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Effects of supplementation of shrimp head meal, chitin, chitosan and chitosan oligosaccharide in feed on the growth performance and survival in early post larval stages of *Penaeus monodon* (Fabricius 1798)

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Abstract

Supplementation of shrimp shell by-products (shrimp head meal and chitin) and chitin derivatives (chitosan and chitosan oligosaccharide) in shrimp feed on feed characteristics, growth, survival and whole body composition of early post larvae of giant tiger prawns (*Penaeus monodon* Fabricius, 1798) was investigated. Five iso-nitrogenous diets were formulated with feed supplements *viz*., control (diet 1), shrimp head meal (SHM) at 6% (diet 2), chitin 5% (diet 3); chitosan 0.2% (diet 4) and chitosan oligosaccharide (COS) 0.2% (diet 5). Physical properties and proximate composition of the prepared feeds were compared. The experimental feeding trial was carried out for 30 days on early stages of post larvae of *P. monodon.* Growth performance and survival (%) were determined on 15th and 30th days of growth trial. Whereas, wholebody composition of *P. monodon* was determined at the end of the experiment. Physical properties of experimental feeds showed significant difference ($p<0.05$) in expansion ratio and water stability. Whereas, bulk density did not differ significantly. Higher body protein and survival rate were obtained for post larvae fed with diet 2, however, better growth performance was recorded for diet 4 ($p<0.05$). Significantly improved average weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency ratio (PER) values were obtained for diet 4 on the 15th and 30th days respectively ($p<0.05$). Shrimp supplemented with 0.2% of chitosan was found superior compared to other diets. in improving the growth performance, survival, and composition of early post larval stages of *P. monodon.*

Keywords: Shrimp waste derivatives, Giant tiger prawn, Specific Growth rate, Feed conversion factor, and Shrimp whole body composition.

Introduction

Dietary supplementation of certain essential nutrients in the form of additives has been proved to be valuable for improving growth performance and immunity in crustaceans and for augmenting the efficiency of aquafeeds (Ganguly et al., 2013; Chandran et al., 2017, Zhou et al., 2017; Niu et al., 2018). Fishmeal has largely been incorporated in feeds as a protein source due to its better nutritional composition, palatability, growth endorsement, and feed conversion efficiency. Shrimp require high protein feed which has caused increased inclusion percentage of fish meal in shrimp feed and consequent escalated feed costs (Sanchez-Muros et al., 2020). Therefore, other animal protein sources like fish, poultry and livestock by-products were used for replacing fish meal (Davis & Arnold, 2000; Cheng et al., 2002; Forster et al., 2004; Ye et al., 2011; Hernandez et al., 2016; Pranama et al., 2018; Bijoy et al., 2018). Shrimp body discards and their derivatives are used in aquafeeds to improve the growth and immunity of cultured shrimps (Zhu et al., 2010; Koca et al., 2011; Niu et al., 2015; Kumar et al., 2015). However, feeds without fish meal were found to affect aquaculture productivity owing to

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nutritional imbalances (Fasakin et al., 2003). Plant sources as protein alternatives in aqua-feeds were also found inadequate due to antinutritional factors and poor palatability (Tantikitti, 2014), and many such sources were reported inferior when compared to fish meal diets (Egerton et al., 2020). Therefore, the addition of aquafeeds with diverse natural dietary supplements like probiotics, prebiotics, feed enzymes, organic acids, herbal extracts, algal extracts, polysaccharides (eg: glucan and chitin), oligosaccharides (eg: mannan oligosaccharide and fructooligosaccharide), etc. have been investigated in penaeid shrimp feeds (Zhou et al., 2007; Genc et al., 2007; Sanchez et al., 2009; Niu et al., 2011; Zhang et al., 2012; Jasmanindar et al., 2018; Kumar et al., 2019).

Dietary supplements were also used for promoting health, disease resistance, and survival as intensive aquaculture practices are known to cause stress in farmed shrimps (Mir et al., 2017). Towards this, antibiotic additives are also used in farmed animals (Arockiaraj et al., 2015; Chaurasia et al., 2016), which, however, has been reported to affect shrimps negatively and cause human health hazards due to residual accumulation in tissue (Kumar et al., 2019; Kim et al., 2020). Attempts on replacing antibiotics with natural growth promoters *cum* immunostimulator as a dietary supplement are essential to achieve the target of sustainable farming and production of safe farmed aquatic food products. Chitin, protein, and minerals present in crustacean shell wastes especially from shrimp processing discards can be utilized as feed ingredients for better growth of cultured prawns (Williams et al., 2005; Kumar et al., 2006; Abdel-Ghany & Salem, 2020). Chitin is a natural biopolymer, a linear polysaccharide of the amino sugar, N-acetyl glucosamine predominantly present in the exoskeleton of crustaceans (Rasweefali et al., 2022). Kumar et al. (2015) has reported a positive effect of chitin as a feed additive on the growth and immunity of *Machrobrachium rosenbergii*. Chitosan is a deacetylated derivative of chitin which is a glucose-based unbranched polysaccharide (Sabu et al., 2020; Rasweefali et al., 2021; Sabu et al., 2022). Chitosan oligosaccharide is low molecular weight oligomers of N-acetyl glucosamine and D-glucosamine (Naveed et al., 2019; Sabu et al., 2022). Owing to immunostimulant and bacteriostatic properties, chitosan has been used as a shrimp feed additive (Khoushab & Yamabhai, 2010).

Studies on the effect of dietary supplementation of chitin and chitosan on growth, survival, and immune function of *Litopenaeus vannamei* and *Penaeus monodon* were reported by Niu et al. (2011; 2013; 2015). However, the same were conducted on juvenile shrimps having bodyweight above 1.0g. To the best of our knowledge, the supplementing effect of shrimp shell by-products and chitin derivatives on growth performance and survival of early post-larval shrimp having body weight up to 1.0g was not investigated to date. Thus, the present study aimed at delineating the effect of dietary inclusion of shrimp head meal, chitin, chitosan, and chitosan oligosaccharide on growth performance and survival of *P. monodon* early post larvae (starting at PL 20 stage), during a 30-day experimental trial.

Materials and Methods

Fish meal was prepared using silver belly fishes (*Leiognathus* spp.) sourced from a local fishing harbor (Vypin, South India) by sun drying for 3 days, before being pulverized using a mixer grinder (Maharaja Co. Ltd., India) and kept in airtight containers, till use. Fresh shrimp head waste was brought from a local seafood processing unit in Ernakulam, South India to the laboratory in iced condition and was washed, cleaned, dried, and pulverized before stored in air-tight containers as shrimp head meal. Chitin and chitosan were sourced from a commercial manufacturer, M/s India seafoods Pvt. Ltd, Cochin, South India. Chitosan was converted to chitosan oligosaccharides (COS) using diluted hydrogen peroxide following Mourya et al. (2011). Other ingredients used for diet formulation such as groundnut oil cake, soybean meal, rice bran, wheat flour, and vitamin- mineral mix (Becadexamin-capsule, GlaxoSmithKline Pharmaceuticals Ltd., India) and cod liver oil (Seacod soft gelatine capsule, Sanofi India Ltd., India) were sourced from a local market. Analytical grade chemicals and reagents supplied by M/s Merck, India were used for chemical analyses.

Five iso-nitrogenous diets (Table 1) including control (diet 1) and four experimental diets incorporating shrimp head meal 6% (diet 2), chitin at 5% (diet 3), chitosan 0.2% (diet 4) and chitosan oligosaccharide 0.2% (diet 5) were formulated following Navinchandran et al. (2014) with slight modifications. The diets were prepared as described by Bijoy et al. (2018). The percentage composition of shrimp head meal was decided as 6% for

conforming to equivalent nitrogen content in other experimental feeds. All dried ingredients were mixed according to formulations as given in Table 1 using potable water and the mix was steamed for 10 min in a pressure cooker (SS, Prestige Co., Ltd., India) before pelletizing by a laboratory (rotary) model hand pelletizer with die having 1 mm diameter. The diets were then broken to 4–5 mm size and were dried at 50°C for 18 h. The prepared diets were stored at room temperature in air-tight food-grade plastic containers until use. Before feeding the pellets were further broken and sieved to achieve an average of 0.5 mm sized crumbles.

Crude protein, crude lipid, ash content and gross energy (KJ) of the experimental diets were determined following AOAC (2000) and Su et al. (2014) and are given in Table 2. The protein and fat contents in prepared diets satisfied the approximate dietary protein (45%) and fat (8%) requirements of *P. monodon* post larvae as reported by Glencross et al. (2002).

Water stability (%) was determined according to Obaldo et al. (2002). The experimental diets were immersed in a conical flask containing 50 mL of water. The conical flask was kept on an orbital shaking incubator (CIS-24 plus, Remi laboratory equipments, India) at 100 rpm in room temperature to rouse gentle water flowing situation for periods of 0, 0.5, 1, 2, 4, and 6 h. After each time, the samples were collected by filtration through Whatman filter paper no.1 and dried at 60°C till complete drying then cooled and measured the water stability. Pellet water stability (in terms of dry matter retention) was calculated as the ratio of dry matter recovered after leaching and dry matter of original samples expressed as percentage.

Floatability or sinking property of feed pellets depends on expansion ratio (Kannadhason et al., 2009). Expansion ratios of diets were determined by following Oliveira et al. (1992). A vernier caliper was used for determining the average diameter of the pellet. The expansion ratio was considered as the raise in pellet cross-sectional area compared to die cross-sectional area.

$$\text{Expansion ratio (\%)} = \frac{\text{pellet diameter } (D_{pellet})^2 - 1}{\text{die diameter } (D^{dia})^2} \times 100$$

Bulk density as (gcm^{-3}) was determined according to Misra et al. (2002).

$$\textit{Bulk density } (gcm^{-3}) = \frac{\textit{mass of the pellet in g (M)}}{\textit{cross sectional area of the pellet in } cm^2 \textit{ (A)} \times \textit{length of the pellet in cm (L)}}$$

The experimental trial was carried out in the hatchery cum grow out complex of School of Industrial Fisheries, Cochin University of Science and Technology, South India. Post larvae of giant

Table 1. Ingredients and formulation of experimental diets.

Ingredients (g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	46.00	46.00	46.00	46.00	46.00
Ground nut oil cake	21.00	17.00	14.50	20.80	20.80
Soybean meal	7.00	7.75	9.35	7.00	7.00
Rice bran	2.00	2.00	2.00	2.00	2.00
Wheat flour	20.00	17.25	19.15	20.00	20.00
Vitamin-mineral mix	2.00	2.00	2.00	2.00	2.00
Cod liver oil	2.00	2.00	2.00	2.00	2.00
Shrimp head meal	-	6.00	-	-	-
Chitin	-		5.00	-	-
Chitosan	-	-	-	0.2	-
Chitosan oligosaccharide	-	-	-	-	0.2

*All ingredients expressed in grams per 100 g feed. Diet 1 = control feed, Diet 2 = shrimp head meal, Diet 3 = chitin, Diet 4 = chitosan and Diet 5 = chitosan oligosaccharide

Table 2. Proximate composition and gross energy of experimental diets.

Diets	Protein	Fat	Ash	Gross Energy
Diet 1	49.21 ± 0.76^{b}	8.17 ± 0.10^{ab}	11.18 ± 0.12^{a}	19.10±0.08^{b}
Diet 2	47.47 ± 0.49^{a}	8.55 ± 0.40^{b}	12.92 ± 0.10^{d}	18.60 ±0.02^{d}
Diet 3	48.12 ± 0.35^{ab}	8.25 ± 0.22^{ab}	10.68 ± 0.11^{b}	19.40 ±0.13^{a}
Diet 4	47.43 ± 1.39^{a}	8.01 ± 0.18^{a}	11.34 ± 0.22^{bc}	18.50 ±0.16^{d}
Diet 5	47.90 ± 1.02^{ab}	8.06 ± 0.14^{a}	11.45 ± 0.05^{c}	18.80 ±0.12^{c}

*Values expressed as mean ± S.D. (n = 3). Means in the same column with different letters are significantly different ($p < 0.05$). Protein, fat and ash contents are expressed in dry weight basis.

Note:- Carbohydrate (%) = 100- (moisture % + protein % + fat % + ash %), Gross energy ((KJ.(g dry matter)$^{-1}$) = 23.4 × protein % + 39.2 × fat % + 17.2 carbohydrate % . (Su et al., 2014)

tiger prawn *Penaeus monodon* (PL 15) were procured from Rajiv Gandhi Centre for Aquaculture (RGCA), Govt. of India, Cochin. The post larvae were held under quarantine conditions for 5 days to acclimatize them to experimental conditions. During the domestication period, the post larvae were fed thrice daily with a commercial feed (Tigeron, 500).

The experiments were conducted with a total of 300 *P. monodon* post larvae (PL-20) in 15 cylindrical tanks of 125 L capacities, filled with approximately 70 L sand bed filtered seawater mixed with de-chlorinated tap water for maintaining 15 ppt salinity. Twenty pre-weighed shrimps each were stocked and were fed with experimental diets for continuous 30 days. Fecal matter, unconsumed food materials, molts, etc., were removed from the tanks every morning by siphoning without disturbing water, and from each tank, 20% of water was exchanged daily, till the completion of the experiment. Continuous aeration was provided in all tanks using a 1.5 HP compressor through air stones to maintain optimum dissolved oxygen. Each tank was covered with small-mesh nets to prevent animals from escaping.

Shrimps were fed at the rate of 6% of the body weight, thrice daily with pre-weighed experimental and control diets. The uneaten feed was collected daily and was gently washed, dried, and weighed to calculate feed intake and feed conversion ratio (FCR). During the feeding trial, the amount of diet given was progressively changed and adjusted according to the appetite of the shrimp by checking for excess feed in the tank bottom. In this way, overfeeding was minimized and shrimps were fed close to satiation.

Water quality parameters such as pH, temperature, salinity, dissolved oxygen, total NH^{4+} -nitrogen, total NO_2^- / NO_3^- - nitrogen and total NO_3^- - nitrogen levels were maintained optimum (7.16 ± 0.05, 29.14 ± 0.30ÚC, 15.5 ± 0.89 ppt, 6.5 - 7.0 mgL^{-1}, < 0.5 mgL^{-1}, < 0.5 $mgL^{-1}$and < 5.0 $m.L^{-1}$ respectively) for the growth of *P. monodon* following Niu et al. (2015). The water P^H was measured by portable P^H meter (EcoTestr pH 1, EUTECH, Singapore) and the water temperature was measured using a mercury thermometer. Salinity was measured by a salinity refractometer (MCP portable handheld refractometer, Medicare products Inc., India) and dissolved oxygen by a hand-operated DO probe (Sky technology dissolved oxygen sensor STI-401, Sky Technology, India). Ammonia, nitrite, and nitrate were estimated using a standard testing kit supplied by M/s Nice chemicals private Ltd, India. pH and temperature were checked daily while other parameters were recorded once in three days. The experimental trials were carried out under natural photoperiod.

Fortnightly sampling was done to evaluate the growth performance of Post larvae of giant tiger prawn. The shrimps were starved overnight before taking body weight measurement (using precision balance, CAH-323, CONTECH, India) after they were blotted free of water using tissue paper. The growth parameters *viz.,* average weight gain (AWG) %, specific growth rate (SGR) %, feed conversion ratio (FCR), feed efficiency (FE) %, the protein efficiency ratio (PER), survival rate (SR) % were worked out following Niu et al. (2015) and Bijoy et al. (2018).

$$SR\ survival\ \% = \frac{final\ number\ of\ shrimps\ (N_f)}{initial\ number\ of\ shrimps\ (N_0)} \times 100$$

$$AWG\ average\ weight\ gain\ (\%) = \frac{mean\ final\ body\ weight\ in\ g\ (W_t) - mean\ initial\ body\ weight\ in\ g\ (W_0)}{mean\ initial\ body\ weight\ in\ g\ (W_0)} \times 100$$

$$SGR = \frac{In\ [mean\ final\ body\ weight\ in\ g\ (W_t)] - In\ [mean\ initial\ body\ weight\ in\ g\ (W_0)]}{duration\ of\ culture\ in\ days\ (t)} \times 100$$

$$FCR\ feed\ conversion\ ratio = \frac{mean\ feed\ intake\ in\ g\ (W_f)}{mean\ final\ body\ weight\ in\ g\ (W_t) - mean\ initial\ body\ weight\ in\ g\ (W_0)}$$

$$FE\ feed\ efficiency\ (\%) = \frac{final\ body\ weight\ gain\ of\ shrimp\ in\ g\ (\Delta W)}{mean\ feed\ intake\ in\ g\ (W_f)} \times 100$$

$$PER = \frac{[mean\ final\ body\ weight\ in\ g\ (W_t)] - [mean\ initial\ body\ weight\ in\ g\ (W_0)]}{[mean\ feed\ intake\ in\ g\ (W_f) \times crude\ protien\ in\ diet\ (\%)\ (P)]}$$

The whole body composition of experimental animals was analyzed following AOAC (2000). Moisture content was analyzed using a moisture analyzer (MB25, Ohaus Corporation, USA). Ash content was determined gravimetrically to constant weight in a muffle furnace (Kemi lab equipments, India) at 550°C for 4 h. Crude protein was determined by Microkjeldhal method (total N × 6.25) with Kjeldahl automatic nitrogen distillation system (Kelplus, Pelican equipments, India) and boric acid was used to trap ammonia released. Crude lipid was determined gravimetrically after extraction with petroleum ether using soxhlet apparatus.

$$Moisture\ \% = \frac{loss\ in\ weight\ (g)}{Weight\ of\ sample\ taken\ (g)} \times 100$$

$$Nitrogen\ (\%) = \frac{14.01 \times Normality\ of\ acid \times (Titer\ value\ blank)}{sample\ weight \times 1000} \times 100$$

$$Fat\ (\%) = \frac{Weight\ of\ fat\ (g)}{Weight\ of\ sample\ taken\ (g)} \times 100$$

$$Ash\ (\%) = \frac{Weight\ of\ ash\ (g)}{Weight\ of\ sample\ taken\ (g)} \times 100$$

The feeding trial was undertaken with a completely randomized design, with five dietary treatments and three replicates per treatment. Data from triplicate tanks of each diet were analyzed by one-way analysis of variance using SPSS software (release 18 for Windows). Duncan's multiple range tests were used to determine the differences between the treatment means. Results were considered statistically significant at the level of $p<0.05$.

Results and Discussion

Physical properties of experimental diets such as expansion ratio, bulk density, and water stability are given in Table 3 and 4. Bulk density and Expansion ratio results were statistically significant ($p<0.05$) and were highest in diet 3 (1.15 ± 0.42 and 75.17 ± 10.00 respectively). Water stability was found to gradually decrease from a range of 93.20 ± 0.20 to 97.10 ± 1.70 at 0 min and a range of 55.15 ± 4.65 to 64.87 ± 0.90 at 360 min in experimental diets (Table 4). Significantly higher water stability could be noted in diets 3 to 5 after 240 min ($p<0.05$).

Table 3. Bulk density and expansion ratio of experimental diets

Diets	Bulk density	Expansion ratio
Diet 1	1.00 ± 0.09^{ab}	68.67 ± 10.97^{a}
Diet 2	0.84 ± 0.01^{a}	74.58 ± 19.63^{b}
Diet 3	1.15 ± 0.42^{b}	75.17 ± 10.00^{b}
Diet 4	0.83 ± 0.08^{a}	75.00 ± 0.00^{b}
Diet 5	0.86 ± 0.11^{a}	75.00 ± 0.00^{b}

*Values expressed as mean ± S.D. (n = 3). Different letters within the same column are significantly different ($P < 0.05$).

The growth parameters and survival (%) of the post larvae were measured on the 15th and 30th day of the feeding trial (Table 5). Survival rates (%) were more or less similar (about 95%) and were not significantly different ($p>0.05$) on the 15th day of the experiment. However, on the 30th day, better survival (%) was recorded in post larvae fed with diet 3 (87.23 ± 1.42%) followed by diet 4 (83.30 ± 2.41%) while lowest survival was recorded in post-larvae fed with diet 1 (65.00 ± 8.66%) ($p<0.05$). Results on AWG, SGR, FCR, FE, and PER were significantly different among treatments on the 15th

and 30th day of the experimental trial ($p<0.05$). Diet 4 recorded highest AWG (880.00 ± 190.79), SGR (7.57 ± 0.62), FE (101.25 ± 7.69), PER (2.11 ± 0.16), and lowest FCR (0.99 ± 0.07) values compared to other diets ($p<0.05$).

The whole body composition of *P. monodon* post larvae at the end of 30 days of the experimental trial (Table 6). It could be observed that moisture contents recorded from post larvae fed with various trial diets were not significantly different ($p>0.05$). However, post larvae fed with diet 3 recorded significantly higher ($p<0.05$) crude protein content (18.87 ± 0.00%) while post larvae fed with diet 2 recorded the lowest (17.10 ± 0.69%). The crude fat content was significantly high in post larvae fed with a control diet (2.96 ± 0.10%) when compared to the other four diets ($p<0.05$). Crude fat contents of post larvae fed with diet 2, diet 3 and diet 5 were considerably low compared to diet 1. Ash content of post larvae fed with diet 1 was high (5.50 ± 0.71%) and was lowest in post larvae fed with diet 5 (4.01 ± 0.00%).

In the present, shrimps fed with shrimp shall waste derivatives showed positive growth and survival in *P. monodon.* Shrimp shell waste derivatives containing diets in the present study gave positive results in *P. monodon* growth and survival. Composition and physical properties of the feed play a major role in

Table 4. Water stability of experimental diets.

Diets	Time (in min)					
	0	30	60	120	240	360
Diet 1	96.30 ± 0.60^{b}	72.90 ± 1.50^{a}	67.85 ± 1.85^{a}	66.50 ± 2.00^{a}	60.30 ± 0.70^{a}	56.93 ± 2.25^{a}
Diet 2	97.10 ± 1.70^{b}	73.20 ± 1.20^{a}	64.95 ± 1.85^{a}	63.95 ± 0.45^{a}	63.00 ± 1.40^{b}	55.15 ± 4.65^{a}
Diet 3	94.75 ± 2.05^{ab}	72.95 ± 1.55^{a}	71.10 ± 3.90^{a}	71.20 ± 3.10^{b}	68.35 ± 1.05^{d}	63.47 ± 1.35^{b}
Diet 4	96.50 ± 0.20^{b}	73.60 ± 0.40^{a}	70.80 ± 0.10^{a}	67.80 ± 1.90^{ab}	67.05 ± 1.45^{cd}	64.87 ± 0.90^{b}
Diet 5	93.20 ± 0.20^{a}	74.20 ± 1.56^{a}	67.80 ± 6.15^{a}	66.67 ± 1.78^{a}	65.83 ± 0.67^{c}	62.13 ± 1.08^{b}

*Values expressed as mean ± SD (n = 3). Different letters within the same column are significantly different ($p<0.05$).

Table 5. Growth performance of *P. monodon* post larvae during 15 & 30th days of experimental trial.

Diets	SR	AWG	SGR	FCR	FE	PER
			15th day			
Diet 1	94.50 ± 1.80	126.67 ± 11.55^{a}	5.45 ± 0.34^{a}	1.75 ± 0.15^{d}	57.44 ± 5.24^{a}	1.17 ± 0.11^{a}
Diet 2	95.77 ± 2.37	353.33 ± 61.10^{cd}	10.04 ± 0.89^{c}	1.40 ± 0.23^{bc}	72.57 ± 12.56^{ab}	1.53 ± 0.27^{ab}
Diet 3	97.00 ± 1.80	220.00 ± 20.00^{ab}	7.74 ± 0.42^{b}	1.53 ± 0.14^{cd}	65.71 ± 5.97^{ab}	1.37 ± 0.12^{ab}
Diet 4	95.33 ± 1.04	460.00 ± 121.66^{d}	11.39 ± 1.38^{c}	1.02 ± 0.24^{a}	102.23 ± 27.03^{c}	2.16 ± 0.57^{c}
Diet 5	94.17 ± 1.44	260.00 ± 20.00^{bc}	8.53 ± 0.37^{b}	1.12 ± 0.11^{ab}	89.50 ± 8.21^{bc}	1.87 ± 1.87^{bc}
			30th day			
Diet 1	65.00 ± 8.66^{a}	286.67 ± 11.55^{a}	4.51 ± 0.10^{a}	1.54 ± 0.06^{c}	65.00 ± 2.62^{a}	1.32 ± 0.053^{a}
Diet 2	83.20 ± 3.15^{bc}	873.33 ± 144.68^{c}	7.56 ± 0.48^{c}	1.13 ± 0.17^{ab}	89.71 ± 14.86^{bc}	1.89 ± 0.31^{bc}
Diet 3	87.23 ± 1.42^{c}	493.33 ± 46.19^{b}	5.93 ± 0.27^{b}	1.37 ± 0.14^{bc}	73.66 ± 6.89^{ab}	1.53 ± 0.14^{ab}
Diet 4	83.30 ± 2.41^{bc}	880.00 ± 190.79^{c}	7.57 ± 0.62^{c}	1.00 ± 0.07^{a}	101.25 ± 7.69^{c}	2.11 ± 0.16^{c}
Diet 5	77.00 ± 3.40^{b}	606.67 ± 46.19^{b}	6.51 ± 0.21^{b}	1.05 ± 0.20^{a}	97.79 ± 21.21^{c}	2.06 ± 0.45^{c}

*Values expressed as mean ± SD (n=3). Different letters within the same column are significantly different ($p<0.05$).
SR survival (%), AWG average weight gain (%), SGR specific growth rate (%), FCR feed conversion ratio, FE feed efficiency (%), PER protein efficiency ratio

Table 6: Whole body composition of *P. monodon* post larvae fed with different experimental diets (wet weight basis).

Diets	Moisture	Protein	Fat	Ash
Diet 1	73.02 ± 3.67	17.94 ± 0.00^{b}	2.96 ± 0.10^{c}	5.50 ± 0.71^{b}
Diet 2	74.83 ± 2.59	17.10 ± 0.69^{a}	2.27 ± 0.04^{a}	4.07 ± 0.63^{a}
Diet 3	73.79 ± 1.14	18.87 ± 0.00^{c}	2.37 ± 0.04^{a}	4.23 ± 0.49^{a}
Diet 4	74.35 ± 2.01	17.59 ± 0.24^{ab}	2.50 ± 0.08^{b}	4.64 ± 0.31^{ab}
Diet 5	74.93 ± 3.37	18.09 ± 0.35^{b}	2.30 ± 0.07^{a}	4.01 ± 0.00^{a}

*Values expressed as mean ± SD (n=3). Different letters within the same column are significantly different ($p<0.05$).

the success of shrimp farming operations (Bijoy et al., 2018). Bulk density (1.15 ± 0.42) and Expansion ratio (75.17 ± 10.00) were highest in diet 3 which can be attributed to the physical nature of chitin. Knott et al. (2003) have reported that bulk density and expansion ratio of feeds are considerably influenced by the particle size of ingredients. The present results of the expansion ratio and bulk density of experimental feeds are comparable to Bijoy et al. (2018). The highest water stability is a desirable quality for shrimp feeds as they can be left uneaten for a prolonged time as shrimp feeding habits differ considerably from fish feeding habits (Ninawe & Khedkar, 2009). Among the different diets, highest water stability at 360 min (64.87 ± 0.90) was observed in the diet 4.

Shiau & Yu, (1998) accounted higher body protein content in shrimp fed with 5% chitin diet than those with chitosan diet or control diet. High protein content in post-larvae fed with diet 3 may be due to improved chitin digestibility owing to the appropriate inclusion of chitin at 5 % (Shiau & Chou, 1991; Clark et al., 1993). The low protein composition in post-larvae fed with diet 2 can be attributed to the low absorption of protein and nutrients in shrimp gut epithelium as reported by Kumar at al. (2006). It has been reported that high shrimp waste meal supplementation in diets de-presses crude fat content (Lu & Ku, 2013). Lower body protein and lipid contents were depicted by Shiau & Yu (1998) in shrimp fed chitosan-containing diets than in controls. Chitosan is also known to affect fat metabolism and inhibit fat intake in the gut (Shiau & Yu, 1998; Koushab & Yamabhai, 2010).

It could be observed that post larvae fed with 5% chitin (Diet 3) registered a high survival rate (%) on the 15th and 30th days of the experimental trial which is in full agreement with the findings of Kumar et al. (2006) and Shiau & Yu (1998) who also reported improved survival rate for *M. rosenbergii* and *P. monodon* respectively with 5% chitin diet. According to Nahavandi et al. (2010), the average weight gain by *P. monodon* generally varied from 111.17 to 874.00% in farm trials. On the 15th and 30th day, the AWG in post-larvae fed with diet 4 was 460.00 ± 121.66% and 880.00 ± 190.79% respectively. The AWG obtained in the current study was on the higher side when compared to values reported by Niu et al. (2015). Further, SGR of postlarvae fed with diet 4 and diet 2 on the 30th day was 7.57 ± 0.62% and 7.56 ± 0.48% respectively. It has been reported that *P. monodon* fed with mannan oligosaccharide recorded high SGR values as 2.51% (Zhang et al., 2012) and 4.40% (Navinchandran et al. (2014). Based on the previous studies the percentage inclusion of chitin, chitosan and chitosan derivatives were selected (Shiau & Yu, 1998; Kumar et al., 2006; Powell & Rowely, 2007 and Niu et al., 2011; 2015).

In the present study, significantly lower FCR was recorded both on the 15th (1.02 ± 0.24 and 1.12 ± 0.11) and 30th days (1.00 ± 0.07 and 1.05 ± 0.20) for Diet 4 and Diet 5 respectively compared to diet 1 (1.75 ± 0.15 and 1.54 ± 0.06) ($p<0.05$). The FCR values in respect of Diet 4 and 5 are comparable to the same in chitosan and COS incorporated diets reported by Niu et al. (2013). SHM incorporated diet (diet 3) obtained significantly low FCR on both 15th day and 30th day than control diet ($p<0.05$) which shows better utilization of diet 3 for growth and body weight gain than diet 1. SHM might have acted as an attractant for postlarvae which might be the reason for its lower FCR. Williams et al. (2005) reported that the inclusion of shrimp head meal produces higher feed consumption and growth in *P. monodon*. Inclusion of fermented shell waste in shrimp feeds yielded an FCR of 1.7 in *Fenneropenaeus indicus* (Amar et al., 2006). According to Nwanna

(2003), FCR in shrimps can be anticipated between 2 to 3, because of loss incurred during moulting and order of acceptance of the feeds varies with different species. However, the lowest possible FCR is welcomed in shrimp farming as it positively curtails feed cost (Maheswarudu et al., 2016). In the present study; FCR of chitosan diet was lower (1.00 ± 0.07). The feed efficiency (FE) is reciprocal to FCR and significantly higher values of FE were recorded in chitosan and COS incorporated diets on the 15th and 30th day in contrast to the control diet ($p<0.05$). Similar results could also be recorded in PER. Niu et al. (2015) have reported comparable PER (1.84 ± 0.08) in 0.2% chitosan incorporated diet. On the contrary, PER values were significantly lower in diets 1 to 3 on the 15th and 30th days of observation ($p<0.05$).

The present results revealed that crude chitosan (0.2%) incorporated diet (Diet 4) gave better survival and growth in *P. monodon* postlarvae. It appears that suitable dietary inclusion of chitosan could enhance the digestion and absorption of nutrients, increase body protein synthesis, and thus yielded better growth performance as reported before (Gopalakannan & Arul, 2006). Dietary inclusion of chitosan at higher concentrations (2%, 5%, and 10%) has been reported to have a negative effect on the growth performance of aquatic animals (Shiau & Yu, 1998; 1999). According to Niu et al. (2013), chitosan at elevated concentrations may surpass the absorbable level of juvenile *P. monodon*, nevertheless, chitosan may play an essential role in enhancing the digestion and absorption of nutrients at lower levels. Attasart et al. (2005); Niu et al. (2011; 2013) reported that feed supplementation with chitosan inappropriate levels yielded enhanced growth and survival in commercially cultivated organisms. According to Niu et al. (2015), 0.1% and 0.2% of chitosan supplementation in feed enhanced the growth of *P. monodon* juveniles, while higher inclusion of 0.3% and 0.4% decreased growth and they recommended 0.2% chitosan in the feed as optimum for juveniles of *P. monodon*. The present investigation confirms 0.2% inclusion of crude chitosan in diet enhanced survival and growth performance in *P. monodon* post larvae. Compared to other crustacean shell waste meal / derivatives, chitosan will be ideal for commercial and organic shrimp farming in aspects to improve feed quality, water quality, shrimp health, and the culture system sanitation as stated by Attasart et al. (2005).

The present study further revealed that shrimp head meal can also be used as a good dietary supplement for early postlarvae of *P. monodon* like chitosan and chitin. It may also be pointed out that Williams et al. (2005) and Koca et al. (2011) recommended 5% and 10% inclusion of SHM in shrimp diets. Another important outcome of the present study is that 5% dietary chitin is less effective for *P. monodon* than 6% SHM and 0.2% chitosan, which is in contrast to findings of Shiau & Yu (1998), and Powell & Rowely (2007). Fox (1993) reported that chitin cannot be utilized by *P. monodon* directly. According to Kumar et al. (2006), chitin is better accepted by *Macrobrachium rosenbergii* in its natural form (SHM) than in purified form. In a comparative study conducted by Niu et al. (2013), a chitin-containing diet was found less efficient than a chitosan diet in promoting the growth of *P. monodon* juveniles.

Among the diets under the study, *c*hitin, and chitosan incorporated diets recorded better water stability than shrimp head meal, chitosan oligosaccharide, and control diets. The experimental growth trial revealed that the diet with 0.2% chitosan supplementation significantly improved the growth performance of early stages of post larvae, *P. monodon* compared to other diets without affecting the survival and composition. It could also be recorded that next to the chitosan diet, a diet with shrimp head meal yielded better growth performance. The utilization of shrimp shell wastes and their derivatives especially chitosan would be recommended for feed manufacturers and shrimp farmers as they are found effective in reducing production cost, dependence on fishmeal, and pollution issues from shell waste discard.

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