JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

EFFECT OF PROBIOTIC AND SUPPLEMENTED FEED ON GROWTH, SURVIVAL AND DISEASE RESISTANCE OF WHITE SHRIMP *LITOPENAEUS VANNAMEI* POSTLARVAE

*Vennila Jayaprakash and Arulvasu Chinnasamy**

Address(es):
Department of Zoology, University of Madras, Guindy Campus, Chennai - 600025, Tamil Nadu, India.

*Corresponding author: carulvasu@gmail.com

https://doi.org/10.55251/jmbfs.4096

ARTICLE INFO

Received 5. 8. 2021
Revised 20. 11. 2022
Accepted 28. 11. 2022
Published 1. 2. 2023

Regular article

OPEN ACCESS

ABSTRACT

The present investigation was focused on the effect of *Bacillus megaterium* enriched to the *Artemia* nauplii and sprayed on the supplemented feed was fed to the white shrimp *Litopenaeus vannamei* postlarvae (PL) to obtain high survival and growth. *Artemia* nauplii enriched with different levels of *B. megaterium* (5×10^7, 5×10^9 and 5×10^{11} cfu /mL) fed to postlarvae for a period of 30 days. Similarly, supplemented feed with probiotic of different levels fed to PL for a time period of 15 days. Postlarvae fed with 5×10^9 cfu cells (E2) of *B. megaterium* enriched *Artemia* nauplii and supplemented feed produce better survival and growth when compared to that of other concentrations and control group. The specific growth rate was found to be maximum in postlarvae fed with *B. megaterium* enriched *Artemia* and supplemented feed when compared to the control group. The biochemical constituents, such as protein, lipid and carbohydrate contents were considerably more in PL fed with both feeds, particularly at 5×10^9 cfu cells. In the challenging study, results showed a higher survival rate in postlarvae fed with enhanced *Artemia* nauplii and supplemented feed of E2. Therefore, it is evident that 5×10^9 cfu cells of *B. megaterium* can be considered as an appropriate concentration for the growth and survival of *L. vannamei* postlarvae.

Keywords: Live feed, enrichment, *Artemia*, *Bacillus megaterium*, bioencapsulation

INTRODUCTION

Globally, aquaculture regarded as the fastest-growing food production technology with high production for several decades (**Das *et al*., 2008; Edwards *et al*., 2019)**. In 2016, around 89 per cent of the output originated in Asia, primarily China, which attracts greater attention from global aquaculture production since 62 per cent occurred in China. After China, India takes the second position concerning annual fisheries and aquaculture production. Hence, the country promoting its aquaculture practices for more shellfishes and crustacean's production. In 2017, fish production is expected to reach 12.60 million metric tonnes, with about 65 % coming from the inland sector and about 50% coming from cultural fisheries **(FAO, 2018)**. Aquaculture is a vital source of food, stock augmentation, employment, and profit for millions of people worldwide **(Murillo-Gurrea *et al*., 2001)**. Many microbiological pathogens, including *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*, have been weakened in shrimp farming as a result of infectious disease outbreaks (**MartinezPorchas and MartinezCordova, 2012)**. Because of its excellent survival rate, quick development in intensive culture systems, and disease resistance, the Pacific white shrimp *Litopenaeus vannamei* is widely farmed across the world. With about 3.8 million metric tonnes generated in 2015, intensive culture of Pacific white shrimp *Litopenaeus vannamei* represents substantial aquaculture operations in terms of production value and a high-value commodity **(FAO, 2016)**.

Bioencapsulation is a technology that is currently being explored for the oral administration of carotenoids, chemotherapeutic vaccines, pigments, sterols, vitamins, and vital polyunsaturated fatty acids (PUFAs) via *Artemia* nauplii. The larval stages of *L. vannamei* exhibit complicated trophic alterations during development, particularly for penaeid. The phytoplankton-feeding zoea and carnivorous mysis stages come after the non-feeding naupliar stages. The latter stage is particularly reliant on a consistent supply of live food, which is frequently provided in hatcheries as rotifers and *Artemia* nauplii. *Artemia* is an important live feed used in the production of shrimp larvae. They are continuous filter feeders that eat suspended particles and are not selective. These qualities allow one to increase their nutritional profile by immersing them in a solution rich in nutrients such as docosahexaenoic acid **(Sorgeloos *et al*., 1986)**. The main role of aquaculturists is to provide organisms suited for the feed size to the initial feeding stage, as well as an acceptable quantity of feed organisms to ensure greater survival and quicker growth **(Arulvasu and Munuswamy, 2009)**. Disease in shrimp cultivation was induced by a variety of biotic and abiotic causes. Farmers mostly use antibiotics as a preventative or therapeutic measure to combat illness **(Hoseinifar *et al*., 2017)**. Antibiotics not only disrupt the natural flora of shrimp intestinal tracts but also render diseases resistant to medications; hence, probiotics are the most promising alternative to antibiotics **(Tripathi and Giri, 2014)**. Because of the importance of water quality, nutritional absorption, the immune system, and the survival and growth rates of hosts, probiotics play a key role in aquaculture. *Bacillus* bacterium strains have produced extremely good outcomes in shrimp farming. This bacterium is a non-infectious Gram-positive spore-forming bacteria that has been utilized to ameliorate the health of shrimp **(Keysami *et al*., 2012)**. *Vibrio harveyi* is a scintillating species commonly obtained from marine sources, has been identified as harmful to fish and various crustaceans, most notably *Penaeus* species. **(Lavilla-Pitogo *et al*., 1990)**.

The quest for sustainable, ecologically friendly aquaculture is driving up research into probiotics for marine creatures. The commercial source of probiotic dietary supplementation provided a superior growth, immuno-physiology of *Litopeneaus vannamei* than the indigenous source of *Bacillus* species **(Abdollahi-Arpanahi *et al*., 2018)**. Several research have been undertaken to investigate the effect of various feeding regimens and additives on shrimp growth performance and immunological state **(Bowyer *et al*., 2019)**. Since probiotics have been shown to promote host organism growth, nutrition, and survival. The purpose of this study was to see how enriched *Artemia* nauplii with the probiotic bacteria *Bacillus megaterium* and supplemented diet affected growth, survival, and disease resistance in postlarvae of white shrimp *Litopenaeus vannamei.*

MATERIAL AND METHODS

Experimental animal and Probiotic strain

Litopenaeus vannamei postlarvae were acquired from Royal hatcheries, Chennai, Tamil Nadu, India. The postlarvae were transported in plastic bags containing 5 ppt seawater to the experimental setup kept at our laboratory. Before the trials, postlarvae were cultivated in laboratory tanks filled with aerated seawater at room temperature for 2–3 days. *Bacillus megaterium* was selected as the probiotic, which was obtained from the Central Institute of Brackishwater Aquaculture, Indian Council of Agricultural Research, Chennai, Tamil Nadu, India. The probiotic strain was grown in Tryptone soy broth (TSB) using a shaking incubator at 30˚C for 24 hours. Using a spectrophotometer, the cell densities of the suspensions were calculated at 600nm.

Artemia Cyst Collection and Hatching

Artemia cysts were collected from (100 µm scoop net) Kelambakkam saltpan, Tamil Nadu, India. Cysts were hatched using the standard procedures adopted from previous publications **(Sorgeloos *et al*., 1986)**. *Artemia* cyst of one gram was hydrated in freshwater for a period of one hour in a beaker with vigorous aeration. The cysts were collected after one hour and rinsed with tap water and then transferred into the decapsulating solution (4% sodium hypochlorite). After 5 to 10 min, the entire cysts turned to an orange-pink colour indicating decapsulation. The cysts were collected in a 100 µm sieve and rinsed with fresh water to remove all traces of the hypochlorite solution. The decapsulated cysts were transferred to 1litre seawater (salinity 30 ppt) and vigorously aerated for 24 hours. The hatched nauplii were siphoned out by exposing them to light. The nauplii were washed completely and used for the enrichment trials (Figure 1A - E).

Enrichment Procedure

Enrichment of the second instar nauplii was carried out by the standard procedure of **Sorgeloos and Kulasekarapandian (1984)**. First instar *Artemia* nauplii appeared after 24 h incubation. Cell suspensions of *B.megaterium* were enriched after 12 h incubation. The second instar stage *Artemia* nauplii were segregated from the container through a 120 µm sieve and moved to a glass beaker at a mass of 200,000 nauplii / L of seawater. The three different concentrations of *Bacillus megaterium*, 5 $\times 10^7$cfu / mL (Experiment 1), 5$\times 10^9$ cfu / mL (Experiment 2) and 5 $\times 10^{11}$ cfu / mL (Experiment 3). After 6 hours of enrichment period, the *Artemia* nauplii were picked and rinsed with seawater. Unenriched *Artemia* nauplii served as control. The *Artemia* nauplii were reaped from the enrichment glass containers. They were washed rigorously with fresh water and stored for further use. *Bacillus* strain present in the gut of transparent nauplii was observed under a light microscope (Figure 1F).

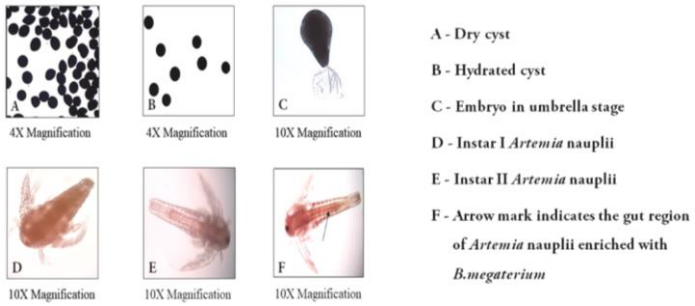

Figure 1 Photomicrographs showing *Artemia* cyst hatching and nauplii enriched with *B.megaterium*

Experimental setup

Experiments were accomplished by dividing the *Litopenaeus vannamei* postlarvae into four groups such as Control, Experiment 1(E1), Experiment 2 (E2) and Experiment 3 (E3). A total count of 180 postlarvae individuals with a weight of 0.58 ± 0.27 mg on average were randomly divided into 12 tanks with 15 individuals per tank placed. Three replicate groups of postlarvae were fed with unenriched and enriched *Artemia* nauplii.

Preparation of the experimental diet

Pellet feed obtained from Central Institute of Brackishwater Aquaculture, Indian Council of Agricultural Research, Chennai, Tamil Nadu, India. After 24 h culture, the probiotic bacteria were collected by centrifuge at 10,000 rpm for 15 minutes. The cells collected were washed and re-suspended in phosphate-buffered saline (PBS pH 7.4). The prepared suspension containing probiotic bacteria sprayed uniformly over the pellet. Then feed was dried and stored at 4°C. Experimental diets E1, E2 and E3 supplemented with *Bacillus megaterium* were 5 $\times 10^7$ cfu, 5$\times 10^9$ cfu and 5 $\times 10^{11}$ cfu cells and control (without probiotic) were prepared once every 15 days.

Experimental Design and Feeding Schedule

After completion of live feed enrichment studies, same postlarvae were used for the supplemented feed experiment. Experiments were accomplished by divided the animals into four groups such as Control, E1, E2 and E3. A total of 40 postlarvae individuals with a weight of 0.17 ± 0.005 on average were randomly divided into 8 tanks with 5 individuals per tank were placed. Feed was given at a ratio of 50% of the postlarvae body weight. The daily ration was split into three equal parts and was fed at 10:00 h, 14:00 h and 18:00 h. Excess feed was removed daily. Residuals removal and seawater exchange was done every day.

Growth parameters

Growth of *Litopenaeus vannamei* postlarvae fed with enriched *Artemia* and probiotic supplemented feed of different concentrations of *Bacillus megaterium* was intended using the formula given by **Nimrat *et al*. (2011)**. The shrimps' postlarvae of each tank were weighed individually and measured to calculate the growth parameters.

Biochemical analysis

The biochemical constituents, comprising protein, lipid, and carbohydrate, were assessed according to protocol. The estimation of protein, lipid and carbohydrates from all experimental groups was quantified using the methods of **Bradford (1976)**, **Barnes and Blackstock (1973) and Roe (1955).**

Challenge study

Postlarvae were exposed to a virulent strain of *Vibrio harveyi* **(Sritunyalucksana *et al*., 2005)** for 10 days. Ten PL were added to aerated seawater that contained 1.4 × 10^7 cfu/mL of *V. harveyi*. During the first 24 hours, there was no water exchange. Post larvae were fed as normal during the challenge experiment. After 10 days, the number of survivals were counted. After the 15-day feeding experiments, three shrimp per tank were challenged. The data is displayed as a relative survival percentage (rps).

Statistical Analysis

One-way analysis of variance was used to conduct statistical analyses of the data (ANOVA). Statistical analyses of the data were carried out, using a one-way analysis of variance (ANOVA). Differences were deemed to be significant when $p < 0.05$. Mean ± standard deviation of the data is displayed.

RESULTS

The present experiment aimed to analyse the growth, survival and disease resistance of *Litopenaeus vannamei* postlarvae fed with different concentrations of probiotic-enriched *Artemia* nauplii and supplemented feed.

Growth and survival of shrimp post larvae

After the 30-day experiment, the growth parameters of shrimp PL in different concentrations of probiotic and control, such as initial length & weight, final length & weight, specific growth rate and survival rates are shown in Table 1. The initial average body length and total body mass of the PL was 5.47mm and 0.58 mg respectively. Initial weight and length did not differ much between experiments (p>0.05). Also, the final lengths of the shrimp PL were 20.98, 23.68, 25.22 and 21.14 mm respectively. The final weights of the shrimps PL were 19.0, 24.8, 27.0 and 22.1mg in control, E1, E2 and E3 respectively. Statistical analysis revealed that shrimps fed with E2-enriched *Artemia* nauplii grew noticeably more than the control group (p<0.05) at the conclusion of the trial. Additionally, significant (p<0.05) differences between the E2 group and control were noted as SGR percentage variances. There were no discernible variations in SGR between the E3 group and control (p>0.05).The survival rates were found at 71.11, 84.44, 93.33 and 80.00 % in control, E1, E2 and E3 respectively. However, the survival rate of the shrimp PL fed with the experimental group was significantly different ($p<0.05$) from that of the control group.

Table 1 Growth and survival of *Litopenaeus vannamei* postlarvae fed with enriched *Artemia* nauplii for 30 days

Experiments	Initial length (mm)	Final length (mm)	Initial weight (mg)	Final weight (mg)	SGR (%)	Survival rate (%)
Control	5.54 ± 1.38	20.98 ±1.02^c	0.56 ± 0.27	19.00 ± 1.22^d	12.44 ± 1.70^c	71.11 ± 3.85^c
E1	5.36 ± 1.12	23.68 ±1.52^b	0.60 ± 0.27	24.80 ± 1.30^b	13.12 ± 1.59^b	84.44 ± 3.85^b
E2	5.28 ± 1.05	25.22±1.14^a	0.52 ± 0.29	27.00± 1.20^a	14.00±1.83^a	93.33 ± 0.00^a
E3	5.76 ± 1.26	21.14 ±0.86^c	0.64 ± 0.27	22.10 ±1.04^c	12.49 ± 1.54^c	80.00 ± 6.66^b

Values represent means ± standard deviation of the samples with different superscript letters that are significantly different from each other ($p < 0.05$). Mean without letter are not significantly different. Control: unenriched *Artemia* nauplii, E1: *Artemia* nauplii enriched with 5 × 10^7 cfu cells, E2: *Artemia* nauplii enriched with 5 × 10^9 cfu cells and E3: *Artemia* nauplii enriched with 5 × 10^{11} cfu cells and SGR: specific growth rate.

Similarly, after 15-days completion all the growth indicators measured in the experiment were significantly different ($p<0.05$). The effects of probiotic *B.megaterium* sprayed pellet feed in *L.vannamei* postlarvae are presented in Table 2. The present investigation is the consecutive process of the previous experiment.

Since the same PL utilized in this experiment, the initial average length and weight showed a substantial variation between the groups ($p<0.05$). The final length of shrimp PL were 31.32, 33.01, 37.30 and 32.00 mm in control, E1, E2 and E3. The data were statistically significant ($p<0.05$) between experimental groups at various doses compared to the control group. Similarly, weight gain in *L. vannamei* post larvae fed with probiotic supplemented feed varied significantly ($p<0.05$) compared to the post larvae fed with pellet feed. The post larvae of *L.vannamei* fed with probiotic supplemented feed attained maximum weight in E2 followed by E3 and E1 were 48.10, 32.41 and 31.52 mg respectively, whereas control showed comparatively less weight of 25.11 mg. The highest specific growth rate found in E2 group followed by E1 and E3 were 3.31, 1.82 and 1.70 % respectively. In comparison to the control and other groups, the E2 probiotic supplemented group demonstrated a significant difference ($p< 0.05$). Compared to the group provided control feed and the probiotic-supplemented feed had a greater survival rate. In probiotic supplemented feed survival rate were ranged from 95.00, 100.00 and 90.00% in E1, E2 and E3 respectively. The highest survival rate of 100% was recorded in shrimp's PL fed with probiotic supplemented feed while the control feed exhibited the survival rate of 80%.

Table 2 Growth and survival of *Litopenaeus vannamei* postlarvae fed with probiotic supplemented feed for 15 days

Experiments	Initial length (mm)	Final length (mm)	Initial weight (mg)	Final weight (mg)	SGR (%)	Survival rate (%)
Control	20.98 ± 1.02	31.32 ±0.23^{b}	19.00 ± 1.22	25.11±0.01^{c}	1.13 ± 0.21^{b}	80.00 ± 3.00^{c}
E1	23.68 ± 1.52	33.01 ±0.26^{b}	24.80 ± 1.30	31.52±0.04^{b}	1.82 ± 0.65^{b}	95.00 ± 3.85^{b}
E2	25.22±1.14	37.30±0.28^{a}	27.00±1.20	48.10±0.03^{a}	3.31 ± 0.33^{a}	100.00± 0.00^{a}
E3	21.14 ± 0.86	32.00 ±0.20^{b}	22.10 ±1.04	32.41±0.05^{b}	1.70 ± 0.37^{b}	90.00 ± 0.00^{b}

Values represent the means ± standard deviation of the samples with different superscript letters are significantly different from each other ($p < 0.05$). Means without letters do not differ substantially. Control: unenriched *Artemia* nauplii, E1: *Artemia* nauplii enriched with 5×10^7 cfu cells, E2: *Artemia* nauplii enriched with 5×10^9 cfu cells and E3: *Artemia* nauplii enriched with 5×10^{11} cfu cells and SGR: specific growth rate.

Biochemical parameters of shrimp PL

The results obtained with biochemical constituents such as protein, lipid and carbohydrate in *Litopenaeus vannamei* postlarvae enriched *Artemia* nauplii with various concentration of probiotics are given in **Figure 2A**. Protein content in postlarvae of E1 (5×10^7 cfu), E2 (5×10^9 cfu) and E3 (5×10^{11} cfu) of probiotic were observed to be 57.40 mg/g, 63.40 mg/g and 56.30 mg/g respectively. The results showed a moderate difference in enriched groups compared to that of control (55.40 mg/g). The total lipid content of post larvae in experimental groups such as E1, E2 and E3 were observed to be 9.20, 11.50 and 8.09 mg/g respectively. However, lipid content was moderately increased in the E2 group than that of the control and other experimental group. The carbohydrate content in postlarvae fed with enriched *Artemia* in E1, E2 and E3 concentrations was recorded as 12.78, 16.32 and 12mg/g. However, the carbohydrate content was less in the unenriched group. Maximum carbohydrate content was recorded in E2 (5×10^9 cfu) of enriched *Artemia* fed to postlarvae followed by the other concentration, such as E1 (5×10^7cfu) and E3 (5×10^{11} cfu) compared to unenriched *Artemia* fed postlarvae group. Among all groups, the E2 is statistically significant group $p<0.05$.

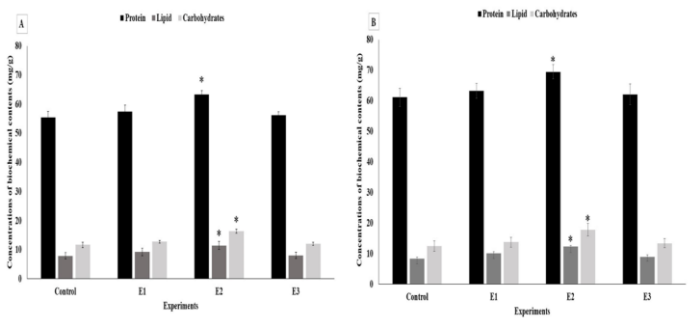

Figure 2 Graph showing biochemical constituents of *L.vannamei* post-larvae fed only with enriched *Artemia* nauplii (A) and probiotic supplemented feed (B) along with various concentration of *B.megaterium*

A- Control: unenriched *Artemia* nauplii, E1: *Artemia* nauplii enriched with 5×10^7 cfu cells, E2: *Artemia* nauplii enriched with 5×10^9 cfu cells and E3: *Artemia* nauplii enriched with 5×10^{11} cfu cells.

B- Control: pellet feed without probiotic, E1: probiotic supplemented feed of 5×10^7 cfu, E2: probiotic supplemented feed of 5×10^9 cfu and E3: probiotic supplemented feed of 5×10^{11} cfu cells.

After 15 days of feeding experiments, the results obtained with biochemical constituents of *Litopenaeus vannamei* postlarvae fed with probiotic supplemented feed are shown in **Figure 2B**. Protein content in *L. vannamei* PL of E1, E2 and E3 of probiotic supplemented feed were observed as 63.21, 69.46 and 62.12 mg respectively. Protein content among the probiotic supplemented feed groups showed moderate difference compared to that of control. The total lipid content of *L. vannamei* in experimental groups such as E1, E2 and E3 were observed as 10.13, 12.35 and 9.03 mg respectively. The variation present in the experimental groups is statistically significant ($p < 0.05$). However, lipid content was moderately increased in the E2 group compared to that of control and other experimental groups. The carbohydrate content of *L. vannamei* PL fed with probiotic supplemented feed sprayed with probiotic of different concentration of E1, E2 and E3 was recorded as 13.81, 17.84 and 13.41 mg respectively. However, the carbohydrate content in control was 12.50 mg compared to that of probiotic supplemented feed. In the biochemical analysis, the protein content in the probiotic supplemented feed is statistically significant when compared to the other two biochemical content of the same experiment. The changes in the biochemical levels in postlarvae fed with enriched *Artemia* and probiotic-supplemented feed were moderately increased in the E2 group compared to that of control group and other experimental group.

Challenge study

After 30 days, the 10-day challenge test against *V. harveyi* was undertaken **(Figure 3A).** The relative percent of survival (RPS) in the E2 group considerably outperformed the control ($p < 0.05$). No significant difference in survival rates was observed among control, E1 and E3 groups when PL were challenged with *V. harveyi* ($p > 0.05$). The survival rate of shrimp's PL against *V.harveyi* from 10 hours challenging study was conducted after 15 days **(Figure 3B)**. The probiotic-supplemented feed showed a higher level of survival ($p>0.05$) than the group fed the control feed after 10 hours of the experimental challenge with *V. harveyi*. No significant difference in survival rates was observed between E1and E3 groups.

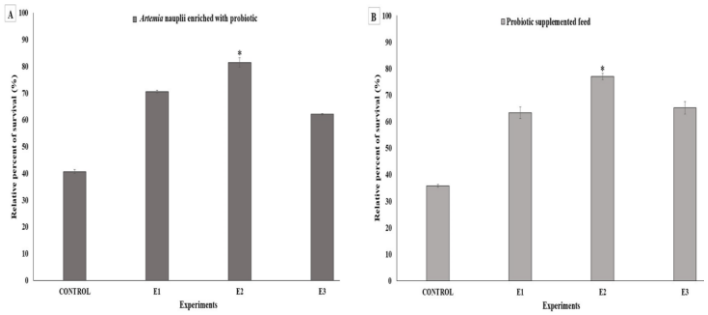

Figure 3 Challenging study of shrimp *L.vannamei* PL fed with enriched *Artemia* nauplii (A) and probiotic supplemented feed (B) with various concentration of *B.megaterium* against *V.harveyi*

A- Control: unenriched *Artemia* nauplii, E1: *Artemia* nauplii enriched with 5×10^7 cfu cells, E2: *Artemia* nauplii enriched with 5×10^9 cfu cells and E3: *Artemia* nauplii enriched with 5×10^{11} cfu cells.

B- Control: pellet feed without probiotic, E1: probiotic supplemented feed of 5×10^7 cfu cells, E2: probiotic supplemented feed of 5×10^9 cfu cells and E3: probiotic supplemented feed of 5×10^{11} cfu cells.

DISCUSSION

Probiotics were used as a growth regulator for aquaculture in order to improve the health of host against diseases. Many studies have shown the advantages of probiotics for aquatic animals, including how they can promote development, improve feed digestion, boost immunological responses, and regulate water quality **(Balcazar *et al*., 2006; Suzer *et al*., 2008)**. In this present study we determined the growth, survival and biochemical of Pacific white shrimp *L.vannamei* PL fed with various ranges of *B.megaterium.*

Probiotics are increasingly being given to aquaculture animals in live form. Minimizing health issues in the host animal, boosting their immunity, and lowering the pathogen in aquaculture are all regarded as beneficial practises. Additionally, instar-II unenriched *Artemia* nauplii have lower energy levels due to nutrient deficiencies. *B.megaterium* supplementation replenishes the energy and nutrients necessary for PL in experimental groups **(Visudtiphole *et al*., 2018)**. Additionally, enriching *Artemia* with other high-HUFA substances, including fish oil, has been demonstrated to improve shrimp development performance **(Immanuel, 2001)**.

According to the results of the current study, varying concentrations of *Bacillus megaterium* have positive impacts on PL growth as measured by length, weight, SGR, and survival of enrichment groups. **Arulvasu *et al*., (2012)** had previously showns that specific growth and survival rate was improved by feeding enriched *Artemia* nauplii to fish fry *Poecilia sphenops.* Analyzing the growth results of experimental groups, *B.megaterium* restored growth of PL fed with enriched *Artemia* was higher than that of the non-enriched group. According to earlier research, crustaceans provided probiotic-treated feed grew more quickly than those supplied untreated feed **(Ziaei-Nejad *et al*., 2006)**. In this study, different concentration of probiotic *B.megaterium* enriched with *Artemia* nauplii administered to post larvae *L.vannamei* led to a significant rise (P<0.05) of length, weight, SGR and survival rates. Comparable results have been revealed in *M. rosenbergii* fed with bio-encapsulated *Lactobacillus cremoris, L. sporogenes* and *L. acidophilus* **(Venkat *et al*., 2004)**.

The current study demonstrates that shrimp PL fed with B. megaterium enriched feed saw increased levels of growth and survival, with improved growth performances seen compared to controls. Similar outcomes were shown in shrimp fed diets containing *B. subtilis* L10 and G1, which had greater survival rates of 100 and 95.5%, respectively, than the control, which had a survival rate of 86.5% (**Zokaeifar *et al*., 2012)**. In the current study, post larvae survival significantly increased, which may be attributable to *Bacillus*' capacity to outcompete other hazardous bacteria. In this study, E1, E2, and E3 supplemented groups outperformed controls in terms of growth and survival rate. Similar outcomes were attained when *Tilapia Oreochromis niloticus* was supplemented with the probiotic *Bacillus coagulans* **(Wang *et al*., 2008)**.

The biochemical composition in post larvae *L. vannamei* fed with *Artemia* nauplii enriched with *B.megaterium* of three different concentration shows maximum level of protein in all experimental groups than control. The results suggest that E2 group shows significant changes when comparing with latter. Similar to this, **Saad *et al.* (2009**) revealed that *M. rosenbergii* PL's biochemical proximate composition was greatly improved by the commercial probiotic. The findings of the current study also demonstrated that L. vannamei's body composition is dramatically impacted by the administration of probiotic *B. megaterium* supplemented feed. Based on our findings, whiteleg shrimp *L.vannamei* post larvae fed dietary probiotic *Bacillus* had higher level of protein, lipid and carbohydrate content.

A variety of infections can be inhibited by probiotics. In order to research the suppression of Vibrio strains, **Decamp *et al*., (2008)** employed a commercial probiotic product that is a combination of particular *Bacillus* strains; the results revealed that probiotics were able to prevent the growth of *Vibrio*, improving shrimp larvae survival rates. A similar outcome was obtained when B. subtilis inhibited Vibrio harveyi and *Vibrio damsela* **(Vaseeharan and Ramasamy, 2003)**. Similar results obtained when *Vibrio harveyi* pathogen challenged against the post larvae *Litopenaeus vannamei* fed with *Artemia* nauplii enriched with three different probiotic concentrations such as E1, E2 and E3 significantly improved the immunity and survival. Additionally, when added to diets, certain *Bacillus* species have been observed to boost shrimp growth **(Dakar and Goher, 2004)**.

The stress tolerance, survival rate, immunological responses, and disease resistance of aquatic animals were all improved by the addition of *Bacillus* probiotics. Additionally, *B. licheniformis* supplementation increased resistance to *V. parahaemolyticus* infection (Gao et al., 2018). **(Gao *et al*., 2018)**. Similar results obtained when *V. harveyi* injected to the post larvae *L.vannamei* fed with probiotic supplemented feed showed a significantly higher level (*P*<0.05) of survival rate than control group. Higher survival of shrimp fed probiotic-supplemented feed may be attributable to the host's immunological response to the probiotics. In our study, bioencapsulation and supplementation of a *B.megaterium* was shown to have positive effects on growth indices of *L.vannamei* PL. Among the experimental groups, PL fed with probiotic in E2 had improved weight, SGR and survival. These results are consistent with the specific *B. megaterium* concentration in shrimp PL, which led to a better survival rate following *V. harveyi* challenge.

CONCLUSION

The potential probiotic *Bacillus megaterium* enriched *Artemia* nauplii and supplemented diet enhance the growth, biochemical composition and disease resistance of *L.vannamei* postlarvae. Therefore, our study suggests that *Bacillus megaterium* can be utilised as a feed enhancer for enriching both live and supplemented diets in order to increase white shrimp, *Litopeneaus vannamei* postlarvae growth and production.

Acknowledgments: The authors acknowledge the infrastructure support offered in the department established under the DST-FIST program.

REFERENCES

Abdollahi-Arpanahi, D., Soltani, E., Jafaryan, H., Soltani, M., Naderi-Samani, M., & Campa-Córdova, A. I. (2018). Efficacy of two commercial and indigenous probiotics, *Bacillus subtilis* and *Bacillus licheniformis* on growth performance, immune-physiology and resistance response of juvenile white shrimp (*Litopenaeus vannamei*). *Aquaculture*, *496*(1), 43-49. http://dx.doi.org/10.1016/j.aquaculture.2018.06.082

Arulvasu, C., & Munuswamy, N. (2009). Survival, growth and composition of *Poecilia latipinna* fry fed enriched *Artemia* nauplii. *Current Science*, 114-119.

Arulvasu, C., Shobana, S., Banu, H. A. A., Chandhirasekar, D., & Prabhu, D. (2012). Bioencapsulation of *Artemia* nauplii with herbal extract for promoting growth of fish fry *Poecilia sphenops* val. *Journal of Modern Biotechnology*, *1*, 37-44.

Balcázar, J. L., De Blas, I., Ruiz-Zarzula, I., Cunningham, D., Vendrell, D., & Muzquiz, J. L. (2006). The role of probiotics in aquaculture. *Veterinary microbiology*, *114*(3-4), 173-186. http://dx.doi.org/10.1016/j.vetmic.2006.01.009

Barnes, H., & Blackstock, J. (1973). Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanilun method for 'total' lipids. *Journal of Experimental Marine Biology and Ecology*, *12*(1), 103-118. http://dx.doi.org/10.1016/0022-0981(73)90040-3

Bowyer, P.H., El-Haroun, E.R., Hassan, M., Salim, H., & Davies, S.J. (2019). Dietary nucleotides enhance growth performance, feed efficiency and intestinal functional topography in European Seabass (*Dicentrarchus labrax*). *Aquaculture Research*,*50*(7),1921-1930. http://dx.doi.org/10.1111/are.14078

Bradford, M.M., (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Das, S., Ward, L.R., & Burke, C. (2008). Prospects of using marine actinobacteria as probiotics in aquaculture. *Applied microbiology and biotechnology*, *81*(3), 419-429. http://dx.doi.org/10.1007/s00253-008-1731-8

Decamp, O., Moriarty, D. J., & Lavens, P. (2008). Probiotics for shrimp larviculture: Review of field data from Asia and Latin America. *Aquaculture Research*, *39*, 334–338. http://dx.doi.org/10.1111/j.1365-2109. 2007.01664.x

Edwards, P., Zhang, W., Belton, & Little, D. C. (2019). Misunderstandinds, myths and mantras in aquaculture: its contribution to world food supplies has been systematically overreported. *MarinePolicy*, *106*,103547. http://dx.doi.org/10.1016/j.marpol.2019.103547

El-Dakar, A. Y., & Goher, T. M. (2004). Using of *Bacillus subtilus* in microparticulate diets for producing biosecure of *Penaeus japonicus* postlarvae. Agriculture Science Mansoura University, *29*, 6855-6873.

FAO. (2016). Contributing to food security and nutrition for all. *The State of the World Fisheries and Aquaculture*. http://dx.doi.org/92-5105177-1

FAO. (2018). The State of World Fisheries and Aquaculture 2018 – Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO; 2018. http://dx.doi.org/10.18356/8d6ea4b6-en

Gao, X., Zhang, M., Li, X., Han, Y., Wu, F., & Liu, Y. (2018). Effects of a probiotic (*Bacillus licheniformis*) on the growth, immunity and disease resistance of *Haliotis discus hannai Ino*. *Fish & shellfishimmunology*, *76*, *143-152.* http://dx.doi.org/10.1016/jfsi.2018.02.028

Hoseinifar, S. H., Sun, Y.Z., & Caipang, C. M. (2017). Short-chain fatty acids as feed supplements for sustainable aquaculture: An updated view. *Aquaculture Research*, *48*(4), 1380-1391. http://dx.doi.org/10.1111/are.13239

Immanuel, G. (2001). Effects of feeding lipid enriched *Artemia* nauplii on survival, growth, fatty acids and stress resistance of postlarvae *Penaeus indicus. Asian Fish. Sci.*, *14*, 377-388.

Keysami, M. A., Mohammadpour, M., & Saad, C. R. (2012). Probiotic activity of *Bacillus* subtilis in juvenile freshwater prawn, *Macrobrachium rosenbergii* (de Man) at different methods of administration to the feed. *Aquaculture international*, 20(3), 499-511. http://dx.doi.org/10.1007/s10499-011-9481-5

Lavilla-Pitogo, C. R., Baticados, M. C. L., Cruz-Lacierda, E. R., & Leobert, D. (1990). Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture*, *91*(1-2), 1-13. http://dx.doi.org/10.1016/0044-8486(90)90173-K

Martinez-Porchas, M., & Martinez-Cordova, L. R. (2012). World aquaculture: environmental impacts and troubleshooting alternatives. *The Scientific World Journal*, *2012*. http://dx.doi.org/10.1100/2012/389623

Murillo-Gurrea, D. P., Coloso, R. M., Borlongan, I. G., & Serrano, A. E. (2001). Lysine and arginine requirements of juvenile Asian sea bass *Lates calcarifer*. *Journal of Applied Ichthyology*, *17*(2), 49-53.

Nimrat, S., Boonthai, T., & Vuthiphandchai, V. (2011). Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (*Litopenaeus vannamei*) larvae and postlarvae. *Animal feed science and technology*, *169*(3-4), 244-258. http://dx.doi.org/10.1016/j.anifeedsci.2011.07.003

Roe, J.H. (1955). The determination of sugar and blood and spinal fluid with anthrone reagent. *Journal of Biological chemistry*, 212, 335-343.

Saad, A. S., Habashy, M. M., & Sharshar, M. K. (2009). Growth response of the freshwater prawn, *Macrobrachium rosenbergii* (De Man), to diets having different levels of Biogen®. *World Applied Sciences Journal*, *6*(4), 550-556.

Sorgeloos, P., & Kulasekarapandian, S. (1984). Production and use of *Artemia* in aquaculture. *CMFRI special publication*, *15*, 1-73.

Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W., & Versichele, D. (1986). Manual for the culture and use of brine shrimp *Artemia* in aquaculture.

Sritunyalucksana, K., Gangnonngiw, W., Archakunakorn, S., Fegan, D., & Flegel, T. W. (2005). Bacterial clearance rate and a new differential haemocyte staining method to assess immunostimulant activity in shrimp. *Diseases of aquatic organisms*, *63*(1), 89-94.

http://dx.doi.org/10.3354/dao063089
Suzer, C., Çoban, D., Kamaci, H. O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., & Küçüksari, H. (2008). *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata, L.*) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture*, *280*(1-4), 140-145.
Tripathi, M. K., & Giri, S. K. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. Journal of functional foods, 9, 225-241. http://dx.doi.org/10.1016/j.jff.2014.04.030
Vaseeharan, B. A. R. P., & Ramasamy, P. (2003). Control of pathogenic *Vibrio* spp. by *Bacillus subtilis* BT23, a possible probiotic treatment for black tiger shrimp *Penaeus monodon*. *Letters in applied microbiology*, *38*(2), 83-87.
http://dx.doi.org/10.1046/j.1472-765X.2003.01255.x
Venkat, H. K., Sahu, N. P., & Jain, K. K. (2004). Effect of feeding *Lactobacillus*-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man). *Aquaculture Research, 35(5), 501-507.*
http://dx.doi.org/10.1111/j.1365-2109.2004.01045.x
Visudtiphole, V., Phromson, M., Tala, S., Bunphimpapha, P., Raweeratanapong, T., Sittikankaew, K., .. & Unagul, P. (2018). *Aurantiochytrium limacinum* BCC52274 improves growth, hypo-salinity tolerance and swimming strength of *Penaeus vannamei* postlarvae. *Aquaculture*, *495*, 849-857. http://dx.doi.org/10.1016/j.aquaculture.2018.06.066
Wang, Y. B., Li, J. R., & Lin, J. (2008). Probiotics in aquaculture: challenges and outlook. *Aquaculture*, *281*(1-4), 1-4.
http://dx.doi.org/10.1016/j.aquaculture.2008.06.002
Zokaeifar, H., Balcázar, J. L., Saad, C. R., Kamarudin, M. S., Sijam, K., Arshad, A., & Nejat, N. (2012). Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish & shellfish immunology*, *33*(4), 683-689. http://dx.doi.org/10.1016/j.fsi.2012.05.027
Ziaei-Nejad, S., Rezaei, M. H., Takami, G. A., Lovett, D. L., Mirvaghefi, A. R., & Shakouri, M. (2006). The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. *Aquaculture*, *252*(2-4),516-524.
http://dx.doi.org/10.1016/j.aquaculture.2005.07.021