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Efficacy of Microencapsulated Diets on the Quality Performance of Larval and Postlarval Stages of *Penaeus vannamei*

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Authors' contributions

This work was carried out in collaboration among all authors. Author SK conceptualized the study, performed the methodology, did investigation, formal analysis and wrote original draft of the manuscript. Author PP supervised the study, wrote, reviewed and edited the manuscript. Author DC did Data curation and prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present study assessed the impact of three different commercial microencapsulated diets (MEDs) on the quality performance of larval and post-larval stages of Pacific white shrimp (*Penaeus vannamei*) by evaluating six key quality indicators. The results showed that at PL₁₀ – mean survival percentage ranged from 59.67 \pm 2.33 to 62.00 \pm 3.21%; average body length varied from 9.11 \pm 0.22 to 9.68 \pm 0.23mm; size variation (CV) ranged from 4.07 \pm 0.52 to 4.53 \pm 0.65%; salinity stress test ranged from 90.11 \pm 1.22 to 92.67 \pm 3.18%, formalin stress from 93.00 \pm 2.52 to 95.39 \pm 1.73%, and swimming rate from 83.00 ± 3.51 to 85.00 ± 3.79 %. Significant improvements were observed in all quality parameters measured in the hatchery for both larvae and post-larvae fed with

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MEDs. These findings suggest that the use of microencapsulation technology can enhance the nutritional value and bioavailability of feed, thereby improving survival and growth outcomes and providing insights into stage-specific nutritional vulnerabilities during the hatchery phase in *P. vannamei.*

Keywords: Hatchery; P. vannamei; microencapsulated diets; nutrition; quality indicators.

1. INTRODUCTION

The Pacific white shrimp (*Penaeus vannamei*) is highly regarded in the global aquaculture industry for its rapid growth rate, high market demand, and adaptability to diverse farming environments (Emerenciano et al., 2022). The hatchery phase is crucial for the cultivation of *P. vannamei*, forming the basis for the success of the entire production cycle (Iba, 2014). The hatchery environment is carefully controlled to optimize conditions for larval development, survival, and growth (Rehman et al., 2017). The quality of post-larvae produced in the hatchery directly impacts their performance in subsequent growout stages, affecting overall production efficiency and profitability (Valenti et al., 2010).

The larval and post-larval stages of *P. vannamei* involve rapid growth and metamorphosis, creating significant nutritional demands. During these critical periods, shrimp undergo substantial physiological changes, requiring a diet rich in essential nutrients to support optimal development (Cuzon et al., 2004, Gamboa-Delgado and Vay, 2009, Suita et al., 2015). Nutritional requirements are crucial for tissue construction, energy metabolism, and immune function. Nutrient deficiencies can lead to growth
retardation. deformities. and increased retardation, deformities, and susceptibility to diseases, resulting in substantial economic losses (Goh et al., 2023, Mente et al., 2019). Therefore, formulating nutritionally balanced diets is essential for ensuring the health and vigor of shrimp larvae and postlarvae.

The nutritional requirements of shrimp larvae and post-larvae are complex and change rapidly as the organisms progress through their developmental stages (Truong et al., 2023, Anger, 2006). Traditionally, live organisms such as rotifers and Artemia nauplii have been the main feed sources in hatcheries (Altaff, 2020, Kandathil, et al., 2020, Das et al., 2012, Suneetha et al., 2024). However, these live feeds have limitations in terms of nutritional consistency, contamination risks, and production costs (Sharma et al., 2023).

Microencapsulated diets (MEDs) offer a viable alternative by providing a controlled and standardized nutrient delivery system. These diets are formulated to meet the specific nutritional needs of different larval and post-larval stages, promoting optimal growth, survival, and uniformity in shrimp populations (Luzardo-Alvarez, 2010, Barreto, 2023). Additionally, MEDs can be enriched with essential fatty acids, vitamins, and immunostimulants to enhance shrimp health and resilience (Kamble, 2024). Hence, the study investigated the impact of MEDs on the percentage survival of larval and post-larval stages of Pacific white shrimp.

2. MATERIALS AND METHODS

2.1 Experimental Design

The present research was conducted with facilities acquired from the BKMN Aqua (shrimp hatchery), Undavalli (16°50'64" N and 80°57'13" E), Guntur, Andhra Pradesh, India. The feed trials for MEDs were conducted under commercial conditions using larval rearing tanks (LRTs) measuring 1.2 m x 7.5 m x 1.8 m in a parabolic structure. At the Nauplii Rearing Hatchery (NRH), the nauplii (N_{V1}) is the first larval stage of shrimp *P. vannamei* reared in the larval tanks at a density of 100 L ¹ and raised until they reached the post-larvae-10 stage (PL_{10}) as PL_{10} is the recommended size for stocking in grow-out farms (FAO, 2007). Experimental feed trials were conducted in triplicates for three consecutive years (2020–22) to investigate the effectiveness of MEDs on the quality indicators of larval and post-larval phases (Z₁ to PL₁₀) of *P. vannamei*.

2.2 Water Management

Prior to stocking LRTs were thoroughly cleaned, disinfected, and washed with freshwater following the standard operating procedures (SOPs) for shrimp hatcheries (FAO, 2007). The tanks had a water-holding capacity of 12 tons with a working volume of 10 tons (80% of tank capacity). They were equipped with the necessary aeration setup for dissolved oxygen (DO) levels. The treated seawater was introduced into the experimental tanks a day before stocking the nauplii. Water management and exchange protocols in the experimental tanks were followed by Panigrahi (2013) with slight modifications (Table 1).

2.3 Feed Management

All experimental treatments included live food (microalgae) in the form of *Thalassiosira weissflogii* to feed protozoea and mysis phases, while Artemia (INVE brand) was used to feed the mysis phase to post-larval stages. Three commercially available MEDs: Treatment–I
(ENCAP), Treatment–II (FRIPPAK), and (ENCAP), Treatment–II (FRiPPAK), and Treatment–III (MACAY) were utilized. The MED feeding protocol was conducted according to the procedures described by Lorenzo et al. (2016) and Suneetha et al*.* (2024), and consists of six daily feedings (once every 4 hours) for the $Z₁$ to PL₁ stage and four times a day (once every 6 hours) for the PL_1 to PL_{10} stages, adhering to manufacturer's recommendations (Table 2).

2.3.1 Treatment–I

The ENCAP MED feed contained crude protein (minimum 50%), crude fat (minimum 10%), crude ash (maximum 18%), and moisture (maximum 8%). The feed particle size for Zoea feed was less than 77 µm, for Mysis feed it was 77-100 $µm$, for early post-larva feed (PL₁ – PL₅) it was 100-250 μ m, and for late post-larvae (PL $_6$ – PL $_{10}$) feed it was 200-350 µm. The larval and postlarval shrimp were fed ENCAP commercial MED to the shrimp as per the manufacturer's recommendations (Sky Aqua).

2.3.2 Treatment–II

FRiPPAK MED (minimum protein content of 52%, minimum lipid content of 14.5%, maximum fiber content of 3%, and maximum moisture content of 10%) was provided to the shrimp following the manufacturer's guidelines (Benchmark Genetics). The feed particle size for Zoea feed was 15-22 µm, Mysis feed was 30-90 $µm$, early post-larva feed (PL $1 - PL$ 5) was 90-200 $µm$, and late post-larvae (PL $_6$ – PL $_{10}$) feed was 200-300 µm.

2.3.3 Treatment–III

MACAY MED was used for all the larval and post-larval phases $(Z_1$ to PL_{10}) as per the manufacturer's instructions (Macay Marine MP feed). The MACAY MED feed contains Crude Protein (50% minimum), Crude Fat (13% minimum), Crude Fiber (3% maximum), Total Ash (8% maximum), and Moisture (6% maximum). The feed particle size, with zoea feed having a particle size of $<$ 70 µm, Mysis feed having a particle size of 70-100 um, early postlarva feed (PL $_1$ – PL $_5$) having a particle size of 100-250 μ m, and late post-larvae (PL $_6$ – PL $_{10}$) having a particle size of 250 - 450 µm.

2.4 Quality Assessment Parameters

Six quality indices i.e., Survival performance, Average Body Length, Size Variation (CV), Salinity stress, Formalin stress, and Swimming rate were employed to evaluate the quality of the post-larvae.

2.4.1 Survival performance

Survival performance (%) was assessed at various larval (Z_{III} and M_{III}) and post-larval (PL₅ and PL_{10}) stages using the calculation method described by Panigrahi et al*.* (2019).

$$
SR (%) = \frac{Nt}{No} X 100
$$

where, $SR =$ survival (%), $N_t =$ the number of shrimps that survived until the end of the experiment, and N_0 = the number of shrimps that were available at the beginning of the experiment.

2.4.2 Salinity stress

Salinity stress tests for PL_{10} were conducted according to the procedures described by de-Lorenzo et al*.* (2016). 100 PLs were exposed to a salinity equal to 50% of the reared tanks' water salinity for 60 minutes to assess larval resistance to severe salinity changes.

2.4.3 Formalin Stress

Formalin stress tests for PL_{10} were conducted according to the procedures described by Racotta et al*.*, (2003). 100 PLs were exposed to a formalin concentration of 100 ppm, and postlarval survival was recorded after one hour of exposure.

2.4.4 Swimming activity

Swimming activity was assessed based on the method determined by Mirzaei et al*.* (2021). 100 PLs (PL₁₀) were placed in a shallow circular pan with a circular flow, and their movements were observed. The number of larvae that were able to

swim against the direction of water flow was recorded as a percentage.

2.4.5 Average Body Length

In the final stage of the experimental period, the total length of PL_{10} (100 no's) was measured and recorded following the standard methods described by Madhukiran et al*.* (2009). The total length of the PLs was measured from the beginning of the rostrum to the end of the telson in millimeters (mm) using an optical micrometer and a caliper.

2.4.6 Size Variation (CV)

The coefficient of variation (CV) was used to assess the length uniformity (SV) percentage, which was computed using the procedure outlined by Balasubramanian (2009).

$$
CV(\%) = \frac{SD}{M} X 100
$$

where, $CV = coefficient of variation (%)$, $SD =$ standard deviation of shrimp's total length; M = mean of shrimp's total length.

2.5 Statistical Analysis

To assess the statistical significance of the data, a one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) was carried out by IBM SPSS software (version 29) at 0.05 level of significance (*P <* 0.05). Additionally, a second-order polynomial was used to find out the R-squared value for mortality rate. All the values are presented as Mean \pm SD (standard deviation) of three replicate analyses.

3. RESULTS AND DISCUSSION

3.1 Survival Performance

The efficiency of three MEDs – ENCAP, FRiPPAK, and MACAY on the survival of larval and post-larval stages of *P. vannamei* is shown in Table 3. The mean survival among the three feed trails during the protozoal stages $(Z_1$ to Z_{III}) was 87.0 – 89.0%, in mysis (M_I to M_{III}) 76.00 – 78.67% whereas in post-larval stages (PL $_1$ to PL₅) 66.67 – 68.67% and (PL₆ to PL₁₀) 59.67 – 62.00%.

The maximum survival of zoea larvae at 89.00 \pm 2.52% was observed in treatment–II (FRiPPAK) followed by 87.33 \pm 1.45% in treatment-III (MACAY) and $87.00 \pm 1.53\%$ in treatment-I (ENCAP). The mean values of different MED feeds showed significant differences (*P <* 0.05) in the Zoea stage of *P. vannamei* (Table 3).

Similarly, in the mysis stage, treatment–II (FRiPPAK) had the highest survival rate at 78.67 \pm 2.60%. The next highest survival of 76.33 \pm 2.33% was observed in treatment–I (MACAY) and the lowest survival of $76.00 \pm 2.89\%$ was observed in treatment–III (ENCAP). The mean values of different MED feeds showed no significant differences (*P >* 0.05) in the Mysis stage of *P. vannamei* (Table 4).

In later developmental stages (PL_1 to PL_{10}) no significant differences (*P >* 0.05) were detected in survival between the larvae fed with different MED feeds. During the early post-larval (PL_1 to PL5) stages, treatment–I (ENCAP) exhibited the highest survival rate at $68.67 \pm 1.20\%$, while treatment–III (FRiPPAK) had the lowest at 66.67 ± 2.91%. Treatment–II (MACAY) remains an intermediate survival rate of $67.00 \pm 3.61\%$ (Table 3).

In the final stage of the post larvae ($PL_6 - PL_{10}$), the average survival rate for all three feeds was

60%. A maximum of 62.00 ± 3.21 % was obtained in treatment–I (ENCAP) followed by the next highest survival of $60.67 \pm 2.33\%$ in treatment–II (FRIPPAK) and the lowest survival of 59.67 \pm 2.30% was recorded in treatment–III (MACAY) (Table 3).

The overall average percentage of survival rates among the treatments showed that in larval stages (Z_l to M_{III}) was Treatment–II < Treatment– I < Treatment–III, whereas in post-larval stages from PL_1 to PL_{10} was Treatment–I < Treatment–II < Treatment–III (Table 3).

P. vannamei experiences mortalities throughout the hatchery phase, but some stages are more critical than others (Mishra et al., 2008). In this study, a comparative analysis of mortality rate trends among three treatments (ENCAP, FRiPPAK, and MACAY) was conducted to identify key developmental stages susceptible to increased mortality.

In the ENCAP treatment, the recorded mortality rates ranged from 05.00% to 12.67%, with an R^2 value of 0.8304 (Fig. 1). The peak mortality percentages of 12.67% and 11.00%, were observed during the Z_{III} and M_{III} of larval stages respectively. In the post-larval stages, the mortality rates were documented at 09.33% for PL₅ and 05.00% for PL₁₀.

For the FRiPPAK treatment, mortality rates were observed between 08.00% and 11.00% (R^2 = 0.8448). Maximum mortality occurred during Z_{III} (11.00%) and M_{III} (10.33%) , while the post-larval stages reported mortality rates of 10.00% at PL⁵ and 08.00% at PL₁₀ (Fig. 2).

In the MACAY treatment, the mortality rates varied from 07.00% to 13.00%, and the $R²$ value was recorded as 0.7784 (Fig. 3). The highest mortalities were detected during Z_{III} (13.00%) and M_{III} (11.00%), followed by 9.33% at PL₅ and 7.00% at PL_{10.}

Larval Stage	Treatment-I ENCAP (%)	Treatment-II FRIPPAK (%)	Treatment-III MACAY (%)
N_{VI}/Z_I Stocking	100 L^{-1}	100 L^{-1}	100 L^{-1}
Zoea \mathbf{u}	$87.33 \pm 1.45^{\circ}$	89.00 ± 2.52 ^{ab}	87.00 ± 1.53 ^a
Mysis _{III}	76.33 ± 2.33 ^a	78.67 ± 2.60^a	76.00 ± 2.89 ^a
PL ₅	67.00 ± 3.61 ^a	68.67 ± 1.20^a	66.67 ± 2.91 ^a
PL_{10}	62.00 ± 3.21 ^a	60.67 ± 2.33 ^a	59.67 ± 2.33 ^a
Δll the values are the mean \pm SD (standard deviation) of three replicate analyses			

Table 3. Survival percentage of larval and post-larval stages of *P. vannamei*

All the values are the mean ± SD (standard deviation) of three replicate analyses.

Data with different superscript letters in the same column indicated significant differences at P < 0.05.

Fig. 2. Percentage of mortality in treatment–II (FRiPPAK)

Fig. 3. Percentage of mortality in treatment–III (MACAY)

The study assessed the impact of using a commercial MED feed (ENCAP, FRiPPAK, and MACAY) on the survival of *P. vannamei* across various larval and post-larval stages $(Z_1 - PL_{10})$. Among the three feed trials, treatment–I with FRiPPAK yielded the highest survival rates for *P. vannamei* during the protozoal and mysis stages $(Z_l$ to M_{III}). Conversely, during post-larval stages

 $(PL₁$ to $PL₁₀)$, treatment-I with ENCAP demonstrated the highest survival rates.

In hatchery operations, the zoea and mysis phases of penaeid prawns present significant rearing challenges (Lemos and Weissman, 2021, Gore 2017). Our study found that treatment–I (FRiPPAK) resulted in the highest survival rate

compared to the other two treatments. We speculate that the feed particle size may have contributed to the higher survival rate. The ideal feed particle size should be suitable for the mouthparts and digestive system of the shrimp at a specific stage (Carter and Codabaccus, 2022). Additionally, Obaldo & Masuda (2006) found that larger feed particles can create increased competition among shrimp, with dominant individuals monopolizing the food source and hindering access for smaller shrimp, thereby impacting their growth and survival. Consequently, in our study, smaller shrimp may have difficulty consuming or digesting larger feed particles, potentially resulting in reduced nutrient intake and impacting their survival.

Enhancing the survival rates of *P. vannamei* post-larvae is crucial for successful shrimp aquaculture (Suneetha et al., 2024). While live food is traditionally used as feed, artificial diets offer a more consistent and controllable alternative (Sangha et al., 2000, Gamboa-Delgado and Le Vay, 2009). This study found that ENCAP with *T. weissflogii* exhibited the highest survival rates, possibly due to the varying nutritional requirements of shrimp larvae at each phase. Aaqillah-Amr (2021) stated that decapods undergo a transition from undeveloped nauplii to stabilized post-larvae, with their feeding behavior and digestive systems becoming more complex during the growing stages. Furthermore, Estévez et al., (2019) emphasized that the growth and development of the digestive system affect digestive capability, mainly due to qualitative and quantitative fluctuations in digestive enzyme production. Consequently, it is evident that shrimp larval stages have distinct nutritional requirements.

3.2 Average Body Length

The effectiveness of three MED feeds (ENCAP, FRiPPAK, and MACAY) on the average body length of *P. vannamei* at PL₁₀ is shown in Table 4. The mean average body length ranged from 9.11 ± 0.22 to 9.68 ± 0.23 mm across the feeding trials, with the highest observed in ENCAP (9.68 \pm 0.23mm), followed by MACAY (9.28 \pm 0.22mm) and FRIPPAK $(9.11 \pm 0.22$ mm).

3.3 Salinity Stress

At PL10 stage of *P. vannamei* the salinity stress test ranged from 90.11 ± 1.22 to $92.67 \pm 3.18\%$ (Table 4). The highest percentages for salinity stress were found in ENCAP (92.67 \pm 3.18%), followed by MACAY $(90.83 \pm 3.03\%)$ and FRIPPAK (90.11 \pm 1.22%).

3.4 Size Variation (CV)

The mean percentage values of the coefficient of size variation (CV) were shown in Table 4. The CV of *P. vannamei* at PL₁₀ ranged from $4.07 \pm$ 0.52 to 4.53 \pm 0.65% among the three feeding trails. MACAY exhibited the highest CV (4.53 \pm 0.65%) followed by FRIPPAK $(4.22 \pm 0.14\%)$ and ENCAP $(4.07 \pm 0.52\%)$.

3.5 Formalin Stress

The formalin stress percentages of *P. vannamei* at PL₁₀ was found between 93.00 ± 2.52 and $95.39 \pm 1.73\%$ among the three feeding trials (Table 4). ENCAP showed the highest values $(95.39 \pm 1.73\%)$, followed by MACAY (93.53 ± 2.03%) and FRiPPAK (93.00± 2.52%).

3.6 Swimming Rate

PL¹⁰ stage of *P. vannamei* exhibited rates ranging from 83.00 ± 3.51 to $85.00 \pm 3.79\%$ across the three feeding trials (Table 4). The maximum percentage was observed at 85.00 \pm 3.79% in ENCAP, the lowest was found at 83.00 ± 3.51% in FRiPPAK, and an intermediate value in MACAY at $84.33 + 5.24\%$

Table 4. Quality indicators of *P. vannamei* **at PL¹⁰**

All the values are the mean ± SD (standard deviation) of three replicate analyses.

Data with different superscript letters in the same column indicated significant differences at P < 0.05.

One-way ANOVA revealed that the mean percentages of average body length, size variation (CV), salinity stress, and formalin stress are not significant different (*P >* 0.05) except for swimming rate (Table 4).

The quality of post-larvae in shrimp hatcheries has a significant impact on the management of *P. vannamei* in grow-out systems. Shrimp farm production is strongly linked to the quality of PL (Ferreira et al., 2011). Fast, inexpensive, and simple standard tests for assessing shrimp postlarval quality can be utilized in hatcheries as a quality control measure (Pedrazzani et al., 2023, Sumarwan et al., 2023).

Among the feed trials, the ENCAP showed the highest average body length $(9.68 \pm 0.23$ mm) and the lowest size variation (CV - 4.07%). It also exhibited a high swimming rate $(85.00 \pm 3.79\%)$, good salinity stress survival (92.67 \pm 3.18%), and formalin stress survival $(95.39 \pm 1.73\%)$ compared to the others. The fluctuation in the quality parameters of the PL in hatchery conditions could be attributed to nutritional intake. Various studies have shown that larger shrimps are more efficient in storing energy and protein in the body than smaller shrimps (Isa et al., 2012, Rosas et al., 2001, Anger et al., 2002). It is noteworthy that there is a direct relationship between the body length status and size variation of the larvae. According to Balasubramanian (2009), a CV of less than 10% of the population is considered excellent for stocking in grow-out farms, and if the CV is greater than 15%, the population may have been infected. Hence, the present study indicates that all the feed trial experiments showed below 5% of CV, which is favourable for seed stocking in the grow-out farms.

Salinity and formalin stress tests are one of the most important environmental factors that affect the growth and survival of shrimp in the Panaeidae family, especially in nursery pond areas that may be exposed to rapid saline changes and environmental conditions (Gamboa‐Delgado, et al., 2003, Álvarez et al., 2004). de Lorenzo (2016) proposed a scale for evaluating larval quality based on the salinity stress test. According to this scale, a survival rate of over 70% is considered acceptable and indicative of good seed quality. In the experimental trials, all showed a survival rate exceeding 70%. The results of both the formalin and salinity stress tests indicated that post-larvae reared in ENCAP exhibited the highest survival

percentage among the three feeding trials. Despite this difference, the study found no significant variations in the experimental trials. except for the swimming rate, between the three feeding trials.

4. CONCLUSION

The present study results demonstrate the effectiveness of various MED feed combinations in enhancing the quality performance of larval and post-larval stages of *P. vannamei*. These findings suggest that the use of microencapsulation technology can enhance the nutritional value and bioavailability of feed, thereby improving survival and growth outcomes and providing insights into stage-specific nutritional vulnerabilities during the hatchery phase in *P. vannamei*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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