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Growth and immune response of pond-reared giant freshwater prawn *Macrobrachium rosenbergii* post larvae fed diets containing *Chlorella vulgaris*

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ABSTRACT

A 70-day feeding trial was conducted to evaluate the efficacy of *Chlorella vulgaris* supplementation in diets of giant freshwater prawn (*Macrobrachium rosenbergii*) post larvae. Resistance of the prawns against *Aeromonas hydrophila* and several immune parameters (total hemocyte count and prophenoloxidase activity) were also assessed in the study. Iso-nitrogenous, iso-lipidic, and iso-caloric prawn feeds were prepared utilizing a fishmeal based positive control diet (F0) and four dietary treatments with *C. vulgaris* inclusion levels of 2 (F2), 4 (F4), 6 (F6), and 8 (F8) %. Post larvae of *M. rosenbergii* were stocked randomly (mean initial body weight of 0.39 ± 0.38 g) in fifteen net cages (8.1 m × 8.9 m with an average depth of 1 m) in an earthen pond for the assessment of growth parameters. Overall growth indices for prawn fed *Chlorella* containing diets were enhanced in comparison to the control treatment. Prawn fed diets with *Chlorella* showed significantly higher ($P < 0.05$) prophenol oxidase activity, total hemocyte counts, and survival rates post bacterial infection. These findings demonstrated that inclusion of 4%, 6%, and 8% *C. vulgaris* in prawn diets provided optimal growth rates and improved immunity of the post larvae.

1. Introduction

Macrobrachium rosenbergii farming is known to be largest in Asia; however, significant producers of the prawn also include Israel, Africa, and South and Central America. Production is also evident for this tropical species in countries that at first seem questionable candidates for its cultivation. The rapid take-off of freshwater prawn farming in India and Bangladesh together with the huge production in China in the last few years resulted in a very rapid expansion of freshwater prawn farming since 1995 (Food and Agriculture Organization of the United Nations, Fisheries Global Information System (FAO-FIGIS, 2009). The yearly production of the giant freshwater prawn was observed to exceed 600,000 tons by the year 2020 where countries like the north-west India, Vietnam, Philippines, Papua New Guinea and Northern Australia are expected to be the main global distributors of the aquatic species because of their fast developing commercial culture methods (Banu & Christianus, 2016).

The culture of *M. rosenbergii* can be conducted in any freshwater body; however, marketable size of the prawn was observed to be easily attainable by rearing the species in their early developmental stages and when the culture system was restricted only to small-size earthen ponds of 200–500 m^2. In earthen ponds, cultured prawns attain marketable size of 30–60 g after five months of feeding with chicken pellets and some females are even egg-bearing upon harvest (Dejarme, 2005, pp. 18–19). Asian countries practice the use of earthen ponds in rearing the giant freshwater prawn and different strategies are utilized to optimize conditions to promote its growth and survival. Embedding plastic film on earthen ponds was shown to keep the warm temperature of the pond water during cold seasons, resulting to the enhanced growth rate of the prawns (Whangchai, Ungsethaphand, Chitmanat, Mengumphan, & Uraiwan, 2007). Moreover, feeding prawns with lower dietary protein in nursing ponds has provided comparable growth performance with prawns fed commercial feeds (Azam & Koroi, 2013). Post larvae of *M. rosenbergii* cultured in ponds also show higher preference to formulated diets as compared to prawns reared in indoor tanks, suggesting that the culture environment can influence their attractablity to the

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feeds (Azam & Koroi, 2013).

Macro invertebrates such as zooplanktons and oligochaetes are natural food sources eaten by freshwater prawns grown in ponds. Live zooplanktons are also consumed by juveniles larger than 2 g. Moreover, insect larvae and earthworms are recognized as natural food items for the prawns. This reliance on macro invertebrates as natural food for prawns presents a challenge in culturing prawns in ponds as it requires increased production of macro invertebrates as the prawn grows. Thus, development and use of good quality feeds is important for uniform production of large prawns (Mitra, Chattopadhyay, & Mukhopadhyay, 2005). Having knowledge about prawn nutritional requirements is considered an essential prerequisite for the formulation of a nutritionally adequate and balanced diet for *M. rosenbergii* post larvae. However, dietary information on effective post larval breeding of the freshwater prawn is still scarce (Kamarudin & Roustaian, 2002). Because of the lack of available prawn feeds, finding an alternative candidate to replace or partially replace conventional prawn feed in the form of fishmeal is necessary.

Another hindrance to the success of the prawn aquaculture industry is problems linked to epizootics (Sahoo et al., 2008). Different strategies are therefore necessary for prophylaxis and disease control. One of the promising techniques is to increase the immune state of the prawn through dietary inclusion of immunostimulants. Administration of immunostimulants through dietary inclusion enhances the growth performance, many innate immune responses, resistance to pathological and environmental stresses, and resistance to viral and bacterial infections in many prawn species (Wang, Chang, & Chen, 2013). Dietary immunostimulants offer a cheap and effective method for increasing the productivity and cost-efficiency of aquaculture operations by enhancing disease resistance and stress tolerance and reducing the need for more radical and costly disease control measures. Healthier shrimps are also more productive and efficient, leading to improved growth rates and feed utilization efficiencies.

Chlorella is one of the most commonly used microalgae in aquaculture due to its low-cost open pond technologies. Together with *Spirulina*sp., *Tetraselmis* sp. and *Chaetoceros* sp., *Chlorella* sp. has been used in aquaculture for nutritional purposes and in prawn culturing, as a direct feed or an ingredient normally incorporated in diets for different stages of the organism (Muller-Feuga, 2000; Sukri, Saad, Kamarudin, & Yasin, 2016). It is reported to contain high concentration of pigments, proteins, lipid, polysaccharides, minerals, vitamins, unknown *Chlorella* growth factors and other nutritional ingredients, which possess great bioactivity that is involved in many physiological responses of different organisms (Henry, 2013, pp. 10–17; Xu et al., 2014). It is utilized in rotifer production, contending with other types of dry feed in the market (Tredici, Biondi, Ponis, Rodolfi, & Zittelli, 2009). *Chlorella* together with *Spirulina.*is commonly integrated into feeds for ornamental fish, where the main market criteria are coloration and healthy appearance (Sergejevova & Masojidek, 2013; Zatkova et al., 2011). It is also reported to enhance the immune system. It is reported that *Chlorella* increased concentrations of immunoglobulins D and M, chemokine ligand-5, and interleukin-22, which regulated the adaptive and innate immunity, when the microalgae was used as an inclusive ingredient in diets of carp (Zhang et al., 2014). In addition, beta-1,3-glucan, which is blood lipid reducer, a scavenger of free-radicals, and an active immunostimulant has been reported to be present within cells of the microalgae. The substance was reported to enhance the immunity of *M. rosenbergii* challenged with the pathogen *A. hydrophila* (Sahoo et al., 2008; Spolaore, Cassan, Duran, & Isambert, 2006).

As published data is relatively few, the present study investigated the potential of the microalgae *Chlorella vulgaris* as a supplement for prawn diets and its ability to improve growth and promote the immunity of pond-reared giant freshwater prawn *M. rosenbergii*.

2. Materials and methods

2.1. Experimental diets

Five iso-nitrogenous, iso-caloric and iso-lipidic experimental diets were prepared in accordance with the nutritional requirements (protein, 35%–40%; lipid, 3%–7%; carbohydrate, 25%–35%) of *M. rosenbergii* post larvae (Mitra et al., 2005). Experimental feeds were computed to have 37% crude protein, 7% lipid and 28% carbohydrate (Table 1). *C. vulgaris* was incorporated in increments with the following percentage inclusions of 0% (F0), 2% (F2), 4% (F4), 6% (F6) and 8% (F8), respectively. F0 served as the control feed. Powdered *C. vulgaris* (Jarrow Formulas, Inc. Los Angeles, California, USA) and other basal ingredients (Southeast Asian Fisheries Development Center SEAFDEC, Aquaculture Department, Tigbauan, Iloilo) were mixed and pelletized with a diameter of 3 mm. The feeds were then cooked at 100 °C for 20 min. The cookes feeds were then finally stored inside the freezer at −25 °C until used (Maliwat, Velasquez, Robil, Chan, Traifalgar, Tayamen, & Ragaza, 2017).

2.2. Experimental animals and feeding trial

Post larvae of *M. rosenbergii* were stocked in fifteen net cages (8.1 m × 8.9 m with an average depth of 1 m), each with fifteen prawns, in an earthen culture pond in the Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (BFAR-NFFTC) in Muñoz City, Nueva Ecija. Diets were randomly assigned to the fifteen net cages. Feeding period was 70 days, from January to March 2015. Prawns were fed at 10% of their body weight. Sample weighing was done every fifteen days, where 30% of the remaining prawns from each net cage were weighed to adjust the amount of feeds to the body weight. Prawns were fed twice daily, at 09:00 and 15:00. Temperature ranged from 28 to 29 °C in the duration of the growth trial. Mortalities were recorded daily. After the 70-day feeding trial, the growth performance indices such as specific growth rate (SGR), survival rate (SR), and average weight gain (%AWG) were calculated.

Table 1
Proportion of ingredients in formulated feeds.

Ingredients (g per 100 g diet)	Experimental Diets				
	F0	F2	F4	F6	F8
Sardine fishmeal[a]	33	31	29	27	25
Chlorella vulgaris[b]	0	2	4	6	8
Corn oil[c]	3	3	3	3	3
Corn flour[d]	17	17	17	17	17
Vitamin and mineral premix[e]	2	2	2	2	2
Wheat bran[f]	15	15	15	15	15
Soybean meal[g]	30	30	30	30	30
TOTAL	100	100	100	100	100
Proximate composition (in % dry matter)					
'Crude protein	37.38	37.40	37.42	37.44	37.46
Crude lipid	6.76	6.63	6.50	6.37	6.24

[a] SEAFDEC, Tigbauan, Iloilo; protein, 65.65; lipid, 0.82 (% dry matter).
[b] Jarrow Formulas, Inc., Los Angeles, California; protein, 65; lipid, <1, carbohydrate 20 (% dry matter).
[c] ACH Food Companies Inc, Brooklyn City, New York region; lipid, 100 (% dry matter).
[d] Bob's Red Mill Natural Foods Inc., Pheasant City, Milwaukie; protein, 6.05; lipid, 3.03; carbohydrate; 69.7 (% dry matter).
[e] SEAFDEC, Tigbauan, Iloilo.
[f] Bob's Red Mill Natural Foods Inc., Pheasant City, Milwaukie; protein, 13.33; lipid, 3.33; carbohydrate, 60.67 (% dry matter).
[g] SEAFDEC, Tigbauan, Iloilo; protein, 44; lipid, 2 (% dry matter).

2.3. Extraction of hemolymph and total hemocyte counting

At the end of the 70-day feeding trial, hemolymph from the ventral sinus cavity of the prawns was collected as described by Bhavan, Kirubhanandhini, Muralinsankar, Manickam, and Srinivasan (2013) at a proportion of one part hemolymph to three parts of prepared anticoagulant solution, using a 1 mL tuberculin syringe (26 gauge) containing chilled (4 °C) anticoagulant solution (pH 7.3, 10 mmol/L KCl, 10 mmol/L EDTA-Na_2, 45 mmol/L NaCl, 10 mmol/L HEPES). Hemocyte counting was done using a hemocytometer (Cole-Parmer 79001–00, LW Scientific, Canada) with dispensed 30 μL of the hemolymph-anticoagulant solution and was viewed under the low power objective of a light microscope (CH20BIMF200, Olympus Optical Co. Ltd., Japan). The assessment of each immunological parameter was done by randomly collecting hemolymph from fifteen prawns of each dietary treatment. All assays were conducted in triplicate.

2.4. Prophenol oxidase activity

The analysis of prophenol oxidase activity was performed as described by Andrino, Serrano, and Corre (2012) with slight modifications. Serum preparation was done by collecting hemolymph from the ventral sinus cavity of the prawns in the absence of anticoagulant solution. The collected hemolymph was allowed to clot and subsequently subjected to a series of freeze and thaw cycle for 5 times to induce cell lysis. Afterwards, the hemolymph was subjected to centrifugation at 10, 000 × *g* for 10 min at 4 °C for the collection of the prawn serum (Shankar et al., 2012). A volume of 25 μL of trypsin in shrimp salt solution (pH 7.3, 45 mmol/L NaCl, 10 mmol/L HEPES, 10 mmol/L KCl) at 0.1 mg/mL was then added to 25 μL of the prepared serum and the solution was incubated for 3 min at 25 C with. Then, addition of 25 μL of L-3,4-dihydroxyphenylalanine at 3 mg/mL in cacodylate buffer was done and the solution was incubated again for another 10 min. By adding 800 μL of cacodylate buffer (0.45 mol/L NaCl, 0.01 mol/L sodium cacodylate, and 0.01 mol/L $CaCl_2$), the solution was diluted and finally, was subjected to spectrophotometry at an absorbance of 490 nm to measure the ProPO activity, using a U-5100 Hitachi (Tokyo, Japan) spectrophotometer.

2.5. Preparation of Aeromonas hydrophila

Pure culture of *A. hydrophila* (UPCC 1309) was purchased from the National Institute of Molecular Biology and Biotechnology – University of the Philippines Los Baños, Laguna. Sub-culture of the bacterium was prepared every week using trypticase soya agar, incubated at 30 °C for 24 h and stored at −20 °C to preserve the bacteria. Prior to challenge test, the bacteria was subjected to serial dilution to achieve an inoculum rate of 10^5 colony forming units/mL.

2.6. Challenge test

After the 70-day feeding trial, the challenge test was conducted by transferring 30 prawns from the hapa nets of each dietary group into separate aquaria. Injection of 50 μL of *A. hydrophila* inoculum at a rate of 10^5 colony forming units/mL was done intramuscularly between the second and third abdominal segments of the randomly selected prawns. Non-infected prawn group was also prepared by injecting 50 μL of phosphate-buffered saline solution into the muscles of the prawns from the control group and served as the control for the challenge test. Documentation of mortalities was done daily for 14 days and bacteria from the hepatopancreas of the succumbed prawn were reisolated to verify the infection of the pathogen (Shankar et al., 2012).

2.7. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Data were subjected to one-way analysis of variance (ANOVA) and to Tukey's multiple comparison test to check significant difference at $P \leq 0.05$. Microsoft Excel 2013 (Microsoft Office, WA, USA, 15.0.4551.1011) was used for statistical analysis (Velasquez et al., 2016).

3. Results

Significant differences ($P < 0.05$) were observed for the percent average weight gain and specific growth rate. Prawns fed F4 obtained the highest weight gain followed by the prawns fed F6, while the least weight gain was observed in prawns fed F0 (Table 2). Weight gain increased significantly with increasing inclusion of *Chlorella*. This significant increase in weight gain, however, halted as inclusion of *Chlorella* reached more than 4%. Prawns fed F6 demonstrated the highest value for specific growth rate while the lowest was observed in prawns fed F0. Moreover, higher specific growth rate was observed in prawns fed F2 to F8 as compared to prawns fed F0.

No significant difference ($P < 0.05$) was observed in the survival rate of prawns. Survival rates of prawns fed F4 showed highest numerical value while prawns fed F6 demonstrated the lowest value. A sharp decline in survival rate was observed as inclusion of *Chlorella* increased to more than 4%.

Significant difference ($P < 0.05$) was observed for prophenol oxidase activity and total hemocyte counts. Prawns fed F8 showed enhanced prophenol oxidase activity while prawns fed F0 exhibited the least activity (Table 3). An apparent increase in prophenol oxidase activity was seen with increased *Chlorella* inclusion. Highest value for total hemocyte count was observed in prawns fed F4 while prawns fed F0 displayed the lowest value. Moreover, total hemocyte count of prawn fed F2 to F8 were significantly increased when compared to prawn fed F0.

Significant difference ($P < 0.05$) was observed for the %survival rate of prawns after their challenge test. Prawns fed F8 showed highest survival rate while prawns fed F0 exhibited the lowest (Table 3). An apparent increase in % survival rate was seen with increased *Chlorella* inclusion. Moreover, % survival rate in prawn fed F4 to F8 were significantly increased when compared to prawn fed F0.

4. Discussion

The present study dealt with the evaluation of *C. vulgaris* as dietary protein source of *M. rosenbergii* post larvae. The results showed that prawns fed *Chlorella* diets at levels of 4, 6 and 8% exhibited significantly enhanced growth performance. This is in agreement with previous studies on fishes like the gibel carp (*Carassius auratus gibelio*), which when fed with *Chlorella* incorporated diets at inclusion levels up to 1.2%, showed a significant increase in growth parameters (Xu et al., 2014). Higher growth rates were also manifested by *Artemia parthenogenetica* fed *Chlorella* combined with rice bran feed (Govindasamy, Srinivasan, Selvam, Ananatharaj, & Krishnaveni, 2012). Improved growth parameters were also evident in shrimps when *Chlorella* is supplemented in their diets like in the case of *Litopenaeus vannamei*, which displayed significantly higher growth rates when diet was supplemented with 97.2 g/kg of the microalgae (Pakravan, Akbarzadeh, Sajjadi,

Table 2
Growth performance of pond-reared post larvae of *M. rosenbergii* fed diets with increasing levels of *C. vulgaris*.

Parameter	F0	F2	F4	F6	F8
%AWG	322.86 ± 99.43 [a]	788.44 ± 54.58 [b]	1382.26 ± 239.43 [c]	1361.41 ± 47.72 [c]	1378.611 ± 203.13 [c]
SGR	0.83 ± 0.16 [a]	1.32 ± 0.04 [b]	1.61 ± 0.11 [b,c]	1.62 ± 0.02 [c]	1.61 ± 0.08 [c]
%SR	84.44 ± 2.22 [a]	77.78 ± 12.37 [a]	86.67 ± 3.85 [a]	44.44 ± 15.56 [a]	60 ± 20.37 [a]

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ($P < 0.05$).

Table 3
Prophenol oxidase activity, total hemocyte count, and % survival (after challenge test) of pond-reared post larvae of *M. rosenbergii* fed diets with increasing levels of *C. vulgaris*.

Parameter	F0	F2	F4	F6	F8
PO activity (OD_{490})	0.41 ± 0.03 [a]	0.47 ± 0.03 [a,b]	0.55 ± 0.03 [b]	0.53 ± 0.01 [b]	0.57 ± 0.01 [b]
THC × 10^5/mL	3.37 ± 0.14 [a]	6.49 ± 0.43 [b]	7.48 ± 0.45 [b]	6.40 ± 0.61 [b]	6.53 ± 0.47 [b]
Challenge Test (%Survival)	43.33 ± 15.28 [a]	63.33 ± 11.54 [a,c]	83.33 ± 5.77 [b]	80 ± 10 [b,c]	86.67 ± 5.77 [b]

Values are means of triplicate groups ± SEM. Means along a row with different letters are significantly different ($P < 0.05$).

Hajimoradlo, & Noori, 2017). Moreover, its efficacy in promoting growth in *M. rosenbergii* was also evaluated by several studies and their results showed that inclusion of *Chlorella* significantly affected different growth parameters of the prawn. In one study, prawn fed diet containing 10% *Chlorella* exhibited significantly higher growth rates than prawn fed control diet (Sukri, Saad, Kamarudin M, & Yasin, 2016). Significant improvement in growth parameters were also displayed by the *M. rosenbergii* when fed with diet containing 50% *Chlorella* when compared to control group (Radhakrishnan, Bhavan, Seenivasan, & Muralisankar, 2016).

The positive growth performance may be attributed to the bioactive ingredients naturally occurring in *Chlorella*, such as *Chlorella* growth factors and considerable amounts of macronutrients such as protein and lipids, which promote growth of fishes and other aquatic species (Badwy, Ibrahim, & Zeinhom, 2008; Yamaguchi, 1997). Moreover, the high digestibility and high concentration of active growth promoters present within the Chlorella cells are two of the contributing factors leading to the apparent growth enhancement of the prawn (Khani, Soltani, Shamsaie, Foroudi, & Ghaeni, 2017). Furthermore, higher inclusion of *Chlorella* in diets is utilized more effectively by *M. rosenbergii* post larvae compared to other aquatic organisms as shrimps are reported to be better in tolerating feeds devoid of fishmeal (Amaya, Davis, & Rouse, 2007).

High survival rates were observed in pond-reared *M. rosenbergii* post larvae, especially in prawns fed 2% and 4% *Chlorella.* This may be due to the optimal conditions present in earthen ponds such as favorable soil composition, topography, and weather – where rainfall can maintain water balance and retention (Anjel, Lara, Morales, De Gracia, & Suarez, 2010, p. 132). Formation of natural food and interaction of the prawns with other organisms may also be a factor that can enhance their growth, increase their capability to assimilate feeds efficiently, and promote high survival rates (Racoky & McGinty, 1989, pp. 1–4). A sharp decrease in survival rate however was evident when prawns were fed diets containing 6% and 8% *Chlorella*. This inconsistency of prawn survival observed in pond-reared *M. rosenbergii* post larvae may not be due to the diets, but to the increase in ammonia levels in the pond during the growth trial. Ammonia is one of the most common environmental stressors that is said to be widespread in farms for shrimp culture (Pakravan, Akbarzadeh, Sajjadi, Hajimoradlo, & Noori, 2017). Factors such as fish excretion, high protein content on feeds, and diffusion of organic matter produced by naturally thriving algae can contribute to the sudden increase in ammonia levels of culture ponds (Hargreaves & Tucker, 2004, pp. 1–3). This stressor may negatively affect crustacean immune system and can impede with the organism's metabolic performance, growth and moulting capacity (Verghese, Radhakrishnan, & Padhi, 2007). In addition, accumulation of ammonia in the pond may cause deleterious effects, such as poor feed conversion ratios and decreased disease resistance, which ultimately result in high mortality rates of cultured aquatic species (Hargreaves & Tucker, 2004, pp. 1–3).

There are limited studies regarding the dietary inclusion of microalgae as a functional ingredient or as an immunostimulant in prawns. *C. vulgaris*, similar to other algal species, is known to contain a significant number of polysaccharides, some of which are active immunostimulants that were reported to induce immunity in different animals (Huang, Zhou, & Zhang, 2006). Several studies have already proven its capacity to improve immune function such as in gibel carp (Zhang et al., 2014) and in *M. rosenbergii* (Maliwat et al., 2017). *Chlorella* extract was also reported to increase resistance of some aquatic species against bacterial infections. It has been reported that *Plecoglossus altivelis* had heightened resistance against *Vibrio anguillarium* (Nakagawa, Kumai, Nakagawa, & Kasahara; 1981) and zebrafish against *Edwardsiella tarda* infection when fed with *Chlorella* incorporated diets (Tello et al., 2017).

The present study revealed a significant increase in total hemocyte count and prophenol oxidase activity in prawns fed *Chlorella* as compared with prawns fed control diet. Enhanced immunity, post challenge test, was also evident from the prawns fed *Chlorella* diets, as reflected by their high survival rates. The higher survival rates is a result of the prawns' resistance against the pathogen *A. hydrophila*, which is an indication that immune function was activated by increasing levels of haemocytes and phenol oxidase (PO). Dietary administration of several algal species were reported to boost immune parameters in different shrimp species, as well as increase their resistance against several viral and bacterial infections. Inclusion of the dried brown algae *Sargassum filipendula* and *Undaria pinnatifida* have reportedly lower mortality rates as compared to control group after being challenged with white spot syndrome virus (Schleder et al., 2018). Red algae in the form of *Porphyra haitanensis* was also reported to improve hemocyte count, phenoloxidase activity, and reduce mortality rates of *L. vannamei* shrimps under WSSV challenge conditions (Niu et al., 2018). This enhanced immunity may be ascribed to the polysaccharides present inside the cells of different algal species. Some of these polysaccharides are known to function as pathogen associated molecular patterns (PAMPs), which are molecules that can induce innate immunity in shrimps. The recognition of PAMPs by the shrimp immune system results to the release of soluble molecules that are required for the instigation of the haemocyte mediated humoral response and cellular immune responses in invertebrates (Yudiati et al., 2016). The humoral responses include the prophenoloxidase (proPO) system that can induce melanization around invading pathogens, which can result to their degradation. On the other hand, encapsulation, apoptosis, nodule formation and phagocytosis are specific examples of cellular immune responses (Yudiati et al., 2016). Several polysaccharides are known to be present in *Chlorella* cells. Spolaore et al. (2006) reported the presence of a substance known as β-1,3 glucan within cells of *C. vulgaris* that function as a PAMP. The substance was already reported to increase phenol oxidase activity and immune response of *M. rosenbergii* against *A. hydrophila* (Sahoo et al., 2008). Moreover, the similar bioactive compound was reported to increase phenol oxidase activity of other shrimp species such as *Penaeus monodon* and *Penaeus vannamei* (Cheng et al., 1999; Solidum, Sanares, Andrino-Felarca, & Corre, 2016; Sritunyalucksana, Sithisarn, Withayachumnarnkul, & Flegel, 1999). Polysaccharide extracts from the microalgae *Chaetoceros mulleri* and the algae *Gracilaria verrucosa* were reported to increase hemocyte counts in shrimps (Maftuch & Risjani, 2012; Manilal, Sujith, Selvin, Seghsl, & Shakir, 2009). In addition to the polysaccharides, *Chlorella* was reported to contain bioactive compounds that can rescind antibacterial activities and bacterial growth such as polyunsaturated aldehydes, glycosides, terpenes and chlorophyll-a derivatives (Ahmad, Shariff, Yusoff, Goh, & Banerjee, 2020; Natrah, Bossier, Sorgeloos, Yusoff, & Defoirdt, 2013).

5. Conclusion

The results of the research indicated that it is possible to incorporate up to 8% of the microalgae *Chlorella vulgaris* in the diets of *Macrobrachium rosenbergii* post-larvae without producing detrimental effects on their growth performance and immunity when cultivated in pond setups.

CRediT authorship contribution statement

Gian Carlo F. Maliwat: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Validation. **Stephanie F. Velasquez:** Investigation, Resources, Formal analysis, Data curation, Validation. **Sheila Marie D. Buluran:** Investigation, Resources, Validation. **Melchor M. Tayamen:** Validation, Supervision, Project administration, Funding acquisition. **Janice A. Ragaza:** Conceptualization, Methodology, Resources, Writing - review & editing, Validation, Supervision, Project administration, Funding acquisition.

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