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Original article

Using of chitosan nanoparticles (CsNPs), Spirulina as a feed additives under intensive culture system for black tiger shrimp (*Penaeus monodon*)

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ABSTRACT

Study conducted to evaluate the impact of dietary incorporation of chitosan nanoparticles (CsNPs) biosynthesis and *Spirulina platensis* on the growth performance and body composition of intensive cultured fingerling of black tiger shrimp (*Penaeus monodon*) in addition to the effect of the diets on heavy metals concentrations in water of culture cages. Six diets were prepared to contain approximately 44% crude protein and 499 k cal./100 g diet and supplemented by *S. platensis* and chitosan as follow: T1: control, T2: 0.05% *S. platensis*, T3: 0.1% *S. platensis*, T4: 1 ml chitosan, T5: 1 ml chitosan and 0.05% *S. platensis* and T6: 1 ml chitosan and 0.1% *S. platensis*. Shrimp fingerlings (1.24 ± 0.01 g) were randomly distributed in 12 hapas at stocking density of 20 fingerlings for each. The hapas were divided into 6 replicate groups and placed in cages. Each group received a kind of the experimental diet twice daily at feeding rate of 5% shrimp biomass for 12 weeks. The results indicated that, the growth parameters; final body weight (FBW g), weight gain (WG g), specific growth rate (SGR%) and survival rate (SR%) were improved significantly all trials compared to the control group. The best values for these parameters were recorded in the group of T5 and T6 without significant differences between them. Shrimp body composition showed the highest protein and fat contents for the group of T5 and T6 with significant differences ($P < 0.05$) among all treatments. No significant differences were noted for the heavy metals concentrations between all treatments. In conclusion, the incorporation of chitosan nanoparticles (CsNPs) and *S. platensis* attained considerable improvement in growth performance of black tiger shrimp and further investigations are recommended to optimize the level of incorporation in the diets for shrimp species.

1. Introduction

Although there are over than 3,000 shrimp species worldwide, only 40 groups are commercially exploited (Whetsone et al., 2002). Shrimp farming is based on a sub-species, mainly selected from the *Penaeidae* family for their good reproductive and growth potential. The *P. monodon* is the second most cultured shrimp species in the world following the white leg shrimp (*Litopenaeus vannamei*) (FAO, 2010). The *P. monodon* species is naturally distributed in the Indo-Pacific region including the eastern coast of Africa, the Arabian Peninsula, south-east Asia, the Sea of Japan, and northern Australia (Holthuis, 1980).

Microalgae consist of high protein and essential amino acid when compared with other reference food proteins (Becker, 2007). For example *Spirulina platensis* has highly protein content (60–70%), and it has been receiving concern as an animal food source and it has also contain a good source of vitamins, minerals, essential fatty acids and antioxidant pigments such as carotenoids. Investigations have been conducted to use Spirulina as a diets supplementation for fish and crustaceans (Jaime-Ceballos et al., 2007; Silva-Neto et al., 2012) and also as a partial substitution for fishmeal in tilapia (Abdel-Warith and Elsayed, 2019), carp and sturgeon (Nandeesha et al., 2001; Palmegiano et al., 2008). In addition, Many researchers (Richmond, 2004; Abdel-Warith and

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Elsayed, 2019) have investigated the microalgae utilization in aquaculture diets. Zooplankters that are commonly used include rotifers (Brachionus *sp.*), copepods (Tigriopus *sp.*), cladocerans (Daphnia *sp.*, Moina *sp.*), and brine shrimp (Artemia *sp.*) (De Pauw and Persoone, 1992). Therefore, diets containing algae may be influenced on growth and the rate of survival (Cohen et al., 1993).

Nanotechnologies have wide range applications in the fishery industry, such as water treatment, sterilization, nano-feed for feeding the fish, and controlling of aquatic diseases. It is thought that nanoparticles of some elements like selenium and iron supplemented in diet could enhance growth of fish and crustacean (Can et al., 2011). Therefore, nanotechnologies can be practically use to create fish ponds that are safe from disease and pollution in addition to providing for the fast growth of fish and Crustaceans including crab, lobster, shrimp, or barnacle (Can et al., 2011). This study aimed to evaluate the effect of chitosan nanoparticles (CsNPs) biosynthesis and *S. platensis* in diets on growth performance and chemical composition of intensive cultured fingerlings black tiger shrimp, *Penaeus monodon* in addition its effect on heavy metals concentration in the aquariums

2. Materials and methods

2.1. Experimental design

Fingerling of *Penaeus monodon* were obtained from a private farm in Burullus, and then transported to the cages in plastic bags filled with water and oxygen. Twelve hapas ($1 \times 2.5 \times 1$ m^3) were placed in cages and divided into six replicate groups (T1 to T6). Twenty fingerlings (1.24 ± 0.01 g) of the black tiger shrimp *P. monodon* were stocked in each hapa (15 fingerlings/m^3). The shrimp fingerlings were fed on sinking pellets diet (2 mm) at feeding rate of 5% of total biomass twice daily for six days per week. Every two weeks, shrimp fingerlings of all groups were weighed and the amount of feed was adjusted according to the changes in body weight throughout the experimental period. The experiments lasted for 12 weeks and at the end of the experimental period five of the *P. monodon* from each hapa were collected. The chemical composition of their whole body was assessed using the methods described by AOAC (2016).

2.2. Diet formulation

The *Spirulina platensis* meal was collected from the algae unit at the National Research Center chitosan (CS) and sodium tri polyphosphate (STPP) was used as a crosslinking agent both were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan was used as a carrier and STPP was used as a crosslinking agent. Chitosan nanoparticles (CsNPs) were prepared by using the ionic gelation method according to Masarudin et al. (2015). Six diets were prepared (Table 1) to provide 44% protein and 499 kcal/100 g diet and supplemented by *S. platensis* and chitosan as follows:

T1: control, T2: 0.05% *S. platensis*, T3: 0.1% *S. platensis*, T4: 1 ml chitosan nanoparticles.

T5: 1 ml chitosan nanoparticles and 0.05% *S. platensis*, T6: 1 ml chitosan nanoparticles and 0.1% S. platensis.

Dietary ingredients were mixed in a food mixer (Legacy, USA) with water (at around 50 °C) to produce a 2 mm pellet. The moist pellets were then oven dried at 105 °C and stored frozen at −20 °C until use. The diets were analyzed for determining their chemical composition (Table 1) according to the methods as described by AOAC (2016).

2.3. Assessment of water quality:

Water temperature was adjusted at the range and checked daily at 9 am. Dissolved oxygen (DO) was checked at 7 am by YSI model (YSI CO., Yellow Springs, Ohio, USA). Total ammonia, nitrate, and nitrite were assessed using a DREL, 2000 spectrophotometer (Hash CO., USA). pH values were estimated daily by Orion pH meter (Texas, USA). Generally, all measures of water quality (temperature, pH value, DO, etc.) were in suitable range for black tiger shrimp (Boyd, 1990).

2.4. Assessment of heavy metals in water of culture hapas

Water samples were collected weekly from various places in each treatment by a PVC tube column sampler at a half meter depth from the water surface. The samples of each treatment were mixed in a plastic bucket and then 1 L was kept in a polyethylene bottle, which was refrigerated and transported to the laboratory for determine the heavy metals concentrations (Fe, Cu, Pb, and Mn) ppm Metals were extracted with concentrated HCl and preserved in a refrigerator until subjected to analysis. The concentrations of the heavy metals as μg/l were assessed using Atomic Absorption Spectrophotometer with air-acetylene mixture as fuel (APHA. 1998).

2.5. Statistical analysis

Results were statistically analyzed by applying the computer program (SAS) ANOVA procedure Statistical Analysis System (SAS, 1996) and Dunncan's (1955). Multiple Range Test were utilized to look at variations between means. Impacts of treatment were viewed as huge at $P < 0.05$.

3. Results and discussion

The average values of initial body weight (IBW), final body weight (FBW), weight gain, specific growth rate (SGR%) and survival rate (SR%) (mean ± SE) of *P. monodon* are presented in Table 2. The results indicated no significant differences in the IBW while, significant differences ($P < 0.05$) were noted in the FBW of shrimp fed various experimental diets (T2–T6) except the average of FBW recorded in T5 and T6. The highest value of FBW (18.49 ± 0.2 g) was observed in shrimp fed on T6 (1 ml chitosan and 0.1% *S. platensis*) and the lowest FBW (12.62 ± 0.17 g) was shown in shrimp fed the control diet.

Data of the present study are in agreement with results obtained by Abdulrahman and Ameen (2014) who showed that, combined dried *S. platensis* had a potential impact on growth at an optimum level of 5 g/kg for white shrimp (*Litopenaeus vannamei*). The mean value of the group receiving 5.0 g/kg was highly significant difference for all the assessed parameters which indicating that the suitable amount in diet of chitosan nanoparticles using *S. platensis* for green tiger shrimp is 5.0 g/kg during the study period. Ibrahem et al. (2013) reported that feed additives with chitosan particles for chlorella enhanced the weight, length, and growth for striped jack, *Pseudocaranx dentex*, and *Oreochromis niloticus*. On the other hand, Badawy et al. (2008) investigated the algae mixture (*S. platensis* and chitosan nanoparticles) as supplemental feeds, results observed that growth performance of *O. niloticus* (FBW, BWG, DWG, RWG, and SGR) increased significantly ($P < 0.05$) when increased with one or both of *S. platensis* and chitosan. Moreover, these data were in accordance with the finding by Abdel-Warith and Elsayed (2019) who found that the possibility of adding *A. platensis* as a protein source fed to *O. niloticus* improves

Table 1
Formulation and proximate composition of experimental diets (g/100 g wet weight) containing *S. platensis* and chitosan nanoparticles fed to black tiger shrimp (*P. monodon*).

Feed ingredients	Experimental diets					
	T1 (Control)	T2	T3	T4	T5	T6
Fish meal (C.P.70%)	48.5	48.5	48.5	48.5	48.5	48.5
Soybean meal (C.P.44%)	20	20	20	20	20	20
Yellow corn meal (C. P. 7%)	10	9.95	9.9	9.9	9.85	9.8
Rice bran (C.P. 14%)	11.5	11.5	11.5	11.5	11.5	11.5
Fish oil	6	6	6	6	6	6
Chitosan (g/kg)	0	0	0	0.1	0.1	0.1
S. platensis (g/kg)	0	0.05	0.1	0	0.05	0.1
Vit*& Min premix**	4	4	4	4	4	4
Total	100	100	100	100	100	100
Proximate analysis (dry matter basis)						
Dry matter	92.16	91.98	93.07	92.55	91.62	91.619
Crude protein	43.79	43.65	43.91	43.22	43.19	43.57
Ether extract (EE)	13.29	13.77	13.91	13.43	13.39	13.58
Ash	10.88	10.79	10.91	10.77	10.82	10.74
Metabolizable energy (Kcal)/100 g	499.2	491.9	492.4	489.9	490.7	494.7

Each 40 g contains
* vit A 200,000 I. U, vit B1 15 mg, Zn 1800 mg.
** Fe 1200 mg, Biotin 100 mg, vit D3 30,000 I. U, vit E 250 mg, vit K3 50 mg, vit B2 12 mg, vit B12 250 mg, Niacin 15 mg, Folic Acid 2 mg, vit B6 20 mg, Bantothonic 80 mg, Mn. 2400 mg, Copper 200 mg, Selenium 10 mg, Sodium 100 mg, Phosphorus 1000 mg.

Table 2
Effect Chitosan nanoparticles using *S. platensis* on body weight (BW), weight gain (WG), specific growth rate (SGR) and survival rate (SR%) of *P. monodon* (mean ± SE).

Treatments	IBW[1]	FBW[2]	WG[3]	SGR%[4]/day	SR%[5]
T1	1.24 ± 0.002[a]	12.62 ± 0.17[e]	11.37 ± 0.0018[e]	2.76 ± 0.017[e]	57.32 ± 1.06[c]
T2	1.25 ± 0.0034[a]	15.28 ± 0.1[d]	14.03 ± 0.001[d]	2.98 ± 0.01[d]	68.07 ± 1.73[b]
T3	1.25 ± 0.01[a]	15.97 ± 0.14[c]	14.72 ± 0.002[c]	3.03 ± 0.014[c]	68.17 ± 1.73[a]
T4	1.24 ± 0.004[a]	17.83 ± 0.23[b]	16.58 ± 0.002[b]	3.17 ± 0.014[b]	71.01 ± 2.082[a]
T5	1.24 ± 0.004[a]	18.48 ± 0.2[a]	17.24 ± 0.002[a]	3.21 ± 0.012[a]	74.19 ± 1.52[a]
T6	1.25 ± 0.004[a]	18.49 ± 0.2[a]	17.25 ± 0.002[a]	3.21 ± 0.011[a]	73.22 ± 3.51[a]

Values in the same column with the same superscript are not significantly different (P > 0.05).
1 IBW: initial body weight (g)
2 FBW: final body weight (g)
3 WG = final body weight - initial body weight
4 SGR = [Ln final body weight (g) - Ln initial body weight (g)]/experimental period × 100.
5 SR: survival rate = (Final fish number/initial fish number) × 100

the overall feed utilization, growth performance, survival rate and body composition.

The highest value of WG (17.25 ± 0.002 g) was found in shrimp fed on diet T6 while the lowest WG (11.37 ± 0.0018 g) were shown by shrimp of the control group (Table 2). The analysis of variance for the averages weight gain was highly significant (P < 0.05) among all trails except the mean WG in trials T5 and T6. Ghaeni et al. (2011) stated that green tiger shrimp (*Penaeus semisulcatus*) fed diets containing *S. platensis* powder showed highly significant (P < 0.05) growth and weight gain when compared to shrimp were fed the control diet. Similar to our results, Carnevali et al. (2006) found that weight gain for black tiger shrimp was significantly higher for the treated groups compared to the control group when *S. platensis* were treated by chitosan nanoparticles and used for 70 days. Al-Dohail et al. (2009) also indicated that African catfish *Clarias gariepinus* fed the combination of *S. platensis* with chitosan showed better growth performance compared to the control fish group. Similarly, application of chitosan nanoparticles and *S. platensis* was found to improve the growth performance of *O. niloticus* (Wang et al., 2008) and *Silurus glanis* (Bogut et al., 2000).

The effect of chitosan nanoparticles biosynthesis and *S. platensis* on SGR is recoded in Table 2. T5 (1 ml chitosan and 0.05% *S. platensis*) and T6 (1 ml chitosan and 0.1% *S. platensis*) showed the highest SGR values (3.21 ± 0.011%/day for both), while shrimp fed on T1 (control) showed the lowest SGR values (2.76 ± 0.017%/day) and the differences between the means were significant (P < 0.05). These might be related to the chitosan nanoparticles and *S. platensis* which might be enhance the digestive enzymes such as protease, amylase, and lipase, which has the potential to increase the value of digestive enzymes in the gut and/or its effect for also improving digestive activity by synthesis of vitamins and cofactors or enzymatic improvement for WG and SGR (Gatesoupe, 1999). Also, Bauer et al. (2012) indicated that *L. vannamei* which fed on diet supplementation by microbial floc showed highly significant differences of all growth parameters including WG, SGR, survival rate and feed utilization when compared to the control group. In addition, Abdel-Warith and Elsayed (2019) found that diets containing *Arthrospira platensis* enhanced the biochemical and hematological parameters and these might be improve immune system and reflect on overall growth parameters.

Survival rate (SR) of *P. monodon* as affected by chitosan nanoparticles biosynthesis using *S. platensis* is presented in Table 2. *Penaeus monodon* of T5 (1 ml chitosan and 0.05% *S. platensis*) and T6 (1 ml chitosan and 0.1% *S. platensis*) recorded the highest SR values (74.19 ± 1.52 and 73.22 ± 3.51%) respectively, whereas, those of T1 (control) showed the lowest value being 57.32% as illustrated in Table 2 with significantly difference (P < 0.05) among treatment groups. These results are in accordance with Moe (2011) who noted that growth performance and the average of survival rate of *Litopenaeus vannamei* fed with 0.05% dietary *S. platensis* and chitosan was higher (100%) than the control group (80%). Jana et al. (2014) also found that Spirulina improves the survival rate of

Table 3
Chitosan nanoparticles biosynthesis using *S. platensis* impact of water heavy metals of *P. monodon* reared in hapas (mean ± SE).

Treatments	Fe ppm	Cu ppm	Pb ppm	Mn ppm
T1	0.413 ± 0.01[a]	0.01 ± 0.0003 [a]	0.1 ± 0.003 [a]	0.2 ± 0.003 [a]
T2	0.43 ± 0.012 [a]	0.01 ± 0.001 [a]	0.1 ± 0.003 [a]	0.2 ± 0.01 [a]
T3	0.42 ± 0.01 [a]	0.01 ± 0.0003 [a]	0.1 ± 0.01 [a]	0.2 ± 0.010 [a]
T4	0.403 ± 0.003 [a]	0.009 ± 0.001 [a]	0.1 ± 0.003 [a]	0.2 ± 0.0145 [a]
T5	0.413 ± 0.003 [a]	0.010 ± 0.001 [a]	0.1 ± 0.01 [a]	0.2 ± 0.003 [a]
T6	0.3 ± 0.12 [a]	0.001 ± 0.0003 [a]	0.1 ± 0.003 [a]	0.2 ± 0.01 [a]

Values in the same column with the same superscript are not significantly different (P > 0.05).

Table 4
Impact of Chitosan nanoparticles biosynthesis using *S. platensis* effect on body composition of *P. monodon* (dry weight basis) (mean ± SE).

Treatments	Moisture	Protein	Lipid	Ash
T1	74.44 ± 0.012[a]	38.27 ± 0.034[f]	10.14 ± 0.08[c]	22.75 ± 0.198[a]
T2	71.38 ± 0.027[d]	39.20 ± 0.04[e]	11.42 ± 0.11[b]	22.22 ± 0.16[b]
T3	71.76 ± 0.02[b]	39.40 ± 0.034[d]	11.53 ± 0.058[b]	22.01 ± 0.17[bc]
T4	71.53 ± 0.012[c]	39.90 ± 0.02[c]	11.90 ± 0.19[a]	21.80 ± 0.2[c]
T5	71.11 ± 0.023[e]	40.34 ± 0.034[b]	12.01 ± 0.19[a]	21.40 ± 0.11[d]
T6	70.90 ± 0.02[f]	40.70 ± 0.05[a]	12.10 ± 0.1[a]	21.22 ± 0.13[d]

Values in the same column with the same superscript are not significantly different (P > 0.05).

Pangus fish *Pangasius sutchi* it was found to be 94% and 80% survival rate, respectively, for the fodder impregnated with spirulina and chitosan at the end of the experiment.

Heavy metals were assessed in the water of black tiger shrimp (*P. monodon*) rearing in hapas placed in three different cages in the same area were recorded in Table 3. The total mean values of iron (Fe) concentrations in water were 0.413 ± 0.01, 0.43 ± 0.012, 0.42 ± 0.01, 0.403 ± 0.003, 0.413 ± 0.003, and 0.3 ± 0.12 ppm for the six different treatments, respectively. The mean values of copper) Cu) concentration ranged from 0.009 to 0.01. The average lead (Pb) concentration was 0.1 ppm for all treatments. The concentration of manganese (Mn) was 0.2 ppm for all trials. The statistical analysis showed that no significant differences (P > 0.05) in concentration of all estimated metals (Fe, Cu, Pb and Mn) among different treatments despite of the variations in the ratio of Chitosan nanoparticles and *S. platensis* added to the diets.

In this study, results indicate that there were significant differences (P < 0.05) in body composition of black tiger shrimp (*P. monodon*) among various treatments of biosynthesis of chitosan nanoparticles and *S. platensis* (Table 4). The moisture content was slightly lower for shrimp fed on T6 (70.9 ± 0.02%) when compared to shrimp fed on the control diet that had higher moisture content (74.44 ± 0.012%). The crude protein content was higher for T6 (40. 70 ± 0.05%) while the lower protein content (38.27 ± 0.034%) was recorded with the control group. In the same way, crude lipid value was also differ among groups, representing the higher amount of lipid in T6 (12.10 ± 0.1%) compared to the other groups. The total ash content was higher (22.75 ± 0.198%) for the control group whereas low ash content (22.01 ± 0.17%) was recorded for T3 where the diet contained 0.1% *S. platensis*. Analysis of variance showed that body composition was significantly (P < 0.05) affected by chitosan nanoparticles biosynthesis using *S. platensis* diets for all incorporation ratios.

Sriraman (1998) reported that protein content was higher for the younger individuals compared to adults for *Penaeus monodon*. The high content of protein for younger individuals might be related to increase the protein structure during the active growth phase as it has been showed for *Penaeus monodon* (Moreno et al., 2015; Mehta and Nayak, 2017). Results of the current study are in accordance with data reported by Hoa (2009), Ambar et al. (2017), and Nesara and Anand (2018) for *Penaeus monodon* and Mahbub (2016) for major prawn and shrimp.

4. Conclusion

Based on results obtained, it can be concluded that microalgae might be a precious, cost effective means of CsNPs for *P. monodon* growth. It can also be concluded that feeding of *P. monodon* on diets containing 44% protein and supplemented with *S. platensis* and chitosan nanoparticles can lead to an increase in shrimp growth performance and improve the body composition of *P. monodon* especially the protein and lipid content thereby improving their production and nutritional value. In addition, results of the current study highlight the possibility of using *S. platensis* and chitosan nanoparticles in *P. monodon* enhanced feed utilization and survival rate. Therefore, the use of *S. platensis* and chitosan nanoparticles in crustacean diets can be included for the large-scale culture of crustacean, particularly for shrimp. The optimum inclusion levels of the *S. platensis* and chitosan nanoparticles in crustacean diets require further investigation to determine its desirable impact on growth, feed utilization, immune response, and infection resistance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdel-Warith, A.A., Elsayed, E.A., 2019. Use of *Arthrospira platensis* as a feed additive to improve growth performance, feed utilization, body composition, and immune response of Nile Tilapia, *Oreochromis niloticus*. J. Sci. Ind. Res. 78, 681–686.

AOAC, 2016. Association of Official Analytical Chemists, Official methods of analysis (16th Ed.) Arlington, VA, USA.

Abdulrahman, N.M., Ameen, H.J.H., 2014. Replacement of fishmeal with microalgae Spirulina on White Shrimp (*Litopenaeus vannamei*) weight gain, meat and sensitive composition and survival. Pak. J. Nutr. 13 (2), 93–98. https://doi.org/10.3923/pjn.2014.93.98.

Al-Dohail, M.A., Hashim, R., Aliyu-Paiko, M., 2009. *Spirulina platensis* in Oscars (*Astronotus ocellatus*) diets, angel animals: a review. Crit. Rev. Food Sci. Nutr. 43, 19–60.

Ambar, A., Fregoso, P., Elisa, M., Valenzuela, S., Ciria, G., Figueroa, S., Alma, B., Peregrino, U., Manuel, O.V., Lilia, L.C., Gloria, Y.P., 2017. White shrimp *Litopenaeus vannamei* recombinant lactate dehydrogenase: biochemical and kinetic characterization. Protein Express. Purificat. 137, 20–25. https://doi.org/10.1016/j.pep.2017.06.010.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.

Badawy, T.E.M., Ibrahim, E.M. Zeinhomm, M.M., 2008. Partial replacement of fish meal with dried microalga (S. platensis and chitosan) in Nile tilapia (Oreochromis niloticus) diets. Internat. Sympo. on Tilapia in Aquacult. pp. 801–811.

Bauer, W., Prentice-Hernandez, C., Tesser, M.B., Wasielesky Jr., W., Luís, S., Poersch, L.H.S., 2012. Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp Litopenaeus vanname. Aquaculture 342–343, 112–116. https://doi.org/10.1016/j.

Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv. 25, 207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002.

Bogut, I., Milakovic, Z., Brikc, S., Novoselic, D., Bukvic, Z., 2000. Effects of *Enterococcus faecium* on the growth rate and intestinal microflora in sheat fish (*Silurus glanis*). Vet. Med. 45 (4), 107–109.

Boyd, C.E., 1990. Water quality in ponds for aquaculture. Agriculture Experiment Station. Auburn University, Alabama. 482 pages.

Can, E., Kizak, V., Kayim, M., Can, S., Kutlu, B., Ates, M., Kocabasl, M., Demirtas, N., 2011. Nanotechnological applications in aquaculture sea food industries and adverse effects of nanoparticles on environment. J. Mater. Sci. Eng. 5, 605–609.

Carnevali, O., De Vivo, L., Sulpizio, R., Gioacchin, G., Olivotto, I., Silvi, S., Cresci, A., 2006. Growth improvement by spirulina Black tiger shrimp (*P. monodonare*), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture 258, 430–438. https://doi.org/10.1016/j.aquaculture.2006.04.025.

Cohen, Z., Reungjitchachawali, M., Siangdung, W., Tanticharoen, M., 1993. Production and partial purification of γ-linolenic acid and some pigments from *Spirulina platensis*. J. Appl. Phycol. 5, 109–115. https://doi.org/10.1007/BF02182428.

De Pauw, N., Persoone, G., 1992. Micro-algae for aquaculture. In: Borowitzka, M.A., Borowitzka, L.J. (Eds.), Micro-algal Biotechnology. Cambridge University Press, Cambridge, United Kingdom, pp. 197–221.

Dunncan, D.B., 1955. Multiple range and Multiple test. Biometerics 11, 1–42.

FAO, 2010. The State of world fisheries and Aquaculture, (SOFIA) FAO Fisheries and Aquaculture. Department Food and Agriculture Organization of the United Nations Rome, 93pp.

Gatesoupe, F.J., 1999. The use of probiotics in aquaculture. Aquaculture 180, 147–165. https://doi.org/10.1016/S0044-8486(99)00187-8.

Ghaeni, M., Matinfar, A., Soltani, M., Rabbani, M., Vosoughi, A., 2011. Comparative effects of pure spirulina powder and other diets on larval growth and survival of green tiger shrimp Peneaus semisulcatus. Iran. J. Fish. Sci. 10 (2), 208–217.

Hoa, N.D., 2009. Domestication of black tiger shrimp (*Penaeus monodon*) in recirculation systems in Vietnam PhD. Thesis. Ghent University, Belgium.

Holthuis, L.B., 1980. "Penaeus (Penaeus) monodon." Shrimps and Prawns of the World. An Annotated Catalogue of Species of Interest to Fisheries. FAO Species Catalogue 1. Food and Agriculture Organization. p. 50. ISBN 92-5-100896-5.

Ibrahem, M.D., Mohamed, M.F., Ibrahim, M.A., 2013. The role of *Spirulina platensis* (Arthrospira platensis) in growth and immunity of Nile tilapia *(Oreochromis niloticus*) and its resistance to bacterial infection. J. Agric. Sci. 5 (6), 109–117. https://doi.org/10.5539/jas.v5n6p109.

Jaime-Ceballos, B., Civera-Cerecedo, R., Villarreal, H., Galindo-López, J., Pérez-Jar, L., 2007. Use of *Spirulina platensis* meal as feed attractant in diets for shrimp *Litopenaeus schmitti*. Hidrobiológica 17, 113–117.

Jana, A., Saroch, J.D., Borana, K., 2014. Effect of Spirulina as a feed supplement on survival and growth of *Pangasius sutchi*. Int. J. Fish. Aquat. Stud. 1 (5), 77–79.

Mahbub, A., 2016. Origin and population structure of major prawn and shrimp species in Bangladesh PhD thesis. University of Iceland.

Masarudin, M.J., Cutts, S.M., Evison, B.J., Phillips, D.R., Pigram, P.J., 2015. Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [(14)C]-doxorubicin. Nanotechnol. Sci. Appl. 8, 67–80. https://doi.org/10.2147/NSA.S91785.

Mehta, N.K., Nayak, B.B., 2017. Bio-chemical composition, functional, and rheological properties of fresh meat from fish, squid, and shrimp: a comparative study. Inter. J. Food Propert. 20 (S1), S707–S721. https://doi.org/10.1080/10942912.2017.1308955.

Moe, P.P., 2011. Effect of Diet Containing *Spirulina platensis* on the Growth and Haematology of White Shrimp, (*Litopenaeus vannamei*). Univ. Res. J. 4 (2), 63–77.

Moreno, J.E., Méndez-Ruiz, C.A., Díaz, G.X., Meruane, J.A., Pedro, H., Toledo, P.H., 2015. Chemical composition of the freshwater prawn Cryphiops caementarius (Molina, 1782) (Decapoda: Palaemonidae) in two populations in northern Chile: reproductive and environmental considerations. Lat. Am. J. Aquat. Res. 43 (4), 745–754. https://doi.org/10.3856/vol43-issue4-fulltext-13.

Nandeesha, M.C., Gangadhara, B., Manissery, J.K., Venkataraman, L.V., 2001. Growth performance of two Indian major carps, catla *(Catla catl*a) and rohu *(Labeo rohi*ta) fed diets containing different levels of Spirulina platensis. Bioresour. Technol. 80, 117–120. https://doi.org/10.1016/S0960-8524(01)00085-2.

Nesara, K., Anand, P.P., 2018. Nutritional requirement of fresh water prawn and shrimps: a review. J. Entom. and Zoo. Stud. 6 (4), 1526–1532.

Palmegiano, G.B., Gai, F., Daprà, F., Gasco, L., Pazzaglia, M., Peiretti, P.G., 2008. Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. Aquacult. Res. 39, 587–595. https://doi.org/10.1111/j.1365-2109.2008.01914.x.

Richmond, A., 2004. In: Handbook of Microalgal Culture: Biotechnology and Appl. Phycology. Blackwell Science Ltd, a Blackwell Publishing Company, p. 577.

Silva-Neto, J., Nunes, A.J.P., Sabry- Neto, H., Sal, M.V.C., 2012. Spirulina meal has acted as a strong feeding attractant for *Litopenaeus vannamei* at a very low dietary inclusion level. Aquacult. Res. 43, 430–437. https://doi.org/10.1111/j.1365-2109.2011.02846.x.

Sriraman, K., 1998. Biological and biochemical studies on the prawns of *Portonova coast* (*Crustacea: Decapoda: Macrura*) Ph.D. Thesis. Annamalai University, India.

Statistical Analysis System (1996). SAS/STAT User's Guide Release 6.03 edn. SAS Institute Inc., Cary.

Wang, Y.B., Li, J.R., Lin, J., 2008. Probiotics in aquaculture: Challenges and outlook. Aquaculture 281, 1–4. https://doi.org/10.1016/j.aquaculture.2008.06.002.

Whetsone, J.M., Treece, G.D., Browdy, C.L., Stokes, A.D., 2002. Opportunities and Constraints in Marine Shrimp Farming, Southern Regional Aquaculture Center (SRAC). SRAC Publication No. 2600.