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Influence of dietary chitosan on the meat composition, amino acid, fatty acid profiles and growth of the black tiger shrimp (*Penaeus monodon*)

Ancy Ashraf¹, Sarasan Sabu^{1*} and Mahadevan Harikrishnan¹

Abstract

The black tiger shrimp (*Penaeus monodon*) is the second best cultivated crustacean species and is a significant exportearning source for South Asian countries. Its meat offers quality protein, and well-balanced essential amino acids. The present investigation is a pioneering attempt to delineate the nutritional composition, amino acid and fatty acid profiles of the meat of the cultured *P. monodon* fed with chitosan diet along with the growth performance. Penaeus monodon juveniles (initial weight of 0.056 g) were fed thrice daily for 105 days to investigate the effect of dietary supplementation of chitosan at 0.2% level. Chitosan supplementation significantly improved growth performances, survival rate and low feed conversion ratio (p < 0.05). The chitosan-fed shrimps had higher compositions of the essential amino acids (tryptophan, phenylalanine, leucine, and lysine), enhanced saturated and monounsaturated fatty acids, chitin nitrogen, total nitrogen, and crude protein content (77.29 \pm 1.91%) but lowered crude fat content (17.02 \pm 3.40%) compared to the control group. The study recommends chitosan supplementation at 0.2% in shrimp feed for enhanced growth and survival of the Penaeus monodon juveniles with superior meat quality and nutritional composition.

Keywords Chitosan, Shrimp feed, Nutritional profile, Natural growth promoter

Introduction

Globally, shrimp is considered a chief economically and nutritionally imperative seafood and protein source. Shrimp culture is one of the constantly growing commercial aquaculture practices around the globe that meet the increasing demand for seafood. Feed accounts for over 50% of the total costs in aquaculture, and aqua feeds should comprise macronutrients and micronutrients [12]. Recently researchers attempted supplementation of growth-promoting and immune-stimulating natural

ingredients in aquafeeds to make aquaculture profitable and sustainable [9, 30, 78]. Additives are supplemented in aquaculture feeds in small amounts in combination to advance the quality of shrimp/aquatic environment or to preserve the feed itself [8]. The search for sustainable and safe ingredients for aquafeed manufacture is gaining importance among aqua culturists and aquafeed industries. Crustacean shell waste extractives such as chitin, chitosan, protein and minerals can be effectively utilized as feed components for ensuring growth in farmed shrimps [83] and fish [1, 8, 9, 41]). Dietary supplementation of essential nutrients and natural organic supplements by including chitosan in feed has been proven to be valuable for enhancing shrimp growth and immunity [9, 54, 95]. Chitosan, the deacetylated form of chitin and

*Correspondence: Sarasan Sabu sabuif@cusat.ac.in

¹ School of Industrial Fisheries, Cochin University of Science and Technology, Lakeside Campus, Cochin, Kerala 682016, India



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a natural carbohydrate polymer, is insoluble in water [8]. Chitosan has potential applications in food, pharmaceutical, agricultural, and environmental industries owing to its antioxidant, antimicrobial, immuno-enhancing, anti-inflammatory, hypocholesterolemic, and anti-tumor effects. Moreover, chitosan is innately biocompatible, non-toxic, and non-allergenic [9, 63, 65, 97].

The black tiger shrimp (Penaeus monodon) is the second supreme cultivated crustacean species and is a significant export-earning source for South Asian countries [60]. Its meat offers quality protein, well-balanced essential amino acids with substantial contents of choline, taurine, omega-3 fatty acids, certain antioxidants, vitamins A, D3 and B12, and minerals like calcium, phosphorus, iron, zinc, copper, magnesium and iodine and has lower calorific value than many terrestrial animal protein sources [4, 31, 73, 89]. Shrimp protein has higher bioavailability than other protein sources, and nutrients in shrimps are mostly absorbed into the human body without any loss, as they are effortlessly digestible [22]. Even though crustacean muscle meat contributes mainly to protein, polyunsaturated fatty acids such as EPA and DHA are also present luxuriously in it [51]. P. monodon meat production recorded 550,000 MT in 2023 through intense fishery exploitation and mariculture/ brackish water aquaculture practices [23]. In India, farmed production of *P. monodon* has been registering a sustainably increasing trend over the years, while P. vannamei yield depressed 12% in 2023 [23].

Application of additives in aquafeeds such as plant/ algal/herbal extracts, prebiotics, polysaccharides, probiotics, organic acids, and their effect on growth, survival and immunity have been attempted in penaeid shrimps by many authors [9, 34, 40, 56, 94, 96]. Fish meal replacement with alternate plant and animal protein sources has revealed the dietary effect of protein sources on growth/ feeding indices, muscle composition, and fatty/amino acid profile of *P. vannamei* [14, 25, 44, 74, 91]. Ragni et al. [59] evaluated the growth performance and fatty acid composition of *P. japonicus* with diets containing fresh seafood discards as a replacement for fishmeal. Shrimp body discards and by-products, including chitosan, have been tried in aquafeeds to enhance the growth and immunity of farmed shrimps [9, 39, 53]. Positive results have been reported in the growth performance, survival and immune function of P. monodon on feeding with chitosan-supplemented feeds [9, 53, 55, 56].

Dietary supplementation effects of natural additives including chitosan derivatives on the meat quality and nutritional composition in pigs [47, 58, 71, 79, 90], and poultry have been investigated recently [10, 42, 80]. However, to the best of our knowledge, the influence of chitosan or its derivatives on the nutritional composition

of meat along with the growth performance of cultured shrimp of any variety has not reported till date. Hence, the present investigation aims to delineate the influence of dietetic supplementation of chitosan on the growth performance and nutritional composition of the cultured *P. monodon* fed with chitosan diet.

Materials and Methods

Feed

In juvenile black tiger shrimp feed, Niu et al. [53] recommended 0.19 – 0.21% inclusion of chitosan after confirming with second degree polynomial analysis. A preliminary investigation by the authors [9] recommended 0.2% chitosan as the best feed supplement for achieving better growth and survival in *P. monodon*, in agreement with the findings (0.2% chitosan) of Niu et al. [53]. Thus, in the current investigation, 0.2% chitosan was selected and included in the experimental diets to investigate the growth and whole-body composition/nutrient levels in experimental shrimps. All the ingredients for experimental feed preparations (Table 1) were procured from a local market in Cochin, Kerala, India.

The dry feed components were assorted (according to proportions, as mentioned in Table 1) then made into dough, by adding wet ingredients and distilled water. Feed noodles were prepared using a kitchen-type hand-operated (rotary) extruder and a 1 mm diameter die after pressure cooking the dough. Dried the feed at 55 °C for 15 h in a hot air oven (Rotek laboratory instruments, India), broken to 2–3 mm and kept at ambient temperature until feeding.

Table 1 Ingredients and formulation of experimental diets

Ingredients (g)	Control diet	Chitosan diet	
Fish meal	46.00	46.00	
Ground nut oil cake	21.00	20.80	
Soybean meal	7.00	7.00	
Rice bran	2.00	2.00	
Wheat flour	20.00	20.00	
Vitamin-mineral mix	2.00	2.00	
Cod liver oil	2.00	2.00	
Chitosan	-	0.2	
Proximate composition (%)			
Crude Protein	49.21 ± 0.76	47.43 ± 1.39	
Crude Fat	8.17±0.10	8.01 ± 0.18	
Ash	11.18±0.12	11.34±0.22	
Energy	19.10±0.08	18.50 ± 0.16	

All ingredients are expressed in grams per 100 g feed

Proximate composition values were expressed as mean \pm S.D. (n = 3). Protein, fat and ash contents are expressed on a dry weight basis. Carbohydrate (%) = 100-(moisture %+ protein %+ fat %+ ash %), Gross energy (KJ/g dry matter) = 23.4 X protein %+ 39.2 X fat %+ 17.2 carbohydrate % [72]

Feeding trial: shrimp and experimental set-up

Juveniles of black tiger shrimp (*Penaeus monodon*) were procured from a local shrimp hatchery (Queen's Hatchery, Kerala, India). They were kept in separate tanks for 15 days to acclimatize them to investigational situations. During domestication, the animals were fed thrice daily with a commercial nursery-grade feed at the rate of 6% of body weight.

During the experimental trial, six 500-L cylindrical tanks were filled with 350 L of filtered seawater and diluted to 15 parts per thousand by combining with dechlorinated tap water. Fifty pre-weighed juvenile shrimps were stocked in each tank and were fed with control and experimental feed containing 0.2% chitosan for 105 days. Pre-weighed experimental and control diets were fed (at 6% of the body weight following Niu et al. [55]) thrice daily. Faeces, leftover food, moults, etc. were siphoned out every morning without upsetting water and water exchange at the rate of 20% was followed every day. To determine feed intake and conversion ratio (FCR), the unutilized feed from the tank was collected daily and carefully cleaned, dried, and weighed. The amount of food given during the feeding trial was gradually adjusted by monitoring the shrimp's appetite and looking for extra feed in the tank bottom. Shrimps were fed almost to satiation in this manner, minimizing overfeeding. The experimental tanks were protected with minor mesh webbings to avoid shrimps from jumping from the water, and the tank water was given continuous aeration using a 1.5 HP compressor.

Water quality indices including temperature, pH, salinity, dissolved oxygen, ammonia, nitrate and nitrite- nitrogen were sustained at optimal level for the growth of P monodon (28.78 \pm 1.78°C, 15.9 \pm 0.57 ppt, 6.5—7.0 mg/L, 7.14 \pm 0.17, < 0.5 mg/L, < 0.5 mg/L, and < 0.05 mg/L respectively) following Ashraf et al. [9]. Temperature and pH (EUTECH, Singapore) were checked daily, while other parameters like salinity (Refractometer, Medicare Inc., India), dissolved oxygen (STI- 401, Sky technology, India), ammonia, nitrite and nitrate (standard testing kit supplied by M/s Nice Chemicals Private Ltd, India) were recorded once in three days. The experimental trials were carried out under a natural photoperiod with proper ventilation.

Sampling and evaluation of growth performance

For the first two months of the trial, the growth performance of the experimental animals was assessed every 30 days; after that, it was assessed every 15 days until the experiment was over. The shrimps were starved overnight and were subjected to measuring body weight (CAH-323 precision balance, CONTECH, India) and blotted free of

water for measuring length (using a vernier calliper). The growth criteria, viz. average weight gain (AWG), average length gain (ALG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency (FE), protein efficiency ratio (PER), and survival % were calculated following Bijoy et al. [12] and Ashraf et al. [9].

Biochemical composition of shrimp

Overnight-starved shrimp were randomly selected from the control and chitosan-fed tanks towards the conclusion of the experimental trials. The biochemical compositions of experimental shrimp meat and whole body were analyzed towards the end of experimental trials following AOAC [5, 6]. A moisture analyzer (MB25, Ohaus Corporation, USA) was used to determine the moisture content. Using gravimetric analysis and a muffle furnace (Kemi lab equipment, India) set at 550 °C, the ash content was estimated. The Microkjeldhal method was used to determine the amounts of total nitrogen and crude protein. A Kjeldhal automatic nitrogen distillation unit (Kelplus, Pelican equipment, India) was used, along with boric acid, to trap ammonia released, and crude protein estimated (conversion factor = 6.25). Crude lipid was estimated gravimetrically after extraction (Soxhlet apparatus) with petroleum ether. The dried sample was digested in a 5% NaOH solution, followed by an estimation of total nitrogen by the Microkjeldhal method [21] for chitin nitrogen. The proximate composition values were expressed on a dry weight basis. The FAO [20] method was used to estimate the energy content, which was computed as follows: proteins, 4.27 kcal/g wet weight, lipids, 9.02 kcal/g wet weight; carbohydrates, 4.11 kcal/g wet weight (1 kcal = 4.184 kJ).

Amino acid Profiling

Amino acid profiling was done following Nimbalkar et al. [52]. In short, 5 mL of 0.1 per cent formic acid in 20 per cent methanol was used to homogenize a known sample to extract free amino acids. Extraction of bound amino acids was done via acid hydrolysis. Then, centrifuged (at 10,000 g for 15 min) and amplified the volume to 10 mL. From that, 0.5 ml sample was made up to 2 ml and filtered through 0.2 μ m nylon filter membrane and injected (temperature (25 °C); flow rate (0.1 mL/min); and injection volume (5 μ L)) to UPLC-MS/MS system (Waters, USA). A PDA detector and a UPLC column effluent pump were used to directly monitor the eluted amino acids, without splitting them into the TQD-MS/MS (Waters, USA) system, designed specifically for analysing amino acids.

Fatty acid profiling

Crude Fat was extracted from a moisture-free sample using the Soxhlet extraction method following AOAC [5, 6] for further analysis. Fatty acid derivatization and

fatty acid profile were determined following AOAC official method 969.33. Methyl esters of fatty acids were prepared by saponification with methanolic NaOH, followed by transesterification with BF₃, and detected by gas chromatography. Briefly, in a round bottom flask, crude fat (100 mg) was weighed, and 4 mL of 0.5 N methanolic NaOH was refluxed until the fat globules disappeared. Added 5 mL of 14% Boron trifluoride (w/v) and refluxed (2 min) 4 mL heptane, poured through the condenser, cooled down, and added 15 mL saturated NaCl. Collected heptane layer injected in GCMS/GCFID (6890N gas chromatograph coupled with 5975 Mass selective detector column & flame ionization detector (Supelco, Inc., Bellefonte, PA, USA).

Statistical analyses

There were two dietary treatments with three replicates for each treatment in the fully randomized feeding trial. A paired sample student 't' test with two tails was used to analyze data from triplicate tanks of each diet using Microsoft Excel software (version 2406, Microsoft, United States of America). Every sample used for nutrient profiling and whole-body composition analysis was examined three times, and the results were presented as mean \pm standard deviations. At p < 0.05, the results were deemed statistically significant.

Results

Growth performance

The average length gain and mean length of juvenile shrimps progressively increased in both control and chitosan diet-fed groups, but the advancement in length gain was slower in control. On the 105th day of the feeding trial, the chitosan-fed shrimps attained a significantly higher mean length of 7.50 ± 0.30 cm compared to 6.30 ± 0.40 cm attained by the shrimps fed with the control diet (p < 0.05) (Fig. 1 (a) and (b)). However, on day 90, the mean length indicated statistical similarity between the control and test sets (p > 0.05). Average length gain on day 60 and day 75 were also statistically similar in the two

groups (p > 0.05). Though the mean final weight increased in both feed groups, it was significantly higher in chitosan-fed shrimps (p < 0.05) except on day 75 (p > 0.05). Further, in contrast to the control group, AWG and SGR were considerably better for chitosan-fed animals in all the sampled days (p < 0.05). On Day 105, the average weight gain of test animals (4318.92 \pm 639.93) was almost double that of control diet-fed animals (2602.48 \pm 439.47). The SGR on the 105th day of the experimental growth trial was 3.60 ± 0.13 and $3.13 \pm 0.16\%$, respectively, for both chitosan and control-fed animals (Table 2).

With the exception of day 105, the shrimps fed chitosan had a significantly better survival rate (p<0.05) than the control group on days 30, 60, 75, and 90 of the feeding study. Feed conversion ratios were statistically lower for the incorporated diet, and on days 30 and 60, the feed efficiency and protein efficiency ratios for the chitosan diet were significantly higher than those for the control diet (p<0.05), although they were comparable to the other days under analysis (p>0.05). The lowest FCR (1.21±0.17) obtained in the investigation was for the chitosan diet on day 105 (Table 2).

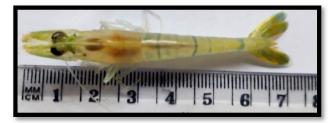
Composition of *P. monodon* juveniles fed with control and chitosan diets

Moisture content was higher in meat for control animals than in chitosan-fed animals (Fig. 2). Shrimp meat possessed the highest crude protein content on a dry weight basis (75.35 \pm 0.29 and 77.29 \pm 1.91%) than the whole body in both groups. The whole body and meat of shrimp fed chitosan had less crude fat than the control. Nonetheless, on a wet weight basis, the difference in crude fat was insignificant in meat between the test and control (p>0.05). Ash content in control and chitosan diet-fed shrimps were comparable and were non-significant in the whole body (p>0.05), very slightly upper in the meat of control animals (p<0.05) (Fig. 2). Chitin nitrogen was marginally higher in chitosan-fed than in control tiger shrimp in the whole body and meat. Even so, it was not significant statistically (p>0.05). Between the control and chitosan, the energy



(a). Control shrimp on day 105

Fig. 1 (a) Control shrimp on day 105, (b) Chitosan-fed shrimp on day 105



(b). Chitosan-fed shrimp on day 105

Table 2 Growth performance of *P. monodon* during experimental trial

Duration	Diet	L _t (cm)	ALG %	W _t (g)	AWG %	SGR%	Survival %	FCR	FE%	PER
Day 30	Control	2.50 ± 0.00	25.00 ± 0.50	0.210 ± 0.00	272.60 ± 3.09	4.38±0.03	91.00 ± 1.00	1.55 ± 0.01	64.48±0.28	1.37 ± 0.01
	Chitosan	2.77 ± 0.06	37.50 ± 0.50	0.227 ± 0.00	303.20 ± 1.26	4.65 ± 0.01	96.33 ± 1.53	1.42 ± 0.00	70.42 ± 0.04	1.48 ± 0.00
Day 60	Control	4.30 ± 0.00	114.17 ± 1.76	0.638 ± 0.00	1033.61 ± 7.35	4.05 ± 0.01	82.00 ± 0.50	1.64 ± 0.00	61.12 ± 0.00	1.30 ± 0.00
	Chitosan	4.50 ± 0.00	125.00 ± 1.00	0.799 ± 0.00	1318.49 ± 5.40	4.42 ± 0.01	88.50 ± 0.50	1.50 ± 0.00	66.51 ± 0.04	1.40 ± 0.00
Day 75	Control	5.23 ± 0.15	161.67 ± 7.64	0.904 ± 0.08	1505.39 ± 136.55	3.70 ± 0.12	77.30 ± 0.46	1.70 ± 0.17	59.38 ± 5.81	1.26 ± 0.12
	Chitosan	5.70 ± 0.20	185.00 ± 10.00	1.148±0.13	1938.37 ± 223.85	4.01 ± 0.15	82.47 ± 0.47	1.52±0.18	66.44 ± 7.91	1.40 ± 0.17
Day 90	Control	6.07 ± 0.50	203.33 ± 25.17	1.226 ± 0.28	2075.30 ± 490.09	3.40 ± 0.26	73.43 ± 0.40	1.50 ± 0.38	69.45 ± 16.87	1.47 ± 0.36
	Chitosan	6.70 ± 0.30	235.00 ± 15.00	1.898 ± 0.27	3269.53 ± 475.88	3.90 ± 0.16	76.60 ± 1.51	1.26 ± 0.18	80.43 ± 11.99	1.70 ± 0.25
Day 105	Control	6.30 ± 0.40	215.00 ± 20.00	1.523 ± 0.26	2602.48 ± 439.47	3.13 ± 0.16	69.07 ± 0.93	1.76±0.31	57.90 ± 10.18	1.23 ± 0.22
	Chitosan	7.50 ± 0.30	275.00 ± 15.00	2.489 ± 0.37	4318.92 ± 639.93	3.60 ± 0.13	70.43 ± 0.40	1.21 ± 0.17	84.17 ± 12.76	1.78 ± 0.27

The table displays the Mean \pm SD of three replicates for each result. On the first day of the growth trial, the average starting body weight (W0) and length (L0) were 0.056 ± 0.00 and 2.00 ± 0.00 , respectively

L_t: mean final body length in cm; W_t: mean final body weight in g

ALG: Average length gain (%) = $100 \times [\text{mean final body length in cm } (L_t) - \text{mean initial body length in cm } (L_0)] / \text{mean initial body length in cm } (L_0) = 100 \times [\text{mean final body weight in g } (W_t) - \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0) / \text{mean initial body weight in g } (W_0)] / \text{mean initial body weight in g } (W_0) / \text{mean initial bod$

FE: feed efficiency (%) = $100 \times \text{final bodyweight gain of shrimp in g } (\Delta W)$ /mean feed intake in g (W_t)

PER: protein efficiency ratio = [mean final body weight in g (Wt)—mean initial body weight in g (W_0)] / [mean feed intake in g (W_0) x crude protein in the diet (%) (P)]

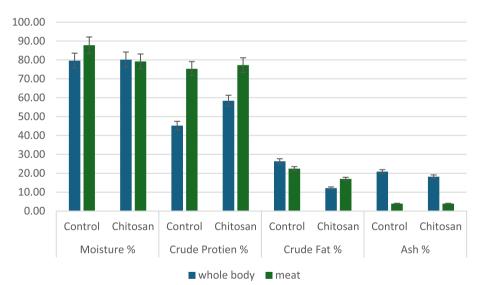


Fig. 2 Proximate composition of whole body and meat of shrimp. Protein, fat, and ash are stated on a dry weight basis. Moisture (%) = 100 × loss in weight (g) / weight of sample taken (g). Protein (%) = [Total Nitrogen (%) – Chitin Nitrogen (%)] x protein conversion factor (6.25). Fat (%) = 100 × Weight of Fat (g) / weight of sample taken (g). Ash (%) = 100 × Weight of Sample taken (g)

values were not significantly different in the whole body (p>0.05). The meat of chitosan-fed shrimps possessed higher energy values than the control (p<0.05) (Table 3).

Amino acid composition of meat

A total of 21 amino acids were detected in LC-MS analysis, of which eight are essential, ten are nonessential, and three are non-proteinogenic (Table 4). It

could be noticed that the composition of essential amino acids such as valine, histidine, and threonine, nonessential amino acids like arginine, serine, and proline, and three non-proteinogenic amino acids like phenylalanine, citrulline, beta 3–4 dihydroxy and ethionine were not significant between test animals and control (p>0.05). In contrast, statistically higher compositions were observed for leucine (20.975 \pm 0.03 mg/g), lysine

Table 3 Total energy, chitin nitrogen and total nitrogen of shrimp

	Total Energy (kJ/g)	Chitin Nitroge	n %	Total Nitrogen 9	%
	Control	Chitosan	Control	Chitosan	Control	Chitosan
whole body	393.76±28.59	335.89±31.27	1.55±0.28	1.68±0.21	8.79±0.56	11.03 ± 0.01
meat	268.10 ± 5.20	427.61 ± 11.22	0.70 ± 0.06	0.82 ± 0.08	12.06 ± 0.05	12.37 ± 0.31

The table displays the Mean ±SD of three replicates for each result. Dry weight is used to express both total nitrogen and chitin nitrogen

Nitrogen (%) = 100×14.01 x Normality of acid used x Titer value/ (sample weight \times 1000)

Total Energy (kJ/g) = $4.184 \times (4.27 \times \text{protein } \% + 9.02 \times \text{fat } \% + 4.11 \times \text{carbohydrate } \%)$

Wet weight basis is used to express total energy

Carbohydrate (%) = 100- (moisture % + protein % + fat % + ash %)

Table 4 Amino acid composition of *P. monodon* juveniles fed with control and chitosan diets

Amino acids	Control (mg/g)	Chitosan (mg/g)	Sig	
Essential amino acids				
Histidine	1.487 ± 0.20	1.735 ± 0.03	0.096	
Leucine	20.655 ± 0.03	20.975 ± 0.03	0.000	
Lysine	44.987 ± 0.90	51.407 ± 0.27	0.000	
Methionine	7.545 ± 0.05	5.452 ± 0.28	0.000	
Phenylalanine	17.382 ± 0.07	22.545 ± 0.38	0.000	
Threonine	3.148 ± 0.20	3.472 ± 0.23	0.138	
Tryptophan	8.422 ± 0.46	9.171 ± 0.02	0.047	
Valine	5.527 ± 0.06	5.950 ± 0.52	0.236	
ΣΕΑΑ	109.152 ± 1.01	120.709 ± 0.60	0.010	
Nonessential amino a	cids			
Alanine	4.226±0.16	6.000 ± 0.01	0.000	
Arginine	43.506±0.67	41.428 ± 1.80	0.135	
Asparagine	5.782 ± 0.32	4.145 ± 0.19	0.002	
Aspartic acid	1.830 ± 0.24	2.454 ± 0.30	0.048	
Cysteine	0.428 ± 0.03	0.554 ± 0.05	0.018	
Glutamic acid	47.241 ± 0.26	41.755 ± 3.14	0.039	
Glycine	0.430 ± 0.02	0.574 ± 0.08	0.036	
Proline	26.536 ± 0.84	27.336±1.29	0.418	
Serine	16.817±0.35	17.859 ± 0.69	0.080	
Tyrosine	38.397 ± 0.12	49.784 ± 0.77	0.000	
ΣΝΕΑΑ	185.191 ± 0.36	191.890 ± 1.97	0.008	
ΣΕΑΑ/ΣΝΕΑΑ	0.589 ± 0.00	0.629 ± 0.00	0.025	
Other non-proteinog	enic amino acids			
Citrulline	0.387 ± 0.03	0.357 ± 0.00	0.122	
Beta 3–4 dihydroxy phenylalanine	0.047 ± 0.00	0.052 ± 0.00	0.078	
Ethionine	0.044 ± 0.00	0.040 ± 0.00	0.238	
ΣΑΑ	294.821 ± 1.37	313.048 ± 2.57	0.019	

Where Σ EAA Total essential amino acids, Σ NEAA Total nonessential amino acids, Σ AA Total amino acids. The table presents the Mean \pm SD of three replicates with a significance level of p < 0.05

 $(51.407\pm0.27~mg/g)$, phenylalanine $(22.545\pm0.38~mg/g)$, and tryptophan $(9.171\pm0.02~mg/g)$; alanine $(6.000\pm0.01~mg/g)$, aspartic acid $(2.454\pm0.30~mg/g)$, cysteine

 $(0.554\pm0.05~{\rm mg/g})$, glycine $(0.574\pm0.08~{\rm mg/g})$, and tyrosine $(49.784\pm0.77~{\rm mg/g})$ in chitosan fed shrimps than control (p<0.05). However, the meat of chitosan-fed shrimps was low in asparagine, glutamic acid (NEAA) and methionine compared to control shrimps. The total amino acids $(313.048\pm2.57~{\rm mg/g})$, complete essential amino acids $(120.709\pm0.60~{\rm mg/g})$, whole nonessential amino acids $(191.890\pm1.97~{\rm mg/g})$, and proportion of whole indispensable amino acids to summation of dispensable amino acids (0.629 ± 0.00) were found superior in shrimp meat fed with chitosan incorporated feed than control feed (p<0.05).

Fatty acid composition of meat

The fatty acid profile of shrimp meat (control and chitosanfed) is given in Table 5. The total fat obtained was 2.58 g per 100 g shrimp meat. Caproic acid, capric acid, caprylic acid, heneicosanoic acid, tridecanoic acid, cis-11,14-eicosatrienoic acid, and cis-11.14.17-eicosatrienoic acid were lower than detectable value (< 0.005) in percentage of sample. Saturated fatty acids like stearic acid and heneicosanoic acid, monounsaturated fatty acids including cis-10-heptadecenoic acid, oleic acid, erucic acid and nervonic acid, as well polyunsaturated fatty acids such as linoleic acid, cis-11,14-ecosadienoic acid, arachidonic acid, EPA, and DHA content in shrimp meat did not differ statistically by the chitosan supplementation. The meat of shrimp fed chitosan had a somewhat higher composition of other saturated fatty acids than the control, including lauric acid, palmitic acid, myristic acid, heptadecanoic acid, pentadecanoic acid, tricosanoic acid, and lignoceric acid. Arachidic acid and behenic acid content in P. monodon meat were lowered by chitosan intake. In monounsaturated fatty acids, palmitoleic acid and cis-11-eicosenoic acids were significantly elevated by dietary chitosan in contrast to the control.

Discussion

Improving the growth and nutritional content of meat through dietary supplementation can bring about substantial economic gains for the farming sector. Additives, like antibiotics, are incorporated into the diets

Table 5 Fatty acid composition of *P. monodon* juveniles fed with control and chitosan diets

Fatty acid	Control (% of total Fat)	Chitosan (% of total Fat)	Sig		
Lauric acid	C12:0	0.108 ± 0.01	0.360 ± 0.02	0.000	
Myristic acid	C14:0	0.583 ± 0.00	0.927 ± 0.04	0.000	
Pentadecanoic acid	C15:0	0.426 ± 0.03	0.637 ± 0.02	0.000	
Palmitic acid	C16:0	24.765 ± 0.14	28.725 ± 0.88	0.001	
Heptadecanoic acid	C17:0	1.800 ± 0.02	2.105 ± 0.02	0.000	
Stearic acid	C18:0	22.145 ± 0.34	22.380 ± 0.32	0.436	
Arachidic acid	C20:0	3.210 ± 0.03	2.945 ± 0.01	0.000	
Heneicosanoic acid	C21:0	0.250 ± 0.00	0.170 ± 0.07	0.119	
Behenic acid	C22:0	5.085 ± 0.18	4.465 ± 0.00	0.004	
Tricosanoic acid	C23:0	0.374 ± 0.00	0.395 ± 0.01	0.036	
Lignoceric acid	C24:0	0.832 ± 0.02	0.904 ± 0.02	0.006	
Palmitoleic acid	C16:1	1.350 ± 0.02	1.775 ± 0.01	0.000	
cis-10-Heptadecenoic acid	C17:1	1.030 ± 0.75	1.211 ± 0.01	0.799	
Oleic acid	C18:1n-9c	15.790 ± 0.39	16.360 ± 0.24	0.097	
cis-11-Eicosenoic acid	C20:1n-9	1.155 ± 0.01	1.475 ± 0.01	0.000	
Erucic acid	C22:1n-9	0.308 ± 0.00	0.283 ± 0.01	0.060	
Nervonic acid	C24:1n-9	0.880 ± 0.03	0.861 ± 0.02	0.360	
Linoleic acid	C18:2n-6c	2.380 ± 0.12	2.365 ± 0.10	0.873	
cis-11,14-Eicosadienoic acid	C20:2	0.466 ± 0.00	0.476 ± 0.01	0.282	
Arachidonic acid	C20:4n-6	3.870 ± 0.11	3.653 ± 0.07	0.074	
cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n-3	5.850 ± 0.22	5.397 ± 0.10	0.058	
cis-4,7,10,13,16,19-Docosahexaenoic acid	C22:6n-3	2.085 ± 0.01	1.970 ± 0.06	0.061	
Σ SFA		59.577 ± 0.07	64.011 ± 1.38	0.005	
Σ MUFA		20.513 ± 0.30	21.965 ± 0.50	0.021	
Σ PUFA		14.651 ± 0.03	13.861 ± 0.51	0.093	
Σ n-3		7.935 ± 0.23	7.367 ± 0.16	0.047	
Σ n-6		6.250 ± 0.20	6.018 ± 0.01	0.185	
Σ n-3/ Σ n-6		1.272 ± 0.08	1.224 ± 0.03	0.460	
DHA/EPA		0.357 ± 0.01	0.365 ± 0.01	0.430	
Σ PUFA/ Σ SFA		0.246 ± 0.00	0.217 ± 0.01	0.005	

Where Σ SFA Total saturated fatty acids, Σ MUFA Total monounsaturated fatty acids, Σ PUFA Total polyunsaturated fatty acids, Σ n-3 Total n-3 fatty acids, Σ n-6 Total n-6 fatty acids, DHA/EPA Ratio of cis-4,7,10,13,16,19-Docosahexaenoic acid to cis-5,8,11,14,17-Eicosapentaenoic acid. Results in the table are expressed as Mean \pm SD of three replicates (p < 0.05)

of livestock for the purpose of enhancing their health, boosting their resistance to illness, and increasing their survival chances. Unfortunately, this practice may pose risks to human health due to the gradual accumulation of these substances in the animal's tissue [38, 40, 50]. Achieving the goal of sustainable aquaculture and the production of safe animal meat products requires efforts to replace antibiotics with organic feed additives that boost growth and activate innate immunity [9] & [8]. Owing to immunostimulant, anti-inflammatory, anti-oxidant, digestive modulatory and bacteriostatic properties, chitosan can replace antibiotics in feed [37, 58]. Chitosan supplementation in feed enhanced growth performance, concentration of fatty acids and amino

acids, and overall meat quality of lambs [47, 58]. Further, chitosan oligosaccharide or chitosan nanoparticles improved growth performance, amino acid composition, meat quality, immune function, antioxidant capacity and intestinal mucosa morphology in pigs [71, 79]. Likewise, supplementing chitosan, chitosan oligosaccharides, or its nanocomposite in diet brings about positive effects in growth performances like body weight, feed efficiency, crude protein content, plus antioxidant capacity, liver lipid catabolism, lipase activity and meat quality concerning amino acid and fatty acid configuration but lowered abdominal and liver fat of poultry animals like broiler chicken and geese [10, 11, 42, 49, 80].

In the current research, the shrimps with an initial weight of 0.056 ± 0 g could attain 0.227 ± 0.00 g final weight within 30 days of nursing by feeding with 0.2% chitosan, which also recorded 303.20 ± 1.26% AWG, $4.65 \pm 0.01\%$ SGR and $96.33 \pm 1.53\%$ survival. Analogous trial for 56-day by Shiau and Yu [68] showed a weight gain of 234.20% and 67.83% survival. As demonstrated by Niu et al. [56], L. vannamei post larvae with an initial weight of 1.2 mg fed for 60 days with a diet containing 0.2% chitosan reached a final weight of 165 ± 15 mg, weight gain of 13,633 ± 1291%, SGR of 21.4 ± 0.4%, and survival (%) of 45.5 ± 4.0 %. Whereas 1.16 ± 0.00 g weighed P. monodon juveniles fed on 0.2% dietary chitosan attained 6.27 ± 0.02 g final weight and AWG of $432 \pm 0.94\%$ within 60 days [53]. Niu et al. [55] also reported that with a 0.4% chitosan incorporated diet, P. monodon juveniles with 1.49 ± 0.02 g average weight gained a final weight of 6.23 ± 0.06 g and 318.51 ± 5.42% AWG in 70 days feeding trials. In the present study, chitosan-fed shrimps attained 2.489 ± 0.37 g within 30 days (i.e. from the 75th to 105th day of trial) from an average initial weight of 1.148 ± 0.13 g. The overall AWG and SGR observed on the final day of the feeding trial were 4318.92 ± 639.93 and 3.60 ± 0.13%, respectively, and were comparatively higher than previous reports [53, 55, 68]. The survival ratio observed in the present study $(70.43 \pm 0.40\%)$ was comparable to 77.78 ± 4.01%, as reported by Niu et al. [55] (2015). Furthermore, FCR recorded in the studies by Niu et al. (53, 55) were 1.09 ± 0.02 for 0.4% chitosan and 1.32 ± 0.01 for 0.2% chitosan included diet. In the present study, FCR was slightly lower (1.21 ± 0.17) to 1.32 ± 0.01 reported by Niu et al. [55]. PER values recorded in the present study (1.78 ± 0.27) were also comparatively lower than values reported by Niu et al. [53] (1.84 ± 0.08) . FE and PER values reported by Shiau and Yu [68] for 2% chitosan were even lower (39.37% and 1.15, respectively) than current results in giant tiger prawns.

The moisture content of the whole body and shrimp meat obtained in the present study was comparable to $75.61 \pm 0.62\%$ reported by Niu et al. [53]. However, lower per cent compositions of protein could only be recorded in the present study, which could be attributed to the smaller size range attained by the shrimps at the end of feeding trials (average 2.489 ± 0.37 g) when compared to the average weight of 6.27 ± 0.02 g reported by Niu et al. [53]. It has been reported that protein synthesis and growth rate in individuals of the same species may diverge in different age or size groups [2]. The lower crude fat content recorded in the whole body and meat of chitosan-fed shrimp in the present study can be attributed to the well-known property of chitosan to disturb fat metabolism and hinder fat assimilation in the gut through electrostatic interaction between lipids and amino-polysaccharides. Chitosan is reported to bind to lipid (cholesterol) micelles and inhibit their absorption [37, 67]. It may also be noted that the ash content in the whole body was comparable to Niu et al. [53], though the same was true for shrimp meat, which was lower than in their findings. It has been reported that the dietary changes will significantly influence the whole-body composition of shrimp [4].

The protein was found as the principal constituent of the P. monodon meat and elevated further by chitosan intake, which can be a superior source of amino acids. Amino acids occupy a fundamental role in the growth of aquatic animals. Furthermore, they govern metabolism, modulate feed intake, cell signalling, immune response and the healthiness of farmed animals [86]. Lysine is vital in growth and essential to produce hormones, immune cells, and carnitine, which are required to convert fatty acids to energy. Leucine is a branched-chain amino acid required for growth, neuron functioning, and healing skin and bone. Aromatic amino acids, viz. phenylalanine, tryptophan, and tyrosine, precursors of neurologically active compounds, were found elevated in shrimp meat fed on a chitosan diet in the present study. Alanine is the energy source for the central nervous system and muscles and is used to break down vitamin B-6. Aspartic acid helps in hormone production and release. Cysteine, a sulphur-containing amino acid, is required all over the body. Glycine contributes to the sweet flavour of shrimp meat [46]. Histidine composition observed in the present study $(1.735 \pm 0.03 \text{ mg/g})$ was reasonably lower than that testified in meat of P. monodon fed on diet incorporated phyto-stimulants for growth and health improvement (12.748 mg/g) [26] but comparable with P. monodon shrimp meat fed on formulated feed (2.8 mg/g) [62]. Moreover, lysine $(51.407 \pm 0.27 \text{ mg/g})$ and methionine content (7.545 ± 0.05) observed were similar to Hardi et al. [26] (51.634 and 8.500 mg/g, individually). The quantity of phenylalanine (22.545 ± 0.38 mg/g) observed in the present study was comparable to 25.928 mg/g by Hardi et al. [26]. Threonine content $(3.472 \pm 0.23 \text{ mg/g})$ obtained in the present investigation is quite lower than Hardi et al. [26] (27.163 mg/g) but was comparable to Rajaram et al. [62] (5.1 mg/g). Chen [17] reported 9.2 mg/g of tryptophan content (9.2) in shrimp meat, similar to the present research $(9.171 \pm 0.02 \text{ mg/g})$. For valine, the current result $(5.527 \pm 0.06 \text{ mg/g})$ is slightly higher than that of Karthikeyan et al. [35] (4.18 mg/g). However, it was low compared to reports of Rajaram et al. [62] (7.3 mg/g) and Hardi et al. [26] (25.367 mg/g).

Concentrations of nonessential amino acids alanine and aspartic acid obtained $(6.000 \pm 0.01 \text{ and } 2.454 \pm 0.30 \text{ mg/g})$ in shrimp meat after 105 days feeding trial was lesser than that recounted by Hardi et al. [26] (34.077 and

52.336 mg/g) yet in covenant with Karthikeyan et al., [35] (7.4 and 2.4 mg/g). The amino acids arginine and proline $(41.428 \pm 1.80 \text{ and } 27.336 \pm 1.29 \text{ mg/g})$ were upper than Karthikeyan et al. [35] (24.2 and 19.4 mg/g). However, Hardi et al. [26] and Chen [17] mentioned slightly better outputs for arginine in shrimp meat (52.698 and 65.7 mg/g). Amounts of cysteine, glycine, glutamic acid and serine in shrimp meat $(0.554 \pm 0.05, 0.574 \pm 0.08)$ and 17.859 ± 0.69 mg/g) were inferior to that in Hardi et al. [26] (56.466, 44.871, 90.620, and 24.633 mg/g) and Karthikeyan et al., [35] (24.5, 38.6, 60.3 and 86.5 mg/g). Relatively superior values were acquired for tyrosine after a feeding trial in shrimp meat $(49.784 \pm 0.77 \text{ mg/g})$ Rajaram et al., [62] (2.96 mg/g) and Hardi et al., [26] (19.491 mg/g). The total amino acid content in shrimp meat declared by Sriket et al. [70] (298.08 mg) is in hand in hand with that in control-fed animals of the present study (294.821 ± 1.37 mg) but lower than that in chitosanfed shrimps (313.048 ± 2.57 mg). Amino acid makeup of shrimp can be impacted by several exterior features, counting sexual maturity, diet, size, season, salinity, and water temperature [45].

The human body cannot synthesize essential amino acids; thus, they are necessary through diet for metabolism, especially in growing children and pregnant ladies [16]. P. monodon meat featured high levels of lysine and an optimally balanced amino acid configuration with all EAAs. A chitosan-incorporated diet further enhanced the lysine content of shrimp meat. Plasma aspartate aminotransferase and alanine aminotransferase are two critical enzymes that participate in the synthesis of nonessential amino acids, which were significantly depressed by 0.2% chitosan intake in *P. monodon* [53]. Kumar et al. [41] noticed no significant differences in aspartate aminotransferase activities, and the rate of nonessential amino acid synthesis was not touched by the incidence of purified or natural chitin [41]. According to Cha et al. [15], olive flounder (Paralichthys olivaceus) fed chitosanlayered feed pellets had decreased aspartate aminotransferase and alanine aminotransferase activities. Similar outcomes were stated for tilapias fed on diets containing 4 g kg⁻¹ chitosan [85]. However, in edible tissue of chitosan-fed shrimp, essential and nonessential amino acids (leucine, lysine, phenylalanine, tryptophan, alanine, aspartic acid, cysteine, glycine, and tyrosine) were found preeminent in feeding trial under discussion. The addition of plant proteins and shrimp hydrolysate mixture to the food of largemouth bass supported the apparent digestibility coefficients of protein and amino acids. It helped to activate the rapamycin pathway's amino acid-sensing loci, which improved the fish's growth presentation [43]. However, shrimp hydrolysate in the diet lowered muscle composition of nonessential amino acids, namely aspartic acid, serine, glycine, and alanine, yet did not affect essential amino acids except phenylalanine. Furthermore, shrimp hydrolysate intake did not affect the total essential amino acids and nonessential amino acid values of fish muscle [43]. Lamb meat supplemented with chitosan showed higher levels of creatine, choline, arginine, carnosine, histidine, glutamate, arginine, 2-oxoglutarate, and 3-hydroxybutyrate [58]. Similarly, Su et al. [71] depicted the positive influence of chitosan oligosaccharide in finishing pig's amino acid composition like inosinic acid and some umami amino acids, namely glutamate, phenylalanine and alanine. The addition of 200 mg/kg of chitosan, in turn, enhanced the concentrations glycine, valine, lysine, glutamic acid, total amino acids and total nonessential & essential amino acids in growing Huoyan geese [49].

Supplementation with functional oligosaccharides affects energy levels in fish through carbohydrate, lipid, and amino acid metabolism [84]. According to Ambasankar et al. [4], the fatty acid configuration of the diet will be echoed in the body proportions of shrimps. Heu et al. [27] reported that the fatty acid composition of P. monodon has a high proportion of oleic acid, followed by palmitoleic acid and cis-10-heptadecenoic acid. In the present study, 22 fatty acids were detected with high compositions of palmitic acid (28.725 ± 0.88%), stearic acid $(22.380 \pm 0.32\%)$ and oleic acid $(16.360 \pm 0.24\%)$ which were comparable with the results of Rosa and Nunes [64], Yanar and Ceilk [88], Sriket et al. [70] and Gayathri et al. [22]. It could be noted that the total saturated and monounsaturated fatty acid compositions were significantly elevated in 0.2% chitosan-fed shrimp meat (64.011 and 21.965 g /100 g total fat, respectively) than in control (59.577 and 20.513 g/100 g total fat correspondingly). However, the polyunsaturated fatty acid composition of shrimp meat was not statistically affected by chitosan supplementation in diet. Karthikeyan et al. [35] reported a similar total saturated fatty acid composition (57.25 g /100 g total fat) in cultured P. monodon. In contrast, Jaseera et al. [33] reported low saturated fatty acid percentages (35.4 and 44.88) for P. monodon fed on artemia. The total monounsaturated fatty acid composition (14.96) indicated by Karthikeyan et al. [35] is lower than the current results.

The polyunsaturated fatty acid compositions reported by Jaseera et al. [33] (29.83) and Karthikeyan et al. [35] (25.63) were higher than the present values. However, in the present study, only low compositions of n-3 fatty acids could be detected in the control and chitosan-fed $P.\ monodon\ (7.935\pm0.23\ and\ 7.367\pm0.16\%)$. Gonzalez-Felix et al. [24] perceived shrimp's preferential utilization of specific fatty acids. Once the intake is low in omega-3 fatty acids, the body retains an extra

fraction of n-3 fatty acids [32, 36]. Omega-6 percentage in P. monodon observed in the current investigation $(control - 6.250 \pm 0.20; chitosan - 6.018 \pm 0.01\%)$ was lesser than that in P. semisulcatus (9.89% \pm 0.08%), and Metapenaeus monoceros (18.95% ± 1.09%) but upper than that in Aristaeomorpha foliacea $(4.48\% \pm 0.07\%)$ [92]. The fatty acid n-3/n-6 ratio can manipulate an animal's inflammatory, metabolic and homeostatic status [28]. Optimal ratios range from 4:1 to 1:4 [69]. From the perspective of human nutrition, a diet rich in omega-3 polyunsaturated fats are progressively more valued [4]. In the current investigation, the omega-3/ omega-6 ratio of shrimp was superior $(1.272 \pm 0.08 \text{ and } 1.224 \pm 0.03)$ to the ideal endorsed percentage of 0.25 of the Department of Health of the UK [66]. The consumption of microalgae loaded with PUFAs results in a better composition of n-3 fatty acids than ω -6 fatty acids in marine shrimps [3]. Moreover, compared to deep-sea shrimp, shrimp in shallow-water environments tend to be more abundant in ω -3 fatty acids. [13, 77]. In the current study, P. monodon reared as control and test had higher n-3 fatty acids $(7.935 \pm 0.29 \text{ and } 7.367 \pm 0.16)$ than n-6 fatty acids $(6.250 \pm 0.20 \text{ and } 6.018 \pm 0.01).$

Furthermore, the essential ω -3 long-chain PUFAs that are most appropriate for human health are cis-5,8,11,14, and 17-EPA and cis-4,7,10,13,16, and 19-DHA [81, 82] (a) & (b)). Their ratio (DHA / EPA) in control and chitosanfed shrimp meat (0.357 ± 0.01) and 0.365 ± 0.01 , respectively) were comparable. Toyes-Vargas et al. [75] reported higher contents of linoleic acid (26.8%), DHA (4.2%), PUFAs (30.6% of total fatty acids), palmitic acid (20.6%), oleic acid (16.5%), EPA (4.3%) and total MUFAs (24.8%) but lower stearic acid (11.3%), arachidonic acid (1.3%), total saturated fatty acids and ratio n-3/ n-6 fatty acids (0.36%) in marine co-product meal fed L.vannamei muscle than present study. Crustaceans, including shrimp, have crude fat compositions of less than 2 per cent. They include phospholipids (65-70 per cent), cholesterol (15–20 per cent), and acylglycerols (10–20 per cent) that are high in unsaturated fatty acids. The current findings, however, are incoherent.

Little information is known about how chitosan supplementation affects the essential nutrient composition of shrimp, including its amino acid and fatty acid composition. Chitosan can form ionic bonds at low pH, hence could hinder lipid absorption in the gastrointestinal tract by binding with fatty acids plus surge faecal lipid excretion as these bound triacylglycerols would escape hydrolysis by lipase [67],and [19]. Similarly, feeding 2% chitosan reduced the serum cholesterol and triacylglycerol values of rabbits, hens and broilers [37]. Hossain et al. [29] and Niu et al. [53] reported that there was a decrease in tissue fat contents accompanied by a decrease in plasma whole

triacylglycerol and cholesterol percentages, respectively, in rat and *P. monodon* fed with chitosan-containing diet assemblies. In rats fed with 2% chitosan, higher levels of oleic acid, linoleic acid, arachidonic acid, n-3 docosapentaenoic acid, n-3 docosahexaenoic acid, total saturated fatty acids, total unsaturated fatty acids, and the ratio of total unsaturated fatty acid / total saturated fatty acid were found. However, chitosan intake did not affect the amounts of palmitic and stearic acid in plasma [29].

Further, the effect of dietary chitosan on the breakdown of food fat and their excrement was faster in shrimps due to their shorter food retention periods since the guts of shrimps vary considerably from fishes and rats have longer intestines and functional liver and pancreas [53]. Cholesterol-lowering effect of chitosan oligosaccharide has also been well established, and serum LDL-cholesterol levels were found to be depressed in L.vannamei groups that received 0.3 g kg⁻¹ chitosan oligosaccharide but enhanced alkaline phosphatase and acid phosphatase activity [61]. Dietary water-soluble chitosan optimistically transformed the intestinal short-chain fatty acids in L.vannamei [18]. Digestive enzymes like proteases and amylases activity of the red tilapia hybrids increased with the levels of chitosan supplementation, which also resulted in higher alkaline phosphatase but low serum alanine aminotransferase and aspartate aminotransferase levels [48].

In dairy cow milk, dietary chitosan in free-fat diets has been shown to increase the concentration of polyunsaturated and long-chain fatty acids [76]. The amount of unsaturated fatty acids, palmitoleic and conjugated linoleic acid, in the meat of feedlot lambs, was elevated by chitosan and ground cotton seed [47]. Supplementation of chitosan in diet upgraded the concentration of oleic-cis-9 acid, linoleic acid, linolenic-trans-6 acid, arachidonic acid and eicosapentaenoic acid in lamb meat [58]. Dietary supplementation of chromiumloaded chitosan nanoparticles amplified fatty acids and lipase activity of serum in finishing pigs [79]. Chitosan oligo-saccharide in diet augmented intestinal fatty acid content in weaned piglets [90]. Chitosan oligosaccharides caused a reduction in liver and abdominal fat in chickens [80]. According to Lan et al. [42], chitosan oligosaccharide decreased the contents of palmitic acid, stearic acid, and total saturated fatty acids in the thigh muscles of broilers.

Chitosan fortification improved the growth traits, body weight gain, and polyunsaturated fatty acid content in loaches (*Misgurnus anguillicaudatus*) [87]. The addition of 0.3 per cent chitosan oligosaccharide to the diet improved the growth characteristics, intestinal digestive-enzyme activities, body protein content, and total polyunsaturated fatty acids in the meats of

Paramisgurnus dabryanus loaches but decreased the percentage of saturated fatty acids in the muscle [93]. Diets supplemented with astaxanthin and fish oil had a significant impact on the concentrations of DHA, omega-3 PUFA, HUFA, and maturation in the muscle of *P. monodon* shrimp [57]. The addition of krill meal to diets improved growth performance, and the contents of myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid, cis-4,7,10,13,16,19docosahexaenoic acid, n-3/n-6 ratio, and all other n-3 fatty acids of Penaeus vannamei were all significantly impacted [4]. Furthermore, the internal lipid and protein levels of Pacific white shrimp (P. vannamei) were elevated by diets containing chitosan-ZnO nanocomposite (10 mg kg-1) [7]. In line with the present findings, dietetic subjunction combined with chitosan oligo-saccharide enhanced the intestinal fatty acid content, serum calcium, nitrogen, and amino acid content in weaned piglets [90]. Consistent results for growth performance and biochemical composition of 0.2% chitosan-fed P. monodon were demonstrated by [53].

Conclusion

Dietary supplementation of 0.2% chitosan enhanced growth performance measures like average weight gain, average final length, specific growth rate, survival rate, feed conversion ratio, feed efficiency, and protein efficiency ratio in black tiger shrimp under experimental conditions. It also enhanced chitin nitrogen, total nitrogen, crude protein in the meat and whole body, essential and nonessential amino acids, and the composition of saturated and monounsaturated fatty acids in shrimp meat. However, compared to the control, the chitosanfed shrimp meat had a lower crude fat content. Compared to the control group, the shrimp fed chitosan had higher compositions of the essential amino acids (tryptophan, phenylalanine, leucine, and lysine), which is advantageous for human nutrition. Chitosan (0.2%) supplementation is recommended in Penaeus monodon feed preparations that aim to provide a safe, growth-promoting diet with the advantage of increasing the overall nutritional quality of shrimp meat.

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Authors' contributions

1. AA: Investigation, writing original draft. 2. SS: Conceptualisation, supervision, review and editing. 3. MH: Overall supervision, Methodology, formal analysis, review and editing

Data Availability

Data will be made available upon request.

Declarations

Competing Interest

The authors declare no competing interests.

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