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Research Article

EFFICACY OF DIETARY SUPPLEMENTATION OF CYANOBACTERIAL MEAL ON GROWTH PERFORMANCE OF BLACK TIGER SHRIMP *PENAEUS MONODON* (FABRICIUS)

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ABSTRACT
The potential of cyanobacterial meal as an alternative protein source to fish meal in practical diets for the juvenile tiger shrimp, *Penaeus monodon*, was studied under laboratory condition. Five different experimental diets were formulated to contain 25% protein. A portion of fish meal (5%) was replaced with four different cyanobacterial meal such as *Phormidium tenue* (C 1), *Phormidium* sp. (C 2), *Synechococcus elongates* (C 3) and *Spirulina* sp. (C 4). A diet with no replacement of fish meal was used as control. Fifteen shrimp juveniles with an average weight of 0.245 ±0.024 g were randomly assigned in fifteen 50-L plastic tanks equipped under controlled laboratory conditions. The shrimp were fed the formulated diets at a daily feeding rate of 10% body weight for 60 days in three replicate samples. *Spirulina* meal supplemented diet (C4) showed highest growth in terms of weight gain (1.68 ± 0.012), specific growth rate (SGR of 3.074 ± 0.026), feed conversion ratio (FCR) (2.054 ± 0.014) with highest survival (68 % ± 4). There were no significant differences in whole body proximate composition (edible flesh weight, dry matter content, protein, total lipid, ash and fiber) of shrimp fed the diets with different cyanobacterial meal substitution. These preliminary results have demonstrated that *Spirulina* meal has a potential protein substitute of fish meal in shrimp diet.

Keywords: *Penaeus Monodon, Cyanobacteria, Spirulina, Growth Performance, Feed Formulation*

INTRODUCTION
Shrimp aquaculture is one of the most profitable and fast growing economic oriented aquaculture industry in India and other South Asian countries (FAO, 2001). In 2005, total aquaculture production reached 63.0 million tonnes, valued at US$78.4 billion (FAO, 2007). In India, the brackish water aquaculture development is mostly contributed by shrimp, *Penaeus monodon* culture (Ponniah and Sundaray, 2008). Marine shrimp farming operations depend on fishmeal as sole or major dietary protein ingredients in farm-made or commercial aquafeeds (Tacon, 1996; Tacon and Akiyama, 1997). In the last few decade, there has been a growing interest in finding and developing alternative protein resources for aquaculture, as fishmeal has a negative impact on global fishing resources (Naylor *et al.*, 2000; Dernekbasi *et al.*, 2010; Tongsiri *et al.*, 2010). Protein rich alternative feed ingredient is needed for better performance of aqua feeds. A cyanobacterium has proven value added products into its own. *Spirulina* has emerged as the most potent and nutritious food, which has the highest protein content (55-65%) as compared to other algal (Venkataraman, 1983). Besides it use as protein source for feed or food, the presence of β-carotene, B-complex vitamins, and minerals contributes to its overall quality. Spirulina can be used as a partial or complete replacement protein source in aqua feed (Stanly and Jones, 1976; Matty and Smith, 1978; Koru, 2009; Dernekbasi *et al.*, 2010; Tongsiri *et al.*, 2010). Several studies have been conducted using dried spirulina as a supplement in diets of crustaceans. *S. platensis* meal is available at a commercial scale; thus its use in feeds for aquaculture is possible (Jaime-Ceballos, 2005; Hanel *et al.*, 2007).

Number of reports revealed that the beneficial growth or survival effect of different algal diets in shrimp culture system (Behanan *et al.*, 1990; Albentosa *et al.*, 1997). High dietary protein of marine algae provided best growth for juveniles (Knuckey *et al.*, 2001). However, there has been no clear data to indicate whether the effects of different cyanobacterial meal as protein supplement for growth and survival of shrimp *Penaeus monodon* (Fabricius). Hence the present study was aimed to evaluate the dietary supplementation of four different cyanobacterial meals (*Phormidium tenue*, *Phormidium* sp., *Synechococcus elongates* and *Spirulina* sp.) on growth and survival of juvenile shrimp *P. monodon* (Fabricius).

MATERIALS AND METHODS

Experimental Diets

The basic diet ingredients used in this study were obtained from local market of Thiruchirappalli, Tamil Nadu, India. Cyanobacteria such as *Phormidium tenue*, *Phormidium* sp., *Synechococcus elongates* and *Spirulina* sp. were isolated from shrimp culture ponds of Cuddalore District, Tamil Nadu, India. Cyanobacterial strains were grown autotropically and axenically in 25 L of transparent plastic vessel provided with proper aeration, and artificial light illumination of 3000 Lux in seawater enrichment medium (Wu *et al.*, 1994) for 25 days. The fresh cyanobacterial biomass was air-dried before use. Five experimental diets were formulated to be approximately isonitrogenous (Table-1). The experimental diets were prepared with 5% (w/w) replacement of the fish meal by cyanobacterial meal such as *Phormidium tenue* (C 1), *Phormidium* sp. (C 2), *Synechococcus elongates* (C 3) and *Spirulina* sp. (C 4). A control diet was prepared with the basic ingredients without any cyanobacterial meal. All the ingredients were dried and finely powdered in an electric grinder and sieved through a 0.5 mm mesh. The predetermined quantities of dry ingredients were thoroughly mixed with water and to made stiff dough. The dough was steamed in a pressure cooker for 15 minutes. Cooled dough was extruded through a 2 mm diameter sieve plate, and pellets were dried in an oven overnight at 45^{o} C. The dry pellets were cut in to required size (5 mm) and packed in airtight plastic containers and stored inside a cold room at -20 ^{0}C until used in the feeding trials (Sudaryono *et al.*, 1995). Proximate analysis of both ingredients and experimental feeds were conducted by the standard method of Association of Official Analytical Chemists (AOAC, 1990).

Feeding Trial

Juveniles of *P. monodon* (n=500) were obtained from a commercial shrimp hatchery in Thanjur, India and were acclimatized in fiber tank (75 cm diameter and 50 cm height) at laboratory conditions (salinity 25ppt, pH 7.9 and temperature 29 ±2^{o}C) for a period of 10 days prior to the experiment. During the acclimation period, shrimp were fed once in daily with the control diet. The shrimp growth trial was carried out in a plastic troughs (60cm height and 75cm dia) containing 25 liters of filtered salt water (25 ppt) with a temperature of 28± 2°C, pH of 7.5 and controlled photoperiod (12D:12L) for a period of 60 days in three replicate samples. Fifteen healthy juveniles shrimp (0.245 ± 0.024 g shrimp^{-1}) from acclimatized tanks were stocked in each experimental trough. Continuous aeration was provided to maintain dissolved oxygen near saturation. The shrimp were fed daily (09.00 hours) with their respective diet at 10% of body weight throughout the experimental period. The leftover diet and waste materials were collected daily before every feeding and calculated the diet intake. One third of the water was replenished every day to avoid accumulation of unutilized food and metabolic wastes. Survival of shrimp was estimated visually at every day. Growth of *P. monodon* juvenile was calculated as mean body weight expressed in grams, Specific Growth Rate (SGR %), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and Feeding Efficiency (FE%) by using standard methods (Henson, 1990; Hanley, 1991). At the end of the feeding trial, all shrimp samples from each tank were pooled and dried (at 45^{0}C in Hot air oven) for proximate analyses of whole body composition following standard methods of AOAC (1990).

The physico-chemical parameters of the tank water such as temperature, salinity, dissolved oxygen, and pH were measured daily and the nitrates, sulphates and ammonia were estimated twice in a week following the standard methods of AOAC (1990).

Statistical analysis: All data were analyzed by the Duncan's Multiple range test to determine the differences between the treatment means (Duncan, 1955). Results were measured statistically significant at the level of $P < 0.05$.

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RESULTS

Table -1 shows the proximate composition of the formulated shrimp diets. The crude protein level was high (25.81 % g on dry weight basis) in C4 diet (5% (w/w) of *Spirulina* meal supplemented diet) and it was low in control diet (24.5%), but total lipid was low in C4 diet. There was no significant difference observed in dry matter, ash, fiber and moisture content of the formulated diets.

Table 1: Composition of formulated cyanobacterial meal supplemented experimental diets (g 100g^{-1} in dry matter)

Ingredients	**Control**	**C1**	**C2**	**C3**	**C4**
Fish meal	25	20	20	20	20
Soya bean meal	15	15	15	15	15
Prawn waste	10	10	10	10	10
Mulberry leaf meal	5	5	5	5	5
Mangrove leaf meal	5	5	5	5	5
Groundnut oil cake	10	10	10	10	10
Gingili oil cake	10	10	10	10	10
Rice brawn	10	10	10	10	10
Wheat flour	5	5	5	5	5
Maida flour	5	5	5	5	5
Cyanobacteria	-	5	5	5	5
Chemical composition (g 100 g^{-1} in dry matter)					
Moisture	9.43	10.12	10.26	10.18	11.41
Dry mater	90.57	89.88	89.74	89.82	88.59
Crude protein	24.5	24.94	25.38	24.94	25.81
Crude fiber	5.36	5.68	5.48	5.48	5.5
Crude fat	5.06	5.3	5.2	5.4	5.03
Ash	11.69	10.54	10.54	10.53	11.21
NFE*	43.96	43.42	43.14	43.47	41.04

C1- Phormidium tenue (%): 54.56, crude protein; 5.7, fiber; 7.09, crude fat and 2.08, ash.
C2 - Synecococcus elongates (%):49.88, crude protein; 3.8, fiber; 6.97, crude fat and 1.8, ash
C3 - Phormidium sp. (%): 54.06, crude protein; 5.4, fiber; 7.12, crude fat and 2.1, ash.
C4 - Spiulina sp.(%): 62.7, crude protein; 4.3, fiber; 6.32, crude fat and 0.83, ash
** Nitrogen free extract calculated as : 100- (moisture+Protein+Crude fat+Crude fiber+ash)*

The results of the feeding trial were summarized in the Table 2. Total feed intake was high in shrimp fed with C4 diet (3.45 ±0.024 g/shrimp), and it was low in C2 diet (2.87 ±0.056 g/shrimp) (Fig. 1A). The shrimp fed with C4 diet showed significantly ($p<0.05$) higher mean body weight (1.68 ±0.012g) than those fed on control (1.54 ±0.041g) and other cyanobacteria supplemented diets (Fig. 1B). Feed conversion ratio (FCR) was high in shrimp fed with C3 diet (2.991 ± 0.022), but best FCR was recorded in C4 diet (Table-2). SGR of the diet group C4 was significantly differ ($P<0.05$) from other cyanobacterial meal substitution, but it was not significantly differ ($P>0.05$) from control diet. Further, feeding efficiency and protein efficiency ratio of the shrimp fed with C4, control and C2 diets were higher (Table-2) than C1 and C3 diets. Survival of the shrimp (Fig - 2) was high in C4 diet (68 ± 4.0 %) and low in diet C3 (41.33 ± 6.11%) group. Mortality rate of the shrimp was significantly low in experimental diet C4 ($P<0.05$).

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Table 2: Growth performance of *P. monodon* fed with cyanobacterial meal supplemented diet (60 days)

Diet	FCR[1]	SGR [2]%	FE[3] %	PER[4]
Control	2.124[d] ± 0.003	2.985[a] ± 0.029	47.28[a] ± 0.063	1.863[a] ± 0.002
C1	2.815[b] ± 0.095	2.276[c] ± 0.08	35.574[c] ± 1.197	1.485[b] ± 0.046
C2	2.056[c] ± 0. 008	2.787[b] ± 0.031	47.046[a] ± 0.176	1.793[a] ± 0.033
C3	3.094[a] ± 0.022	2.136[c] ± 0.011	33.601[d] ± 1.050	1.341[b] ± 0.009
C4	2.027[c] ± 0.014	3.074[a] ± 0.026	45.312[b] ± 0.290	1.887[a] ± 0.014

C1 – Phormidium tenue; C2 – Synechococcus elongates; C3 – Phormidium sp; C4 – Spirulina. All values are mean of triplicate samples with ± SD. Values with the different superscript letters in the same column are significantly different (P< 0.05) from each others.

[1] Feed conversion ratio (FCR) = Feed intake/ shrimp weight gain

[2] Specific growth rate (SGR% d^{-1}) = [Ln (final weight) - Ln (initial weight)] X 100/ time (days)

[3] Feeding efficiency (FE%) = Wet weight gain (g) x 100/ Dry feed consumed (g)

[4] Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g)

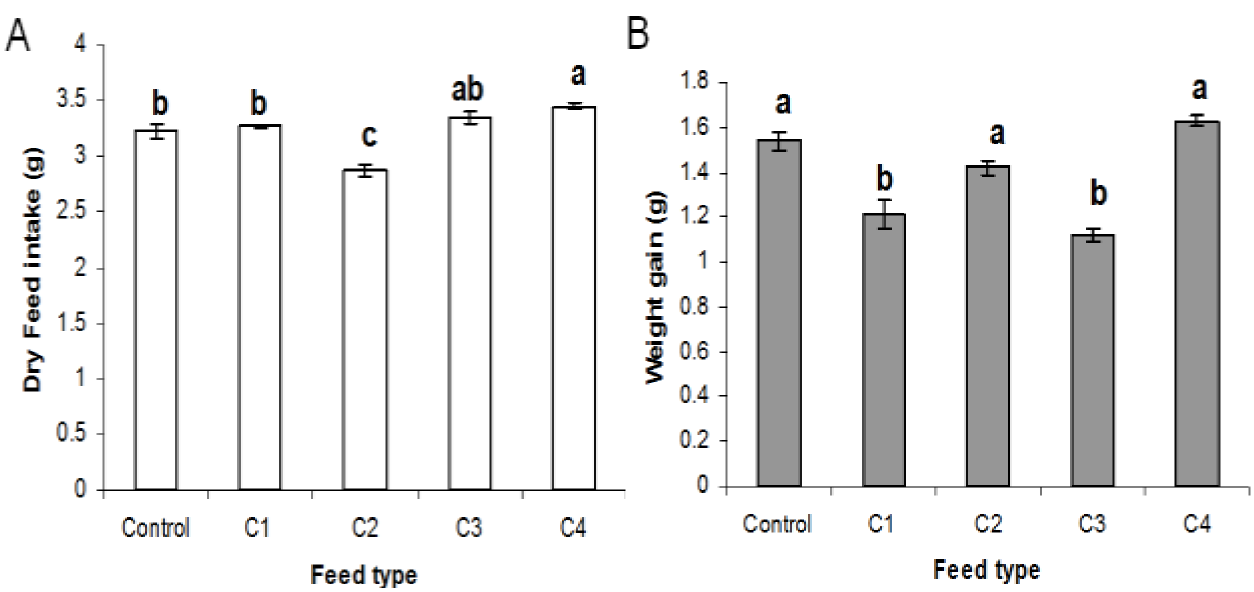

Figure 1: Dry feed intake and mean weight gains of shrimp *P. monodon* fed with cyanobacterial meal supplemented diet. All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.05). A. Dry feed intake (g/animal); B. Weight gain (g/animal) = (final weight - initial weight)

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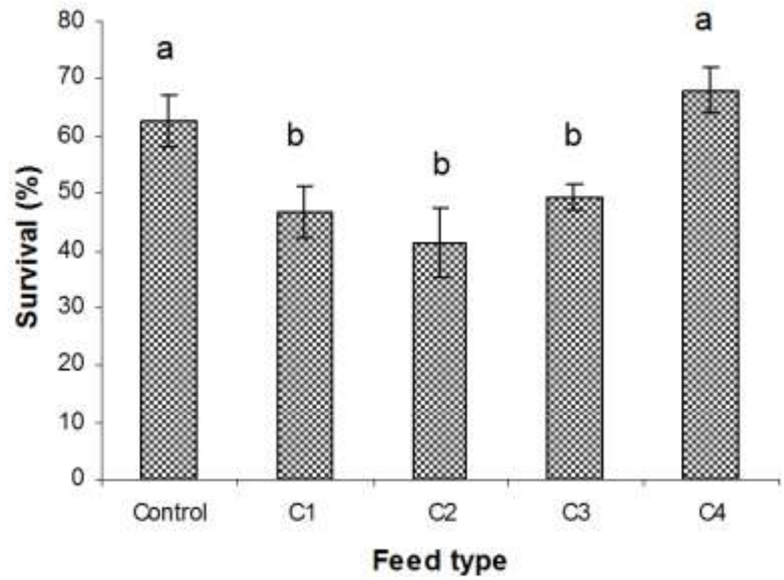

Figure 2: Survival (%) of the shrimp *P. monodon* fed with experimental diets (after the 60 days feeding trail). All values are mean of triplicates with standard deviation. Values indicated with the same letter are not significantly different (P< 0.05). Survival (%) = 100 X (final count /initial count)

The shrimp carcass proximate composition is summarized in Table 3. There was no significant difference (P>0.05) on percentage of edible flesh, ash content and total lipids. Interestingly, crude protein and total lipid content was significantly high (P<0.05) in shrimp fed with C4 diet. Shrimp fed with control diet showed low crude protein and crude lipid (Table-3). Physico-chemical parameters such as dissolved oxygen, salinity, temperature were not significantly differing and they were within the limit, but little difference was observed in pH, NO_3 (ppm) and accumulation of ammonia.

Table 3: Proximate composition of *P. monodon* fed with cyanobacterial meal supplemented diet (60 days) (g % on dry weight basis)

Proximate components	**Control**	**C 1**	**C 2**	**C 3**	**C 4**
1) Waste weight (%)	39.9[b] ± 0.2	39.25[c] ± 0.05	41.8[a] ± 0.3	40.45[ab] ± 0.25	37.55[d] ± 0.15
2) Edible flesh (%)	60.1[c] ± 0.2	60.75[b] ±0.05	58.2[d] ± 0.3	59.55[cd] ± 0.25	62.45[a] ± 0.15
Body composition					
1) Moisture (%)	75.9 ± 0.2	76.5 ± 0.4	75.5 ± 0.03	75.45 ± 0.05	75.35 ± 0.75
2) Protein (%)	44.63[b] ± 0.44	43.76[b] ± 0.86	45.94[b] ± 0.44	45.28[b] ± 0.22	49.0[a] ± 0.44
3) Ash (%)	4.79 ± 0.14	4.96 ± 0.25	5.05 ± 0.12	4.7 ± 0.02	5.04 ± 0.13
4) Lipid (%)	6.1[b] ± 0.2	6.45[b] ± 0.35	6.5[b] ± 0.1	6.35[b] ± 0.15	7.45[a] ± 0.35
5) NFE* (%)	20.38[a] ± 0.32	21.33[a] ± 0.24	18.01[b] ± 0.64	19.12[ab] ± 0.44	14.04[c] ± 1.22

C1 – Phormidium tenue; C2 – Synechococcus elongates; C3 – Phormidium sp; C4 – Spirulina. All values are mean of triplicate samples with ± SD. Values with the same superscript letters in the different column are significantly different (P< 0.05) from each others.
** Nitrogen free extract calculated as: 100- (moisture+Protein+Crude fat+Crude fiber+ash)*

DISCUSSION

Recently there has been great interest in microalgal meals as in the feeds of different aquatic species. As referred in previous literatures, only small number of microalgal strains are used in aquaculture based on practical considerations of strain availability, ease of culture, nutritional composition, digestibility and absence of toxins (Muller-Fuega *et al.*, 2004; Tredici *et al.*, 2009; Guedes, Malcata 2012). But cyanobacterial meal as alternative protein source for fish meal replacement in shrimp diet. In this study, to explore the possibility of using four cyanobacterial meals as partial substitute of fish meal in shrimp diet. Furthermore, this study highlights the palatability of cyanobacteria supplemented diet and their effect on shrimp growth was addressed. Algal diets have been used extensively for the aquaculture (Knuckey *et al.*, 2005; Lee *et al.*, 2006; Milione and Zeng, 2007) as a dietary protein source in fish diet (James *et al.*, 2006; Azaza *et al.*, 2008). High nutritive value algae and cyanobacteria are a good alternative protein source of aqua feed (Nakagowa *et al.*, 1987; Chow and Woo 1990). In this study, four different cyanobacterial meals were used for the shrimp diet. The proximate composition of diets has little variation because of the cyanobacterial meal substitution. All four cyanobacterial meals contain different concentrations of crude protein and crude fat (Table 1). It is well known that cyanobacteria contain large quantities of proteins (Nagle *et al.*, 2010), lipids and fatty acids (Sharathchandra and Rajashekhar, 2011). Nagle *et al.*, (2010) reported that *Spirulina platensis* has high protein (66.7%) in compared that *Phormidium tenue* (46.6%) and *Synecococcus elongates* (45.9%). *Spirulina* sp. has been identified as an excellent alternative protein source for aqua feed (James *et al.*, 2006).

All the formulated diets with 5% fish meal replacement by cyanobacterial meal were highly acceptable and were noted to be consumed by the shrimp. Partial substitution of the fishmeal by cyanobacterial meal was affecting the shrimp growth except *Spirulina* incorporated diet C4. *Spirulina* meal diet has increased body weight and survival rate of *P. monodon*. Similarly, *S. platensis* in shrimp diet showed weight gain in *P. japonicus* (Tsai, 1981) and green tiger shrimp *P. semisulcatus* (Ghaeni *et al.*, 2011). Dry feed intake was higher in shrimp fed with *Spirulina* supplemented diet C4 and it was lower in *Phormidium* sp. supplemented diet C2. Result of the feeding study clearly shows the acceptance of *Spirulina* diet for shrimp *P. monodon*. Jones (1998) indicated that the acceptance of formulated artificial diet is related to the growth and survival of the animal. James *et al.*, (2006) found that effect of *Spirulina* in the diet enhances feed intake and growth in red swordtail. Dietary supplementation of 40% spirulina in the diet of guppy has a positive effect on growth and FCR (Dernekbasi *et al.*, 2010). The *Spirulina* diet C4 showed better FCR, high SGR and PE than the other cyanobacterial substitution, revealed that *Phormidiuma* sp. and *Synecococcus elongatus* were less susceptible for making shrimp diet. The survival of the shrimp was higher in C4 diet. Similarly the survival rates of green tiger shrimp larvae fed with Spirulina supplemented diet was also higher than that of control (Ghaeni *et al.*, 2011). Addition of spirulina in the diet significantly improved growth, survival and feed utilization of prawn *Macrobrachium rosenbergii* (Nakagawa and Gomez-Diaz, 1995) and shrimp *Litopenaeus vannamei* (Hanel *et al.*, 2007). Cyanobacteria such as *Spirulina* sp. and *Phormidium* sp. are suitable live feed for shrimp *P. monodon* (Sivakumar *et al.*, 2011). The results of the study clearly indicated that *Spirulina* sp. in diet is more acceptable for shrimp *P .monodon*. Previous studies suggested that cyanobacterial (live) exposure through the gastrointestinal system could cause toxic effects on many aquatic animals (Fischer and Dietrich, 2000; Mohamed *et al.*, 2003; Mohamed and Hussein, 2006; Xie *et al.*, 2007). But, this study results positively support the uses of cyanobacterial meal as alternative protein source in shrimp diet preparation.

Shrimp carcasses proximate composition was influenced by dietary treatment. The moisture level of shrimp was not significantly varied in all groups, total protein was higher in shrimp fed with *Spirulina* sp. (C4) and it was lower in shrimp fed with *Phormidium tenue* (C1). Higher dietary protein and carbohydrate content of formulated diet resulted in increased level of tissue protein and nitrogen free extract (NFE) of shrimps. Compared to control group, diet with cyanobacterial meal substitution were posse’s higher tissue protein. This result agrees with the concept that increasing concentration of tissue protein depends on the protein source present in the diet. Mohanty and Dash (1995) reported that the body protein concentration of the fish was increased by *Azolla* diets. Dietary algae increase lipid deposition in

the shrimp tissues (C4). Small amount of algae added to the fish diet significantly improves growth, lipid metabolism, body composition and disease resistance (Yones *et al.,* 1989; Clarke 1988; Xu *et al.*, 1993).
In conclusion, a partial substitution of *Spirulina* meal has proved as alternative protein ingredient in *P. monodon* diet. In addition, cyanobacterial meal substitution did not give any harmful effect on growth and body composition of the shrimp. Further, concentration (*Spirulina* at various level) based study is required to formulate a suitable shrimp diet.

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