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Effect of Chitosan Nanoparticles Biosynthesis Using *Spirulina platensis* on Growth Performance and Blood Parameters of the Black Tiger Shrimp (*Penaeus monodon*)

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ABSTRACT

The aim of this work was to investigate the effect of chitocanitosan nanoparticles (CsNPs) biosynthesis using *Spirulina platensis* as supplemented food in black tiger shrimp (*Penaeus monodon*) diets. Therefore 6 diets were formulated to provide 45% crude protein and 499k cal./100 g diet and supplemented using *S. platensis* and chitosan as follow: T1: control, T2: 0.05% *S. platensis*, T3: 0.1% *S. platensis*, T4: 1 ml chitosan nanoparticles, T5: 1 ml chitosan nanoparticles with 0.05% *S. platensis* and T6: 1 ml chitosan with 0.1% *S. platensis*. Each diet was offered at 5% feeding rate twice, 6 days/ week in duplicate shrimp groups (1.24 g/fingerling) stocked at 15 fingerlings per m^3 for each of 12 (1×2.5×1m³) hapas for 12 weeks. Growth performance, some hematological parameters and blood components of *P. monodon* were determined after 84 days. Supplementation of the basal diet using each of spirulina and chitosan nanoparticles (T5 and T6) significantly improved the final body weight (BW), body length (BL) and Daily weight gain (DWG) and *P. monodon* fed the diet T5 (supplemented using 1ml chitosan and 0.05% S. platensis) and T6 (supplemented using 1ml chitosan and 0.01% *S. platensis*) showed the highest serum protein, cholesterol and lactate of black tiger shrimp (*P. monodon*).

Accordingly, it could be concluded that feeding *P. monodon* with diets containing spirulina and chitosan nanoparticles diets improves the growth performance, blood biochemical parameters of the *P. monodon* and therefore improves their production.R).

INTRODUCTION

Algae (microalgae besides macroalgae) play an important role in aquaculture, pharmaceutical and medical faces implementations. Algae are valued source for various profitable products like normal dyes besides biofuels (Lee, 2008; Johansen, 2011; Borowitzka, 2013; Fon-Sing *et al.*, 2013). Until today, for the biosynthesis of metallic NPs, algae unlike group such as *Phaeophyceae, Chlorophyceae, Cyanophyceae, Rhodophyceae* then others (diatoms besides euglenoids) have been used (Sharma *et al.*, 2015). The facility of algae to accrue metals besides decrease metal ions makes them the greater contender for the biosynthesis of nanoparticles.

Additionally, algae are relatively convenient and easy to handle, along with some other advantages such as synthesis at a little temperature with greater energy productivity, less toxicity, and environment freindly. In physical besides chemical technique, different commercially obtainable surfactants were used as templates besides plugging agents in CsNPs synthesis with diverse morphologies (Deepika *et al*. 2013).

Several nutritional requirements and dietary supplies are well recognized in shrimp culture. Most previous studies were on *Penaeus semisulcatus, P. japonicas P. monodon* and *Litopenaeus vannamei*. There were less data about their nutritional needs and commercial diets (Lavery *et al.*, 2004; Ma *et al.*, 2011). The development of a high-quality diet has been a very important factor for tiger shrimp farming. Various nutritional besides dietary needs are identified to be decent. Most studies were on *monodon sp.*, *P. japonicas* in addition to *Litopenaeus vannamei*. But there were less evidence about their dietary supplies and commercial diets. Growing of tiger shrimp, *P. monodon* has been extensively practiced in recent years in some countries as Egypt, Kuwait, Turkey besides the Gulf countries (Flegel, 2007). In spite of imperfect food used for p. monodon, most studies offered existing economical foods. Several research developments have been carried out for improve the growth of green tiger shrimp in a lot of countries (Bowen *et al.*, 2014).

This study aimed to investigate the effects of chitosan nanoparticles biosynthesis using *S. Platensis* on growth performance of black tiger shrimp (*P. monodon*) in order for determine the maximum benefit of dietary *Spirulina platensis* and chitosan nanoparticles for black tiger shrimp (*P. monodon*) reared in cages.

MATERIALS AND METHODS

Location:

This work was conducted during the period of 20th February to 8th May 2019 in the region of Bourg Magaza-Ibeana, Al Gazirah Al Khadraa, Metobas, Kafr El Sheikh Governorate, for studying the effect of chitosan nanoparticles biosynthesis using *S. platensis* for Black tiger shrimp (*P. monodon*) reared in hapas placed in 3 different cages (4 hapas per cage – 2 hapas per treatment).

Experimental design:

Twelve hapas ($1\times2.5\times1m^3$) were placed in cages, divided into six treatments. The Black tiger shrimp were stocked in these hapas (each hapa contains 15 fingerlings per m^3) to investigate effect of chitosan nanoparticles biosynthesis using *Spirulina platensis* on growth performance, hematological promoters and biochemical analysis of *P. monodon*.

***S. platensis*, chitosan nanoparticles and *P. monodon* source:**

S. platensis meal was obtained from algae unit of National Research Center was used. The condition of cultivation *S. platensis* cells in Zarrouk's medium (Zarrouk, 1966).

Chitosan (CS) with molecular weight (MW) = 50,000–190,000 De acetylation degree (Da) was used as carrier and sodium tri polyphosphate (TPP) with MW= 367.86 Da was used as crosslinking agent, both were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chitosan nanoparticles (CsNPs) was prepared by using ionic gelation method as reported by Masarudin *et al*. (2015).

P. monodon were obtained from a private farm in Burullus. The experimental shrimp were transported in plastic bags filled with water and oxygen to the cages. *P. monodon* (1.24±0.01 g/fingerling) were adapted and distributed randomly into 12 hapas. The *P. monodon* were weighed and the initial weight for each hapa was recorded. Each hapa was stocked with 15 shrimp per m^3.

Estimation Preparation of diets and feeding practices:

The experimental black tiger shrimp were conducted to 6 treatments in 12 hapas (1×2.5×1m^3) placed in 3 cages according to adding rate of chitosan nanoparticles and *S. platensis* algae to the diets as found in table 1 during the experimental periods (12 weeks). The black tiger shrimp (1.24±0.01 g/fingerling) fed commercial sinking diet to keep the diets available for *P. monodon* fingerlings (as pellets 2 mm in diameter) at a daily rate of 5% of total biomass for 6 days / week twice daily at 9.00 am and 3.00 pm (Table 1).

Every 14 days, *P. monodon* groups were randomly obtained from each hapa then weighted and amount of feed was adjusted according to the changes in body weight throughout the experimental period.

Preparation of diets and feeding practices:

Six isonitrogenous and isocaloric diets were formulated to provide 45% protein and 499 kcal/100 g diet and supplemented using *S. platensis* and chitosan as follow:

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*.

Table 1: Composition of groups diets used during the experimental period.

Feed ingredients	Experimental diets					
	T1	T2	T3	T4	T5	T6
Fish meal (72%)	48.5	48.5	48.5	48.5	48.5	48.5
Soybean meal (44%)	20	20	20	20	20	20
Yellow corn	10	9.95	9.9	9.9	9.85	9.8
Rice bran	11.5	11.5	11.5	11.5	11.5	11.5
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
Fish oil	6	6	6	6	6	6
Vit. & Min. mixture	4	4	4	4	4	4
Total	100	100	100	100	100	100
Proximate analysis (dry matter basis)						
Crude protein (CP)	92.16	91.98	93.07	92.55	91.62	91.619
Ether extract (EE)	43.79	43.65	43.91	43.22	43.19	43.57
Crude fiber (CF)	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/100g)	7.09	7.12	7.02	6.97	7.18	6.94

Water quality:

Water temperature was recorded daily at 9:00 am using a mercury thermometer. Dissolved oxygen (DO) was measured at 07:00 am using YSI model 56 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia, nitrate and nitrite were measured weekly using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). pH values were estimated on morning using using a pH meter (Orion pH meter, Abilene, Texas, USA). Water temperature ranged from 16.7 to 17.4°C; dissolved oxygen (DO) ranged between 4.7 and 5.9 mg/l and pH values it was about a neutral ranged between 6.7:7.4 for the different treatments during the experimental period (84 days) of this study.

Generally, all tested water quality criteria (temperature, pH value DO) were suitable and within the acceptable limits for rearing Black tiger shrimp (Boyd, 1990).

Biochemical components:

At the end of the experimental period, six Black tiger shrimp were chosen at random from each replicate (hapa). Blood samples of three shrimp were collected from the vein in clean tube with 10% EDTA solution to determine hematocrit (Ht), hemoglobin (Hb) and total hemosytes. Blood samples of the other three shrimp were collected also from the vein in clean dry centrifuge tubes, kept for 15 minutes and centrifuged at 3000 rpm for 10 minutes, then kept frozen at -20°C for determination of glucose. Total protein was determined using the method of Bradford (1976), albumin content using the method of Doumas *et al*. (1971). Serum cholesterol and Lactate were measured using the method of Trinder (1969) method. Hematocrit (Ht) was determined using the microhematocrit method as described using Reitman and Frankel (1957).

Growth performance parameters:

Records of live body weight (g) and body length (cm) of individual Black tiger were measured in 20 shrimp for each hapa and registered every 14 days (two weeks) during the experiment period.

Daily weight gain (DWG) was calculated using the following formula:

DWG= (Final Weight-Initial weight)/ (experimental period, 84days).

Statistical analysis:

The statistical analysis of data carried out using applying the computer program SAS (1996). Differences among means were tested for significance according to Duncan (1955) using using the following model: -

$$x_{ij} = \mu_{..} + \alpha_i + \Sigma_i.$$

Where:

μ = Overall mean.

α_i = The effect of treatments.

e_i= Random error.

RESULTS AND DISCUSSION

Results of the effect of chitosan nanoparticles biosynthesis using *S. platensis* on body weight (BW) of *P. monodon* are presented for table 2. At the start of the experiment, the initial BW of *P. monodon* within all treatment groups was nearly similar (ranged between 1.24 and 1.25 g) with no significant differences (Table 2) indicating that the experimental groups at the start of the experiment were randomly distributed. At the end of experiment (after 84 days), the highest final BW (18.49 g) was recorded using shrimp of T6 (basal diet 45% crude protein with 1ml chitosan and 0.1% *S. platensis*) while the lowest one (12.62 g) was shown using shrimp of T1 (control). The analysis of variance of averages for body weight were highly significant ($P<0.05$).

The results of the current study are in accordance with Abdulrahman and Ameen (2014) who observed that, the combined of dried *S. platensis* with chitosan nanoparticles found to remain of potential effects on growth at an optimum concentration of 0.5 g/kg for white shrimp (*Litopenaeus vannamei*). The mean value level for the group received 0.5 g/kg is higher for all the tested parameters with significant difference indicates the optimum dietary level of *S. platensis* treated using chitosan nanoparticles for green tiger shrimp is 0.5 g/kg during studying period.

Ibrahem *et al.* (2013) found that fodder supplemented with chitosan particles biosynthesis for chlorella improved the body weight, length and growth rates for striped jack, *Pseudocaranx dentex* and *O. niloticus*.

Table 2: Effect of chitosan nanoparticles biosynthesis using *S. platensis* on growth performance of black tiger shrimp (*P. monodon*)..

Treatment	No.	Initia1 weight	Fina1 weight	DWG g/d*	Initia1 1ength	Fina1 1ength
T1	40	1.24±0.002	12.62±0.17e	0.135±0.001e	2.52±0.04	13.50±0.104e
T2	40	1.25±0.003	15.28±0.1d	0.167±0.001d	2.57±0.04	14.77±0.1d
T3	40	1.25±0.01	15.97±0.14c	0.175±0.002c	2.51±0.04	15.03±0.17c
T4	40	1.24±0.004	17.83±0.23b	0.197±0.003b	2.51±0.04	16.54±0.2b
T5	40	1.24±0.004	18.48±0.2a	0.205±0.002a	2.44±0.05	17.32±0.1a
T6	40	1.25±0.004	18.49±0.2a	0.205±0.002a	2.50±0.043	17.39±0.14a

+ Means with the same letter in each column are not significantly differences (P < 0.05).
Daily weightgain (DWG)= (Final Weight-Initial weight)/ (experimental period, 84days)

Results of the effect chitosan nanoparticles biosynthesis using *S. platensis* on daily weight gain (DWG) of *P. monodon*are shown for Table (2). The highest DWG (0.205g) was recorded by *P. monodon*are of T5 and T6, while the lowest ones (0.135g) were shown by *P. monodon*are of T1 (control) (Table 2). The analysis of variance of averages for body weight were highly significant (P<0.05).

Gatesoupe (1999) reported that *S. platensis* treated by chitosan nanoparticles have been shown to improve digestive enzymes as amylase, protease, lipase which may enrich the concentration of intestinal digestive enzymes or its effect for improving digestive activity by synthesis of vitamins and co-factors or enzymatic improvement for WG or SGR.

Noveirian and Nasrollahzadeh, (2012) found that green tiger shrimp (*Penaeus semisulcatus*) fed diets supplemented with *S. platensis* and chitosan nanoparticles showed significantly (P<0.05) better growth performance and weight gain than shrimp fed the control diet.

Results presented for Table (2) showed that the differences for initial body length (BL) of *P. monodon* among different treatments were insignificant. At the end of the experiment *P. monodon* of T6 (1ml chitosan and 0.1% *S. platensis*) showed the longest final BL (17.39 cm), whereas those of T1 (control) recorded the shortest one (13.50 cm) and the analysis of variance of averages for body length were highly significant (P<0.05).

Table 3: Effect of biosynthesis of chitosan nanoparticles using S. *platensis* on the average blood components (haematological parameters) and glucose of black tiger shrimp (*P. monodon*).

Treatment	No.	Hb (g/dl)	Total haemocytes (1×10^7cell ml^{-1})	Ht (%)	Glucose mg/100ml
T1	3	10.21±0.011c	2.60±0.03e	0.80±0.01d	0.51±0.012c
T2	3	10.51±0.12b	2.90±0.022d	0.98±0.01c	0.60±0.011b
T3	3	10.83±0.09a	3.10±0.040c	1.12±0.01b	0.63±0.014a
T4	3	10.95±0.11a	3.70±0.04b	1.70±0.014a	0.64±0.010a
T5	3	10.88±0.21a	4.30±0.033a	1.70±0.03a	0.70±0.011a
T6	3	10.97±0.19a	4.40±0.037a	1.70±0.041a	0.71±0.012a

+ Means with the same letter in each column are not significantly differences (P < 0.05).

In accordance with Jana *et al*. (2014) observed an enhancement in the growth green tiger shrimp (*Penaeus semisulcatus*) with chitosan nanoparticles biosynthesis using *S. platensis* supplement. The highest average body weight and length values of the shrimp were observed with the diet supplemented with 0.5% spirulina which came out to remain 30.4 g and 19.67 cm while the lowest average body weight and length values of the shrimp were observed with the diet without spirulina,22.7 g and 12.16 cm. On the other hand, Jarolowicz *et al*. (2012) reported that, white shrimp, fed commercial fodder supplemented with spirulina supplement did not have an impact on final body length.

Averages of Hemoglobin (Hb) as affected by chitosan nanoparticles biosynthesis using *S. platensis* were increased from 10.21 to 10.97, g/dl for *P. monodon* fed the diets supplemented with chitosan nanoparticles using *S. platensis* (Table 3). Analysis of variance showed that the supplementation of *P. monodon* diets chitosan nanoparticles using *S. platensis* levels significantly (P<0.05) increased Hb content.

The total haemocytes values were found to be 2.6 to 4.4 10^7cell ml^{-1} for black tiger shrimp fed diets supplemented with chitosan nanoparticles biosynthesis using spirulina. Analysis of variance showed that differences in haemocytes among dietary groups due to different levels were highly insignificant (P>0.05).

Results presented in Table 3 also outlined the effect of chitosan nanoparticles biosynthesis using *S. platensis* on hematocrit (Ht). At the end of the experiment, Ht was higher for shrimp fed the diet supplemented with chitosan nanoparticles using *S. platensis* than those fed the other diets.

Values of blood glucose are presented in Table 3. Glucose values found to remain 0.51, 0.60, 0.63, 0.64, 0.70 and 0.71 mg/100 ml blood for the different six diets used for the present study (T1, T2, T3, T4, T5 and T6, respectively). Analysis of variance showed that blood glucose was significantly (P<0.05) affected with chitosan nanoparticles biosynthesis using *S. platensis* used for the present study.

Haematological evaluation is a routine practice for determining health status for aquaculture, crustaceans and also other terrestrial animals (Tavares and Ruas, 2006). Blood is a suitable means of indicating and identifying the effects of stress, environment and health status of shrimp for a given area. Blood cell count is a stable index and normally shrimp tries to maintain it between certain limits. Blood composition is usually altered during diseases or malnutrition conditions. So shrimp blood is very significant to accurately evaluate the health of species (Celik, 2004). Using spirulina and chitosan for fish and shrimp diet improves these haematological parameters and makes the cultured fish healthy and disease resistant.

Table 4: Effect of chitosan nanoparticles biosynthesis using *S. platensis* on the total protein (TP), cholesterol (CHO) and lactate (LACT) of black tiger shrimp (*P. monodon*).

Treatment	No.	TP mmol/L	CHO mg/ml	LACT mg/ml
T1	3	1.80±0.033d	0.30±0.003b	1.26±0.02c
T2	3	2.43±0.1c	0.32±0.01a	1.40±0.003b
T3	3	2.83±0.03b	0.31±0.012a	1.40±0.014b
T4	3	3.30±0.1a	0.32±0.01a	1.42±0.03ab
T5	3	3.43±0.1a	0.34±0.01a	1.50±0.031a
T6	3	3.43±0.12a	0.32± 0.011a	1.51±0.011a

+ Means with the same letter in each column are not significantly differences (P < 0.05).

Averages of Total protein mmol/L as affected by chitosan nanoparticles biosynthesis using *S. platensis* were increased from 1.8 to 3.43 mmol/L for *P. monodon* fed the diets supplemented with chitosan nanoparticles using *S. platensis* (Table 4). Analysis of variance showed that the supplementation of *P. monodon* diets supplemented with chitosan nanoparticles using *S. platensis* significantly (P<0.05) increased total protein content.

These results are for agreement with Meharbi *et al.* (2012) reported that diet supplemented with spirulina increase the serum protein level of white shrimp (*Litopenaeus vannamei*). in addition, Jha *et al*. (2007) found that, increase for the serum total protein levels is thought to remain associated with a stronger innate response for fish. Zhou *et al*. (2010) reported that application of spirulina as a nutrition additive enhanced the immune and health status of tilapia (*O. niloticus*).

Dietary cholesterol content (CHO) mg/ml averages were presented in Table (11). The highest value of CHO was recorded using T5 (0.34 mg/ml) while, the lowest value was 0.30 mg/ml for the control. Analysis of variance showed that CHO was significantly ($P<0.05$) affected by chitosan nanoparticles biosynthesis using *S. platensis* used for the present study.

Our study supports the conclusion of Teshima (2007) that, the whole body cholesterol content was a better indicator of requirement than growth response. Smith *et al.* (2011) found that, the growth response of shrimp indicated that the cholesterol requirement of *P. monodon* could remain with a dietary cholesterol content of less than 2.3 g kg^{-1}. The growth responses measured suggest that the requirement would remain a dietary cholesterol content between 0.7 and 1.2 g kg^{-1} of diet. However, the increase for whole body cholesterol content suggests that the requirement was met when the dietary cholesterol content was close to 1.7 g kg^{-1}. As the dietary cholesterol content increased, retention efficiency consistently decreased, with the whole body cholesterol content reaching a maximum when the dietary cholesterol content was 1.7 g kg^{-1}. The authors stated that this maximum level appears to reflect the optimum whole body cholesterol content for structural and metabolic purposes, and so indicates the requirement.

Values of lactate mg/ml are presented for Table 4. The average lactate values found to remain 1.26, 1.40, 1.40, 1.42, 1.50 and 1.51mg/ml for the different six diets used in the present study (T1, T2, T3, T4, T5, and T6, respectively). Analysis of variance showed that lactate mg/ml was significantly ($P<0.05$) affected by chitosan nanoparticles biosynthesis using *S. platensis* used in the present study. In the shrimp *L. vannamei*, accumulation of lactate occurs during spirulina add at 0.5% in basal diet for a few weeks (Racotta *et al*., 2002) also, during severe hypoxia of 0.4 mg DO (Perez-Rostro *et al.*, 2004). Lactate dehydrogenase -LDH (EC 1.1.1.27) is the terminal enzyme for anaerobic glycolysis for many organisms and catalyzes the inter-conversion of lactate and pyruvate using NAD as coenzyme (Racotta *et al.*, 2003).

CONCLUSION

Based on results obtained in this study it could be concluded that, algal live or meal may be a precious, cost effective means with of CsNPs for *P. monodon* growth. Also, it could be concluded that, feeding of *P. monodon* on diets contained 45% protein and supplemented with S. platensis and chitosan nanoparticles lead to increase shrimp growth performance and improve its blood biochemical parameters.

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