Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Journal of Fisheries and Aquatic Science

ISSN 1816-4927
DOI: 10.3923/jfas.2017.127.133

Research Article
Effect of Red Seaweed *Kappaphycus alvarezii* on Growth, Salinity Stress Tolerance and Vibriosis Resistance in Shrimp *Litopenaeus vannamei* Hatchery

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Abstract

Background and Objective: Several seaweeds have been reported to contain different bioactive compounds with antimicrobial activity, providing protection against certain infectious diseases in aquaculture production. This study aimed to explore the potential of red seaweed *Kappaphycus alvarezii* product as an alternative for anti-infective strategy and enhancement of salinity stress tolerance in shrimp (*Litopenaeus vannamei*) hatchery culture. **Materials and Methods:** Shrimp post-larvae were fed with *Artemia* nauplii, either enriched or not enriched with seaweed paste then challenged with *Vibrio* at day 5 and 8. Comparison of shrimp growth and survival between treatments following salinity stress test and *Vibrio* challenge were done using one-way analysis of variance analysis. **Results:** Shrimp fed with seaweed-enriched *Artemia* resulted in higher survival after *Vibrio* challenge (90.2±7.0%) compared to shrimp fed with non-enriched *Artemia* (77.7±3.1%). Shrimp fed with non-enriched *Artemia* resulted in lower growth after *Vibrio* challenge (9.65±0.20% b.wt., day^{-1}) compared to the non-challenged group (10.34±0.25% b.wt., day^{-1}). In contrast, there was no difference in the growth of shrimp fed with seaweed-enriched *Artemia* with or without *Vibrio* challenge (10.51±0.19 or 10.80±0.28% b.wt., day^{-1}, respectively). The shrimp fed with seaweed-enriched *Artemia* also obtained a higher survival following salinity stress test (94±2%) compared to shrimp fed with non-enriched *Artemia* (79±4%). **Conclusion:** Overall results suggested that red seaweed *K. alvarezii* by-product enrichment on live feed *Artemia* for shrimp post-larvae during the hatchery phase can provide protection against *V. harveyi* infection, improve the growth of shrimp when exposed to pathogenic *V. harveyi* and also allow higher salinity stress tolerance. Further evaluation on the effect of seaweed by-products dietary supplementation in the nursery and grow-out phases are undoubtedly required, to accurately evaluate the potential of seaweed by-product application as a growth and disease resistance promoting agent in those later phases as well.

Key words: *Kappaphycus alvarezii*, vibriosis, growth, survival, salinity stress, disease resistance, white shrimp, *Litopenaeus vannamei*, hatchery

Received: February 20, 2017 Accepted: March 21, 2017 Published: April 15, 2017

Citation: Gede Suantika, Magdalena Lenny Situmorang, Pingkan Aditiawati, Abdul Khakim, Shrikumar Suryanarayan, Sri Sailaja Nori, Sawan Kumar and Ferisca Putri, 2017. Effect of red seaweed *Kappaphycus alvarezii* on growth, salinity stress tolerance and vibriosis resistance in shrimp *Litopenaeus vannamei* hatchery. J. Fish. Aquat. Sci., 12: 127-133.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Aquaculture industry is one of the global fastest-growing food producing bio-industries with great contribution to the world economic growth. It is reported that in 2014, the world aquaculture production has reached 101.1 million tones (live weight), for an estimated value of US$ 165.8 billion for fish and plants in combination[1]. The farmed aquatic plants, mostly seaweed, contributed up to 27.3 million tones by volume and US$ 5.6 billion by value; meanwhile, the total volume of crustaceans farming of 8.3 million tones contributed up to US$ 35 billion by value[1]. After being the most-traded commodity in aquaculture industry for decades, shrimp currently ranks second in terms of value. Asia currently provides 90% of the global shrimp production, with Indonesia as the 2nd top producer following China[1].

The pacific white shrimp (*Litopenaeus vannamei*) has become an important cultured shrimp species and its proportional share in global shrimp production keeps increasing. The world farmed shrimp production volumes, however, was decreased particularly in 2013, mainly due to disease problems. Vibriosis, caused by luminous *Vibrio harveyi* is one of the main serious infectious diseases affected the shrimp culture[2]. Antibiotics have been overused for the pathogenic bacteria control, but their efficacy in general has become very poor due to the rapid development of antibiotic-resistant strains of the pathogens and the risks of the transfer of the resistance to human pathogens[3,4]. Therefore, alternatives to antibiotic treatments, for example immunostimulants, is of major concern in disease management for a more sustainable shrimp aquaculture production.

In recent years, several seaweed species have been extensively studied as immunostimulant agents due to their biocompounds/bioactive molecules. In Indonesia, up to 8.2 million tones of seaweeds are harvested in 2014 and used as human food, as well as in cosmetics, fertilizers and animal feed industry. The red seaweed *Kappaphycus alvarezii* is one of the main seaweed species produced, which contributed up to 90% of the seaweed total production, due to the high demand especially for its carrageenan. Bioactivity of the diverse seaweeds bioactive molecules plays an imperative role in diseases prevention with their antioxidant, antiviral and antimicrobial properties[5]. For example, it was reported that the polyphenol contained in *Kappaphycus* sp., may exhibit reducing power with hydroxyl radical scavenging activity higher than that of standard antioxidants[6]. The antibacterial activity of *K. alvarezii* was also reported against animal borne bacterial pathogens[7].

Several studies had reported that seaweeds contain different kinds of bioactive compounds with antimicrobial activity, including polyunsaturated fatty acids, polysaccharides, carotenoids and other phenolic compounds[8]. It has been shown that seaweeds polysaccharides can modify the activity of the immune system and provide protection against certain diseases in aquaculture production. For example, fucoidans (commonly found in brown seaweeds) had allowed a higher shrimp *Penaeus monodon* growth and survival upon infection by the white spot syndrome virus and also inhibited the growth of pathogenic *V. harveyi*[9]. In addition, the use of fucoidan as shrimp diet supplement was found to enhance the growth and survival of *P. monodon* culture against *V. harveyi* infection[10]. Similarly, Kitikiew *et al.*[11] also reported that fucoidan showed resistance against *V. alginolyticus* and provoked the innate immunity of white shrimp culture.

Following polysaccharide (agar/carrageenan/fucoidan) extractions, seaweed wastes/by-products may still contain some remaining polysaccharides and often still can be considered as good protein sources[12]. However, the produced seaweed by-products are usually not being used anymore and only allowed to pile up in the landfill. Utilization of these seaweed by-products as diet supplement and immunostimulant agent in shrimp production will give an added value in both seaweed and shrimp farming industry. Therefore, the general objective of this study was to explore the potential of red seaweed *K. alvarezii* by-product as an alternative as growth modulator and anti-infective strategy for white shrimp *L. vannamei* hatchery production. This study assessed the effect of seaweed by-product paste enrichment on the shrimp live feed *Artemia* sp., nauplii culture, on the survival, growth, vibriosis resistance and salinity stress resistance of white shrimp culture in the hatchery phase.

MATERIALS AND METHODS

Experimental setting: This study was conducted from December, 2015 to March, 2016. Pacific white shrimp post-larvae (PL) were produced and exclusively fed on the microalgae *Chaetoceros gracilis* (130-150 cells μL^{-1}). About 3,000 individuals of 5 days old post-larvae (PL5) shrimp were collected from commercial hatchery "Suri Tani Pemuka" in Indramayu, West Java, Indonesia and acclimated overnight to seawater of 33 ppt at 28°C in a 220 L (1.1×0.8×0.25 m) polyethylene tank connected to a biological filter. Before stocking, raw natural seawater was treated to reach 5-10 ppm chlorine concentration, 30 min post-treatment and neutralized using natrium thiosulphate. The PL were fed *Artemia* nauplii

(50 per shrimp). Following acclimation period, shrimp PL6 with the average body weight of 0.91±0.19 mg were distributed in 12 rectangular tanks of 90 L (200 PL per tank) with a semi-batch system supplied with dechlorinated, heated seawater. About 10% daily water renewal was done to maintain optimal water quality parameters.

Characteristics of seaweed by-product paste for shrimp feed supplementation: Seaweed paste (SIP), an aqueous extract of *K. alvarezii* was provided by Sea6 Energy Private Limited, Bangalore, India for experimentation. The SIP has 15.8±0.5% w/w solids and the solid matter comprises of 38.2±3.4% w/w sulphated polysaccharides (carrageenan) and 67.8±0.6% ash content.

Experimental diets and feeding regime during 12 days feeding-challenge period: In this study, during the 12 days of experimental period, shrimp PL were fed with *Artemia* nauplii, either enriched or not enriched with seaweed paste (0.5 g L^{-1} seaweed paste *Artemia* enrichment suspension). Bacteria challenge was done twice[13], at day 5 and 8. In total there were four experimental groups in this hatchery study, each tested in three replicates (Table 1).

For production of *Artemia* nauplii, commercial cysts of *Artemia franciscana* (EG® Type, INVE Aquaculture, Belgium) were decapsulated according to the protocol as described by Sorgeloos *et al.*[14]. The dry cysts were first soaked in tap water for 1 h and then decapsulated by reaction with sodium hypochlorite. Decapsulated cysts were harvested and washed with filtered sterilized seawater to remove residual bleach before transferred into 1 L sterile bottles containing 1 L filtered sterilized seawater for hatching at 28°C for 30 h under standardized hatching conditions[14].

Seaweed solution (filtered seawater mixed with seaweed paste) at the concentration of 0.5 g L^{-1} was prepared as the enrichment media for live feed *Artemia* nauplii[9]. Enrichment of *Artemia* nauplii were done by immersion of instar II nauplii (stocked at the density of 100 nauplii mL^{-1}) in enrichment media for 2 h in 1 L glass cone bottle supplied with gentle aeration to maintain the oxygen level and to keep uniform dispersion of the seaweed particles in the medium. After 2 h[14], the enriched (encapsulated) *Artemia* nauplii were sieved, washed carefully and counted prior to feeding to the shrimp post-larvae. *Artemia* nauplii instar II, with or without seaweed enrichment were fed to shrimp PL at the feeding level of 50 *Artemia* nauplii per PL in one equal meal given in the morning (10 am). Daily water renewal was done prior the first daily feeding.

Microorganism: Microorganism pathogenic *V. harveyi* were collected from Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, West Java, Indonesia. Confirmation of species identification was done through bacterial DNA extraction (using commercial Qiagen DNeasy blood and tissue kit), Polymerase Chain Reaction (PCR) amplification (using 27F/1492R primer) and bacterial rRNA 16S gene sequencing (using 785F/907R primer) at the Macrogen Inc., Korea. After two successive transfers of the strain in Luria-Bertani (LB) broth containing 33 ppt seawater at 25°C for 24 h, the activated culture was again inoculated into LB broth at 25°C for 24 h which served as the bacterial inoculum.

Bacterial challenge procedure: Bacterial challenge using *V. harveyi* was done twice on day 5 and 8 of experimental period[13]. Suspension of pathogenic *V. harveyi* culture was harvested by centrifuging at 1000×g for 10 min and washed twice in its culture medium followed by one time washing using shrimp culture water before addition. The density of the bacterial suspension prior to addition was determined based on the McFarland standard (BioMérieux, Marcy L'Etoile, France) by measuring the turbidity with a spectrophotometer (Genesys 20 Thermo spectronic) at 550 nm. For each replicate tank, bacteria were added at the concentration of 10^6 CFU mL^{-1} as suggested by Sivakumar *et al.*[15].

Evaluation of shrimp survival and growth after 12 days feeding-challenge period: Mortality observation and dead shrimp removal were done twice daily. At the end of the experimental period, the shrimp weight gain, Specific Growth Rate (SGR) and survival for individual treatments were calculated. Shrimp survival for individual treatments was determined as the number of surviving shrimp at the end of experimental period (day 30) relative to the number of shrimp at the beginning of the experimental period (day 0).

Table 1: Experimental set up of shrimp feeding and challenge test

Treatments	Diets	Challenge test
Seaweed-unchallenged	Enriched *Artemia* (0.5 g L^{-1})	Not challenged
Seaweed-challenged	Enriched *Artemia* (0.5 g L^{-1})	Challenged at day 5 and 8
Control (+)	Non-enriched *Artemia*	Not challenged
Control (-)	Non-enriched *Artemia*	Challenged at day 5 and 8

The average weight gain of the shrimp in a tank over the 12 days is calculated by subtracting the weight of the shrimp sampled on the final day of the experiment with the average weight of the shrimp as measured at the beginning of the experiment and then taking the average. The Specific Growth Rate (SGR)[16] is calculated as following Eq. 1:

$$\text{SGR (Body weight gain per day) (\%)} = \left[\frac{(\ln W - \ln W_0)}{t}\right] \times 100 \quad (1)$$

where, W is the average body weight after 12 days, W_0 is the average initial body weight and t is experimental period (12 days).

Evaluation of dynamics of shrimp MC profile during feeding-challenge period: Four shrimp PL from each replicate tank were randomly collected every 5 days during 12 days of feeding-challenge period to monitor the dynamics of shrimp Microbial Community (MC) profile. Shrimp were pooled, rinsed and homogenized in 9 g L^{-1} NaCl sterile saline solution. Subsequently, 50 µL of the homogenate was plated on marine agar plates. The inoculated plates were incubated at 27±1°C for 24 h and the total number of bacteria was counted. About 50 µL of the homogenate was also inoculated on Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates to count the total number of *Vibrio*.

Identification of bacteria was carried out using molecular approach. Bacterial DNA extraction (using commercial Qiagen DNeasy blood and tissue kit), Polymerase Chain Reaction (PCR) amplification (using 27F/1492R primer) and bacterial rRNA 16S gene sequencing (using 785F/907R primer) was done at the Macrogen Inc., Korea. Sequence homology search for the test sequences was done using Nucleotide Blast versus GenBank and Ribosomal Database Project (RDP) data. Results were validated with a phylogenetic analysis using the Winclada program and the Ratchet method (Island Hopper).

Salinity stress test: Following the bacterial challenge test, a salinity stress test was performed to the unchallenged shrimp PL to further determine their quality or physiological condition following feeding test with seaweed-enriched and non-enriched *Artemia* nauplii. Both groups were tested in the salinity stress test in triplicates. In this stress test, 50 shrimp was acclimatized in 10 L tanks containing seawater (33 ppt) at the temperature of 27±1°C for 24 h. Shrimp was then abruptly exposed to tap water (freshwater, 0 ppt) for 30 min, before put back into their culture tanks containing seawater (33 ppt). Shrimp survival was then determined every 30 min for 3 h.

Statistical analyses: Normalization of the distribution of the survival data was done using arcsin transformation. Comparison of the shrimp survival, final body weight and length, SGR and FCR were done using one-way analysis of variance (ANOVA) analysis. Grouping of treatments based on significant differences in mean values was done according to Duncan test (0.05 level of confidence). The SPSS statistical software was used for these statistical analyses.

RESULTS

Shrimp survival and growth following 12 days feeding-challenge period: Following 12 days of feeding-challenge period, shrimp fed with seaweed-enriched *Artemia* nauplii had a higher survival compared to the shrimp fed with non-enriched *Artemia* nauplii ($p<0.05$) (Table 2). When challenged with pathogenic *Vibrio harveyi*, shrimp fed with seaweed-enriched *Artemia* nauplii still had a higher survival compared to the challenged shrimp fed with non-enriched *Artemia* nauplii, even though the difference was not statistically significant ($p>0.05$). Shrimp fed with non-enriched *Artemia* nauplii and then challenged with *Vibrio* had the lowest growth among all treatment groups ($p<0.05$). After *Vibrio* challenge, shrimp fed with non-enriched *Artemia* nauplii resulted in a significantly lower growth compared to the non-challenged group ($p<0.05$). In contrast, *Vibrio* challenge did not have any negative effect on the growth of the shrimp fed with seaweed-enriched *Artemia* nauplii. Furthermore, the growth of the shrimp fed with seaweed-enriched *Artemia* nauplii after *Vibrio* challenge was significantly higher compared to the shrimp fed with non-enriched *Artemia* nauplii after *Vibrio* challenged ($p<0.05$) (Table 2).

Table 2: Shrimp survival and growth following 12 days challenge period with *V. harveyi*

Treatments				
Feeding	*Vibrio* challenge	Survival (%)	Final body weight (mg)	SGR (% b.wt., day^{-1})
Fed not enriched-*Artemia*	Not challenged	84.2±4.7[a]	6.7±0.4[a]	10.34±0.25[b]
Fed not enriched-*Artemia*	Challenged	77.7±3.1[a]	5.8±0.5[b]	9.65±0.20[c]
Fed enriched-*Artemia*	Not challenged	95.5±4.8[b]	7.0±0.5[a]	10.80±0.28[a]
Fed enriched-*Artemia*	Challenged	90.2±7.0[ab]	6.6±0.2[a]	10.51±0.19[ab]

[a-c] Different letter between rows indicates significant difference between treatments, SGR: Specific growth rate

Table 3: Bacterial load in shrimp during 12 days of feeding-challenge period

Treatments		Bacterial load (CFU mL^{-1})					
		TBC			*Vibrio* count		
Feeding	*Vibrio* challenge	Initial (day 0)	Middle (day 5)	Final (day 12)	Initial (day 0)	Middle (day 5)	Final (day 12)
Fed not enriched-*Artemia*	Not challenged	8.40×10^{4}	6.45×10^{5}	1.04×10^{7}	3.00×10^{3}	9.80×10^{4}	9.65×10^{5}
Fed not enriched-*Artemia*	Challenged	Na	9.15×10^{5}	1.76×10^{7}	Na	5.75×10^{4}	7.75×10^{6}
Fed enriched-*Artemia*	Not challenged	Na	1.31×10^{6}	7.75×10^{6}	Na	5.75×10^{4}	2.17×10^{6}
Fed enriched-*Artemia*	Challenged	Na	3.65×10^{5}	2.13×10^{7}	Na	1.02×10^{5}	1.64×10^{7}

CFU: Colony forming unit, TBC: Total bacteria count, Na: Not applicable

Table 4: Bacterial load in culture water during 12 days of feeding-challenge period

Treatments		Bacterial load (CFU mL^{-1})					
		TBC			*Vibrio* count		
Feeding	*Vibrio* challenge	Initial (day 0)	Middle (day 5)	Final (day 12)	Initial (day 0)	Middle (day 5)	Final (day 12)
Fed not enriched-*Artemia*	Not challenged	7.35×10^{5}	1.04×10^{6}	1.40×10^{7}	1.79×10^{3}	5.10×10^{3}	2.39×10^{4}
Fed not enriched-*Artemia*	Challenged	8.15×10^{4}	2.30×10^{5}	4.20×10^{6}	1.70×10^{2}	2.00×10^{3}	1.99×10^{4}
Fed enriched-*Artemia*	Not challenged	1.32×10^{5}	1.32×10^{6}	2.44×10^{6}	6.50×10^{1}	3.15×10^{3}	7.85×10^{3}
Fed enriched-*Artemia*	Challenged	5.30×10^{4}	1.23×10^{6}	1.38×10^{6}	3.42×10^{2}	3.65×10^{3}	1.87×10^{4}

CFU: Colony forming unit, TBC: Total bacteria count, Na: Not applicable

Table 5: Shrimp survival following salinity stress test after feeding with *Artemia* culture with and without seaweed enrichment

	Survival percentage (at minutes after stress test)					
Shrimp feeding treatment	30	60	90	120	150	180
Seaweed-enriched *Artemia*	99 ± 1^{a}	99 ± 1^{a}	97 ± 1^{a}	96 ± 2^{a}	95 ± 3^{a}	94 ± 2^{a}
Non-enriched *Artemia*	89 ± 10^{a}	83 ± 3^{b}	80 ± 4^{b}	79 ± 4^{b}	79 ± 4^{b}	79 ± 4^{b}

[a,b] Different letter between columns indicates significant difference between treatments

Microbial community in shrimp during feeding-challenge period: The *Vibrio* counts at the end of the feeding period were relatively similar in all six treatment groups, at a range of 9.65×10^{5} to 1.64×10^{7} CFU mL^{-1} and 7.85×10^{3} to 2.39×10^{4} CFU mL^{-1} in the shrimp and the culture water, respectively. The Total Bacteria Count (TBC) in the shrimp and culture water was in a range of 7.75×10^{6} to 2.13×10^{7} CFU mL^{-1} and 1.38×10^{6} to 1.40×10^{7} CFU mL^{-1} in the shrimp and the culture water, respectively (Table 3, 4). The results of the 16S rDNA gene sequencing analysis showed that the *Vibrio* sp., group was the dominant component in both the shrimp and culture water microflora during the feeding-challenge period. The rest of the bacteria groups existed at a low density, including *Pseudoalteromonas* sp. (closely related to *Pseudoalteromonas piscicida*) and *Alteromonas* sp., that were found in both the shrimp and culture water, *Pantoea* sp. (closely related to *Pantoea anthophila*) that was found only in the shrimp samples and also *Kocuria* sp., that were found only in the culture water samples.

Salinity stress test: In the first 30 min of incubation following the stress test, the shrimp group fed with seaweed-enriched *Artemia* nauplii already showed a higher survival of 99±1%, compared to the shrimp group fed with non-enriched *Artemia* nauplii with the survival of 89±10% ($p>0.05$) (Table 5). During the 3 h of incubation following the salinity stress test, the mortality in the shrimp group fed with non-enriched *Artemia* nauplii was increasing significantly. At the final observation (3 h after the salinity stress test), the shrimp group fed with seaweed-enriched *Artemia* nauplii obtained a significantly higher survival of 94±2%, compared to the shrimp fed with non-enriched *Artemia* nauplii with the survival of 79±4% ($p<0.05$) (Table 5).

DISCUSSION

Shrimp larviculture is still considered as a bottleneck for further industrialization of shrimp aquaculture. Several disadvantages are attributed to this production stage, including its production technique that still most widely uses

relatively static culture system with less attention to water quality and hygiene aspects. This condition often contributes to the unpredictability of both quantity and quality of the PL production (e.g., low survival and contaminated PL culture). To solve this problem, new approaches to increase the shrimp fitness and disease resistance needs to be seriously considered, to prevent and control the detrimental effects of the disease problems including vibriosis, starting from the early stage of shrimp larviculture. Among them, application of seaweed products with antimicrobial/immunomodulatory effect has an excellent potential.

In this study, seaweed by-product paste that was tested as a biocontrol agent through enrichment of the live feed *Artemia* nauplii supplied to the shrimp larvae did not resulted in any negative effect on PL growth in normal (unchallenged) condition. Interestingly, positive effects of seaweed by-product enrichment on PL growth were then observed following *Vibrio* challenge, where significantly higher shrimp growth was obtained in shrimp larvae fed with seaweed-enriched *Artemia* nauplii. This result suggested that the seaweed by-product as an enrichment agent can further improve the shrimp PL growth even in sub-optimum condition due to vibriosis, as similarly reported by several earlier studies[10,15,17-19].

In order to answer the basic needs of shrimp larviculture production in hatchery, it is important to evaluate the effect of seaweed by-product enrichment on PL survival during larviculture process. In this study, the seaweed by-product enrichment can allow a higher PL survival after *Vibrio* challenge, suggesting that seaweed dietary enrichment can provide such protection against *Vibrio* infection. Several studies had reported that seaweeds contain different kinds of bioactive compounds with antimicrobial activity, including polyunsaturated fatty acids, polysaccharides, carotenoids and other phenolic compounds[8]. Similar results in protection of shrimp from vibriosis due to seaweed *Ulva fasciata* utilization was reported by Sivakumar *et al*.[15], where the seaweed extract was found to have antagonism effect against the luminous vibriosis by *V. harveyi* during shrimp *P. monodon* larviculture, possibly by reducing the virulence factors produced by the pathogen, including protease and exopolysaccharide. In this study, however, both TBC or *Vibrio* count in the shrimp and culture water of the treatment group fed with seaweed-enriched *Artemia* nauplii were relatively similar to those of the control groups after the *Vibrio* challenge. Thus, it is suggested that the higher PL survival after *Vibrio* challenge obtained in the seaweed-enriched treatment groups may not be related to the bacterial load in the shrimp or the culture water. On the other hand, the antimicrobial seaweed substances could have affected the communication between the pathogenic *Vibrio*, rather than their growth. In addition to that, the protection against *Vibrio* infection that was provided to the seaweed-enriched treatment groups could have also been related to the physiological factors of seaweed supplementation affecting the shrimp.

The direct effect of seaweed supplementation on shrimp physiology can be indicated by the results of the salinity stress test. Salinity stress tests are commonly performed to estimate the PL quality or physiological condition on a short term, in relation to treatments including diet[20]. Shrimp PL are considered of better quality when reached the survival percentage of higher than 60% following salinity stress test. In this study, shrimp fed with seaweed-enriched *Artemia* nauplii prior to salinity stress test showed a higher salinity tolerance, indicated by the significantly higher survival following the salinity stress test, compared to the shrimp fed with non-enriched *Artemia* nauplii. Palacios and Racotta[20] suggested that higher survival to salinity stress test is correlated with higher energy reserve during the stress test. In this study, seaweed supplementation in the shrimp diet may have increased the digestive energy level of the diet, as suggested by Da Silva and Barbosa[21] and hence increased the energy reserve in the shrimp which could have played an important role in allowing the higher survival to salinity stress test in the group fed with *Artemia* nauplii with seaweed enrichment.

CONCLUSION

This study suggested that seaweed *K. alvarezii* by-product supplementation to the live feed *Artemia* culture fed to the shrimp at the hatchery phase not only can provide protection against *V. harveyi* infection, but can also improve the growth of the shrimp PL culture when exposed to the pathogen. Nevertheless, further evaluation on the effect of seaweed by-products dietary supplementation in the nursery and grow-out phases are undoubtedly required, to accurately evaluate the potential of seaweed by-product application as a growth and disease resistance promoting agent in those later phases as well. Furthermore, a detailed chemical characterization, identification and isolation of the seaweed bioactive compounds through bioassay-guided fractionation of the seaweed by-product still needs to be performed in order to increase the effectiveness and efficiency of the utilization of the seaweed *K. alvarezii* by-product as an alternative of a nutritional tool for PL white shrimp improvement against vibriosis and environmental stress in shrimp hatchery production.

SIGNIFICANCE STATEMENTS

Vibriosis, caused by luminous *Vibrio harveyi* is one of the main serious infectious diseases affected the shrimp culture and antibiotics have been overused to overcome the syndrome. This study provides an alternative approach to address the issue through the use of red seaweed product as an enrichment agent in the production of shrimp juvenile. In addition, the use of seaweed product can also improve the shrimp growth and survival. Furthermore, it can also improve the larvae capability to withstand a stressful culture condition during nursery phase.

ACKNOWLEDGMENT

We thank Sea6Energy Pvt. Ltd., Bangalore, India for the research funding and supply of red seaweed by-product paste for this study.

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