannamei Shrimp Rearing Tanks

The rearing tanks used in this study were rectangular concrete tanks with a minimum base area of 20 m², 548 x 231.5 x 162 cm, and a total volume of 16 tons. Three concrete tanks were used for observation, labeled as Tank C1, Tank C2, and Tank C3. Before use, the rearing areas were fumigated using 100 grams of potassium permanganate (KMnO₄) and 200 mL of formalin placed in a can inside the tanks. After 24 hours, the tanks were washed with 5 mL of iodine and scrubbed using a scouring pad. The cleaned tanks were then sprayed with Virkon disinfectant at 20 ppm. The tanks were left overnight before being filled with up to 70% of their capacity with seawater. The seawater used had been pre-treated with 24 ppm chlorine and

filtered using zeolite stones, silica sand, and coconut shell charcoal. Before stocking nauplii, the tank water was treated with 10 ppm EDTA, five ppm sodium, and 0.02 ppm iodine.

Vannamei Shrimp

The vannamei shrimp used in this observation were nauplii at the N4–N5 stage, produced by Hatchery PT. Suri Tani Pemuka, Singaraja. Prior to stocking, the nauplii bags were rinsed and soaked in 20 ppm iodine solution, then acclimated in a 100-liter fiber tank for 30–60 minutes. Samples were then randomly collected to estimate the number of nauplii, assess nauplii health scores, and evaluate the physical, chemical, and microbiological parameters of the water from the bags and nauplii. To determine the number of nauplii to be stocked, the following formula was used according to (D'Abramo *et al.*, 2006):

$$\frac{A+B+C}{3} = D x \frac{Water volume}{3 mL} = E x Number of bags$$

Where: A = count from the first sample; B = second sample; C = third sample; D = average of the samples; E = total count. The result obtained from the above formula was then divided by 40%. After the calculation, nauplii were stocked into each tank using a 250-mesh scoop and rinsed with a continuous flow of 20 ppm iodine solution. The stocking density for each 15-ton concrete tank was 3,150,000 nauplii.

Monitoring the Growth and Health of Vannamei Shrimp Larvae

Monitoring was conducted through visual and microscopic observations every three days. Visual observations were carried out using a 500 mL beaker glass containing vannamei shrimp larvae samples held up to a light source. Observations included the condition of the larvae, watercolor in the rearing tank, residual waste, uneaten feed, larval size uniformity, and larval movement. In addition, larval length measurements were conducted from the Post Larva (PL) 1 stage up to harvest. Microscopic observations were performed by collecting approximately 250 mL of rearing water and 30 shrimp larvae (benur) from the tank. These observations aimed to assess larval developmental stages, mortality, Gut Muscle Ratio (GMR), gut content (%), lipid droplet (%), Bolitas (hepatopancreas [HP] and gastrointestinal tract [GI]), epibionts (%), necrosis (%), and pigmentation (%).

Natural Feed Administration

Thalassiossira sp. was administered as natural feed by opening the outlet pipe connected via a spiral hose between the mass algae culture tank and the algae pipeline in the larval rearing module. Meanwhile, Artemia was administered as natural feed by adding seawater according to the predetermined dosage.

Vannamei Shrimp Larvae Health Management

Larval health management was conducted by administering probiotic cultures prepared by mixing vitamin C, Epicin D, and sugar in a 1:1 ratio. In addition, daily water exchange was carried out by reducing 5–10% of the total water volume. Bacterial checks in the rearing water were also conducted every three days once the larvae reached the Zoea 3 stage and continued until harvest.

Water Quality Monitoring

Physical parameter were measured daily; temperature (°C), salinity (ppt) and pH (ppm) were assessed using pH Meter Hanna Digital HI98107, clarity were assessed using Secchi disk by observing how far down it can be seen (cm), and water color were assessed visually. Chemical parameter factors were measured every three days; which the alkalinity (ppm), nitrite

(ppm), and nitrate (ppm) were assessed using test kit, and total organic matter (TOM, ppm) were assessed using Titrimetry according to the testing procedure SNI 06-6989.22.2004 by the calculation for KmnO4.

Samples for assessing Total Bacterial Count (TBC) and *Vibrio* levels were collected following the weekly water quality sampling. A volume of up to 100 mL was taken and analyzed at the microbiology laboratory of PT Menjangan Mas Nusantara. For TBC analysis, samples were serially diluted to a concentration of 10^{-3} and then cultured on Tryptic Soy Agar (TSA) medium. For *Vibrio* analysis, the samples were diluted to 10^{-1} and inoculated onto Thiosulfate Citrate Bile Salts (TCBS) agar. Both media were incubated at room temperature for 24 hours. Colony counts were performed based on morphological characteristics such as shape, color, and size, following the Total Plate Count (TPC) method as described by Prescott *et al.*, (2002).

Plankton abundance samples were collected simultaneously with the bacterial samples. These were transferred into 50 mL containers and analyzed at the water quality laboratory. Plankton counts were conducted using a NEUBAUER Haemocytometer under a light microscope, with calculations performed based on the formula outlined by APHA (1980).

$$N = \frac{100 \ (P \ x \ V)}{0.25 \ x \ W}$$

Where: N = amount of plankton per liter; p = amount of plankton; V = volume of the plankton sample; and W = volume of the collected water sample.

Data Analysis

Larval population and survival rate data were collected during the harvesting process using the following formula developed by Nawang *et al.*, (2022):

$$Population = \frac{Number \ of \ larvae}{Volume \ sample \ (mL)} \ x \ volume \ (mL)$$
$$SR = \frac{End \ Population}{First \ Population} \ x \ 100\%$$

All collected data were analyzed using Analysis of Variance (ANOVA) with the GraphPad Prism 9 software. The statistical test applied was One-Way ANOVA followed by a post hoc test.

RESULT

Biochemical Composition of Artemia sp. and Thalassiosira sp.

The combination of *Artemia* sp. and *Thalassiosira* sp. as natural feed was used in the larval rearing of vannamei shrimp at PT. Suri Tani Pemuka contains 45–60% protein, 16–23% fat, 26–27% carbohydrates, 6–8% ash, 80–85% moisture, polyunsaturated fatty acids (PUFA), and omega-3 fatty acids. The hatching rate of natural feed was calculated by collecting samples at the beginning of the feeding period and just before harvesting *Artemia*, using a 1 mL pipette. The hatching rate (HR) was 84.13% for Golden West *Artemia* and 88.07% for instar *Artemia*.

Growth of Vannamei Shrimp Larvae

The results of growth monitoring of vannamei shrimp larvae, measured from PL 1 to PL 11 stages in each rearing tank, are as follows. The larvae length in C! is; PL 1 (4.07 ± 0.31 cm); PL 2 (4.1 ± 0.33 cm); PL 3 (4.42 ± 0.4 cm); PL 4 (5.17 ± 0.24 cm); PL 5 (5.47 ± 0.51 cm); PL 6 (5.8 ± 0.25 cm); PL 7 (6.33 ± 0.3 cm); PL 8 (6.95 ± 0.42 cm); PL 9 (7.03 ± 0.52 cm); PL 10 (8 ± 0.3 cm); PL 11 (8.64 ± 0.24 cm). Larvae length in C2 is ; PL 1 (3.57 ± 0.22 cm); PL 2 (3.95 ± 0.33 cm); PL 3 (4.2 ± 0.36 cm); PL 4 (4.42 ± 0.35 cm); PL 5 (5.48 ± 0.36 cm); PL 6 (5.73 ± 0.37 cm); PL 7 (6.57 ± 0.47 cm); PL 8 (7.47 ± 0.41 cm); PL 9 (7.92 ± 0.35 cm); PL 10 (8.51 ± 0.5 cm); PL 11 (8.63 ± 0.4 cm). While, the larvae length in C3 is; PL 1 (3.5 ± 0.25 cm); PL 2 (4.08 ± 0.32 cm); PL 3 (4.93 ± 0.41 cm); PL 4 (5 ± 0.47 cm); PL 5 (5.33 ± 0.36 cm); PL 10 (8.32 ± 0.5 cm); PL 11 (8.76 ± 0.24 cm). Based on the results of One-Way ANOVA and post hoc test, it was concluded that there were no significant differences (P > 0.05) in the larval length of vannamei shrimp among tanks C1, C2, and C3 (Figure 1)

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Table 2. Feeding Time

	Natura	ıl Feed		Artificial Feed							
Artemia	Instar	Instar	Thalla	RC0-	RC50-	RC100-	RC200-	E0	E1	E2	E3
supreme	E	1		50	100	200	300				
				13.00; 17.00; 19.00; 23.00							
			00.00								
		09.00;	,								
15.00;		15.00;	15.00								
21.00		21.00;									
		03.00									
					01.00;	05.00; 07.0	00; 11.00; 1	13.00; 17	.00; 19.0	0; 23.00	
09.00;	09.00;										
,	,										
	supreme 15.00;	Artemia supremeInstar E15.00; 21.00	supreme E 1 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 03.00 09.00; 09.00; 15.00; 15.00; 03.00	Artemia supreme Instar E Instar 1 Thalla 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 09.00; 09.00;	Artemia supreme Instar E Instar 1 Thalla RC0- 50 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 09.00; 15.00 100 09.00; 09.00; 15.00 100 100 100 09.00; 09.00; 15.00 15.00 100 100 100 09.00; 09.00; 09.00; 15.00 100 100 100	Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 09.00; 15.00 09.00; 15.00 09.00; 15.00 09.00; 15.00 09.00; 15.00 09.00; 15.00 09.00; 15.00 01.00; 01.00; 01.00; <td>Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 RC100- 200 15.00; 21.00; 21.00; 03.00 09.00; 15.00; 21.00; 03.00 15.00 13.0 09.00; 09.00; 01.00; 05.00; 07.0 01.00; 05.00; 07.0</td> <td>Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 RC100- 200 RC200- 300 15.00; 21.00; 21.00; 03.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00; 03.00 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00;</td> <td>Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 RC100- 200 RC200- 300 E0 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 13.00; 17.00; 19.00; 23 09.00; 15.00; 03.00 09.00; 15.00 01.00; 05.00; 07.00; 11.00; 13.00; 17 09.00; 09.00; 01.00; 05.00; 07.00; 11.00; 13.00; 17</td> <td>Artemia supremeInstar EInstar 1Thalla$\begin{array}{cccccccccccccccccccccccccccccccccccc$</td> <td>Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC100- 100 RC200- 300 E0 E1 E2 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 09.00; 15.00 13.00; 17.00; 19.00; 23.00 13.00; 17.00; 19.00; 23.00 09.00; 03.00 01.00; 05.00; 07.00; 11.00; 13.00; 17.00; 19.00; 23.00</td>	Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 RC100- 200 15.00; 21.00; 21.00; 03.00 09.00; 15.00; 21.00; 03.00 15.00 13.0 09.00; 09.00; 01.00; 05.00; 07.0 01.00; 05.00; 07.0	Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 RC100- 200 RC200- 300 15.00; 21.00; 21.00; 03.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00; 03.00 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 17.00; 13.00; 13.00; 17.00; 13.00;	Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC50- 100 RC100- 200 RC200- 300 E0 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 13.00; 17.00; 19.00; 23 09.00; 15.00; 03.00 09.00; 15.00 01.00; 05.00; 07.00; 11.00; 13.00; 17 09.00; 09.00; 01.00; 05.00; 07.00; 11.00; 13.00; 17	Artemia supremeInstar EInstar 1Thalla $\begin{array}{cccccccccccccccccccccccccccccccccccc$	Artemia supreme Instar E Instar 1 Thalla RC0- 50 RC100- 100 RC200- 300 E0 E1 E2 15.00; 21.00 09.00; 15.00; 21.00; 03.00 09.00; 15.00 09.00; 15.00 13.00; 17.00; 19.00; 23.00 13.00; 17.00; 19.00; 23.00 09.00; 03.00 01.00; 05.00; 07.00; 11.00; 13.00; 17.00; 19.00; 23.00

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Table 3. Feeding Dossage

	Natural Feed							Artificial Feed					
Stadia	Artemia supreme (Cell/ml)	Instar E (Cell/ml)	Instar 1 (Cell/ml)	Thalla (Cell/ml)	RC0-50	RC50- 100	RC100- 200	RC200- 300	E0	E1	E2	E3	
Ν													
Zoea 1				5,000	2,25				6.75				
Zoea 2				10,000	8,5				25.5				
Zoea 3			30.0	15,000	12				36				
Mysis 1			50.0	20,000		20.5			61.5				
Mysis 2			70.0	25,000		27.5			82.5				
Mysis 3			100.0	20,000		35			70	35			
PL. 1	125.0	175.0					57.75			173.25			
PL. 2	125.0	175.0					67.25			201.75			
PL. 3	100.0	170.0					91.25			273.75			
PL. 4	100.0	160.0					106			318			
PL. 5	75.0	150.0					126.75				380.25		
PL. 6	75.0	150.0					182.25				546.75		
PL. 7	50.0	135.0						212			551.2	84.8	
PL. 8	50.0	135.0						246.25			640.25	98.5	
PL. 9	25.0	125.0						282.75			735.15	113.1	
PL. 10	25.0	125.0						297.5			773.5	119	

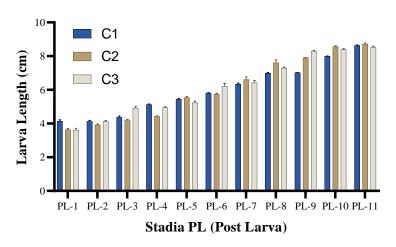


Figure 1. Growth Curve of Vannamei Shrimp Larval Length in Three Different Rearing Tanks

Microscopic observations on the developmental stages of vannamei shrimp larvae, including Gut Muscle Ratio (GMR), gut content (%), lipid droplet (%), bolitas (hepatopancreas [HP] and gastrointestinal tract [GI]), epibionts (%), necrosis (%), and pigmentation (%) in rearing tanks C1, C2, and C3 are presented in Table 4 below.

Stadia	GMR (%)	Gut (%)	Lipid droplet (%)	Biolit HP (%)	as GI (%)	Epibion (%)	Necrosis (%)	Pigmentation (%)
Z-1	-	57	10	0	0	0	0	0
Z-2	-	88	10	0	0	0	0	0
Z-3	-	65	12	10	0	10	0	R
M-1	-	65	10	0	0	+	0	R
M-2	-	70	10	0	0	+	0	20
M-3	-	65	19	0	0	0	0	R
PL-1	-	50	26	0	0	+	0	В
PL-2	10	67	40	0	0	0	0	0
PL-3	40	56	30	0	0	0	0	0
PL-4	60	62	35	0	0	0	+	R
PL-5	65	51	30	0	0	0	+	0
PL-6	70	64	40	0	0	0	0	0
PL-7	90	68	34	0	0	0	0	0
PL-8	90	74	36	0	+	0	+	R
PL-9	90	75	31	0	0	0	0	0
PL-10	90	70	31	0	0	0	0	0

Table 4. Pengamatan Microscopic Observation of Vannamei Shrimp Larval Development

From the Zoea 1 to Zoea 3 stages, variations were observed in GMR, gut content, lipid droplet, and bolitas. For example, stage Z-1 had a GMR of 57%, gut content of 10%, and lipid droplet 10%. In contrast, the stages of Mysis 1 to Mysis 3 showed increased parameters, such as M-1 with a GMR of 65% and gut content of 10%. More advanced development, indicated by higher GMR values and more significant variation in lipid droplets and pigmentation, was observed in vannamei shrimp larvae from the Post Larva 1 to Post Larva 10 stages.

Survival Rate Of Vannamei Shrimp Larvae

The highest survival rate (SR) was recorded in rearing tank C2, with an SR of 76.15% and a harvest population of 1,522,936 shrimp larvae. This was followed by tank C1, with an SR of 72.39% and a harvest population of 1,447,808 larvae. Meanwhile, tank C3 had the lowest SR at 63.62%, with a harvest population of 1,272,384 larvae. Statistical analysis showed no significant difference in survival rate among the three rearing tanks.

Water Quality Parameter

During the observation period, the water quality parameters in each rearing tank remained within the standard thresholds for vannamei shrimp cultivation. Water temperature ranged from 30.1 to 31.4°C, dissolved oxygen (DO) was between 4.0–5.5 ppm, pH ranged from 7.5 to 8.5, ammonia levels were between 0–1 mg/L, nitrite ranged from 0–0.25 ppm, salinity was 30–34 ppt, alkalinity ranged from 100–200 ppm, and total organic matter (TOM) was between 47–78 mg/L. The total viable count (TVC) at the end of the culture period was $5,000 \times 10^2$ CFU/mL, and *Vibrio alginolyticus* density was $4,800 \times 10^2$ CFU/mL. No presence of *Mycobacterium tuberculosis* (TBC) or *Vibrio cholerae* was detected.

DISCUSSION

Artemia sp. is an essential natural feed source for freshwater, brackish, and marine fish hatchery operations. It has high nutritional value and size compatible with the mouth opening of nearly all types of fish and crustacean larvae (El-Sayed *et al.*, 2021). This observation demonstrates innovation in combining natural and artificial feed to enhance the growth and survival of vannamei shrimp (*Litopenaeus vannamei*) larvae.

The hatching rate of *Artemia* sp. cultured in this observation was relatively high (84.13%). A study by (Wijianto *et al.*, 2024), reported a maximum hatching percentage of 50.21%. Another survey of *Artemia* cultured under various salinities showed a peak hatching rate of 62.37% at 60 ppt (Bahr *et al.*, 2021). This indicates that this study's natural feed culturing technique was effective. The high hatching rate also suggests that the genetic strain of *Artemia sp.* used was of excellent quality and rich in nutrients, including proteins, lipids, and vitamins.

The protein content of natural feeds such as *Artemia sp.* and *Thalassiosira* sp. in this study ranged from 40–60%, which is considered high (Albaqami, 2025), especially when compared to other natural feeds such as *Chlorella* sp. (42–50%), *Nannochloropsis oculata* (52.11%) (Zhang *et al.*, 2022), and *Spirulina platensis* 53% (Ahmed *et al.*, 2025). Protein plays a vital role in muscle development and larval growth (Albaqami, 2025), so the high protein content of *Artemia* and *Thalassiosira* supports larval development, particularly in early life stages. Additionally, the lipid content (16–23%) in these natural feeds is a significant energy source for vannamei larvae.

In shrimp hatchery operations, artificial feed is essential as a supplement to natural feed, particularly for vannamei larvae. Artificial diets provide more controlled and specific nutritional content tailored to the larvae's needs at different growth stages. Artificial feeds such as Royal Caviar, Elevia, Epibal, and Frippak significantly improved larval growth and health. An average larval length of 8.67 cm indicated good growth performance, while a survival rate of 70.72% demonstrated that most larvae survived up to the PL-11 stage.

A study by Serihollo (2022), showed that vannamei larvae raised in brackishwater ponds in Pasuruan reached only 3.5 cm in length, with a survival rate of 75.6%. Larvae fed natural diets such as *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, or their combination recorded survival rates of 55.04%, 68.22%, and 70.04%, respectively (Amyda *et al.*, 2017). Natural enrichment of *Artemia sp.* with *Chaetoceros* sp. improved absolute growth to 5.45 cm and survival rate to 83.5% (Perdana *et al.*, 2021). A combined use of natural and artificial feeds in

Penaeus merguiensis larval culture also significantly increased survival rates by up to 89% (Ighwerb *et al.*, 2021).

Artificial feeds are formulated with specific nutrients to meet larval requirements at each developmental stage (Kumar, 2021). The use—especially products like Royal Caviar, Elevia, Epibal, and Frippak—has enhanced larval growth, disease resistance, stress tolerance, and feed conversion efficiency.

Parameters such as GMR, gut content, and lipid droplets from Table 4 provide valuable insights into larval health and physiological conditions. Speed *et al.*, (2022), reported that higher GMR values correlate with better growth in snapper larvae than other species with lower GMR. Furthermore, larvae with gut content exceeding 15% demonstrated higher survival rates than those with less than 10% (D'Abramo *et al.*, 2006).

Water quality parameters in the rearing tanks were consistently within optimal limits for vannamei shrimp cultivation. These optimal water conditions contributed to good larval health, growth, and survival while reducing the risk of disease. Continuous monitoring and proper water quality management are crucial for shrimp and fish larval rearing success.

CONCLUSSION

This study confirms that implementing innovative feed management through a combination of natural feeds (*Thalassiosira* sp. and *Artemia salina*) with artificial diets significantly supports the growth and survival of vannamei shrimp larvae. The optimal nutritional composition—particularly the high levels of protein and fat—proved to play a crucial role in enhancing larval performance from the Nauplius stage to Post Larva 11. Additionally, maintained water quality and regular larval health monitoring contributed to the overall success of the rearing process. With no significant differences in growth or survival rates among the tanks, this approach demonstrated consistency in results under industrial-scale hatchery conditions. Therefore, this integrative feed management strategy is highly recommended for broader application to increase the productivity of vannamei shrimp hatcheries.

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