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Effects of replacing fishmeal with soybean protein concentrate (SPC) on growth, blood biochemical indexes, non-specific immune enzyme activity, and nutrient apparent digestibility for juvenile *Litopenaeus vannamei*

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Abstract
An 8-week feeding trial was conducted to investigate the effects of dietary soy protein concentrate (SPC) levels on growth, blood biochemical indexes, non-specific immune enzyme activity, and nutrient apparent digestibility for juvenile *Litopenaeus vannamei* (initial weight 0.44 ± 0.002 g). They were formulated by replacing 0% (the control), 10%, 20%, 30%, 40%, 60%, and 80% of fish meal (FM) protein with SPC (designed FM, S11, S22, S33, S44, S55, and S66, respectively). With the replacement level of SPC in the diet was higher than 30%, weight gain (WG) and specific growth rate (SGR) for juvenile *Litopenaeus vannamei* significantly decreased ($P < 0.05$) with SPC increasing, but feed conversion ratio (FCR) significantly increased ($P < 0.05$). When the replacement level was above 40%, the protein efficiency ratio (PER), survival rate (SR), apparent digestibility of dry matter, and energy significantly decreased ($P < 0.05$). On the contrary, the content of triglyceride glucose (TG) in serum was increased significantly ($P < 0.05$). Apparent digestibility for crude protein and total lipid were significantly higher at higher fishmeal inclusion (0 and 10% SPC replacement) and significantly lower at lower fishmeal inclusion (< 70%) ($P < 0.05$). In conclusion, with reference to all the parameters, SPC can be used to replace up to 30% of fishmeal protein in shrimp feeds and so the total fishmeal exceeds 70% be used to cater to shrimps' dietary needs.

Keywords Soy protein concentrate · Fish meal · Juvenile *Litopenaeus vannamei* · Apparent digestibility

Highlights
• This experiment was conducted to assess the possibility of replacing fishmeal (FM) with soybean protein concentrate (SPC) at different levels on growth, blood biochemical indexes, non-specific immune enzyme activity, and nutrient apparent digestibility for juvenile Litopenaeus vannamei.
• In the basic diet of juvenile Litopenaeus vannamei with 40% protein and 30% fish meal content, with growth performance as an indicator, the appropriate level of SPC to replace fish meal was 30%.
• When the level of SPC replacement fish meal was higher than 30%, the growth performance and feed utilization of juvenile Litopenaeus vannamei will decrease.

Introduction

Global aquaculture production reached 101.2 million tons in 2016 and was estimated at US$243.5 billion (FAO 2018). Fishmeal (FM) production is experiencing shortages due to its raw material resources, thereby reducing the sum of FM production capacity to support the shrimp farming industry (Katya et al. 2016). Such shortage results in a deficiency in supplies, which increases the FM cost for the prescribed regulation of shrimp. Soy protein concentrate (SPC) is a high-protein soybean product made by purification of proteins from low-temperature-dissolved soybean meal (Bradford 1976). Given its high protein content, rich amino acid composition, and low anti-nutritional factors, SPC is also considered an important plant protein raw material for aquatic animal diets (Oyedapo et al. 2004). Studies have been conducted on the effect of replacing FM with SPC on the growth performance and feed utilization of aquatic animals. SPC was used to replace 50% FM protein in *Penaeus monodon* diet; no significant effect was observed on the weight gain rate (WGR), specific growth rate (SGR), and feed conversion rate (FCR) (Paripatananont et al. 2001). A 44% SPC content in the diet caused no significant effect on the growth of *Eriocheir sinensis* (Li et al. 2006), and no significant change was observed in the WGR and SGR of yellow catfish (*Pelteobagrus fulvidraco*) when the SPC substitution for FM in the diet remained below 20% ($P > 0.05$). The 100% replacement of FM with SPC for yellow mullet diet showed no significant effect on its growth. Studies on gingiva also showed that 25% SPC replacement of FM protein significantly reduced its WG and feed utilization (Deng et al. 2006). Studies on *Monopterus albus* showed that when the replacement ratio was greater than 45%, the WGR of yellow eel was significantly lower ($P < 0.05$) (Zhang et al. 2014). The replacement of FM with SPC results in different effects on aquatic animals. Therefore, research must be conducted on the effects of SPC replacement of FM on the growth performance, blood biochemical indicators, non-specific immune enzyme activity, and apparent digestibility of nutrients in juvenile *Litopenaeus vannamei* (*L. vannamei*). The results will provide academic significance and production guidance. This experiment was conducted to assess the possibility of replacing fishmeal protein with different levels of SPC for *L. vannamei* and explore the appropriate proportion of SPC protein in the diet of this shrimp.

Materials and methods

Experimental diets

With the fish meal, peanut meal and shrimp shell meal were used as the protein source, with fish oil and soy lecithin were used as the lipid source. Eight iso-nitrogenous and iso-lipidic experiment diets were formulated to contain 0%, 3.07%, 6.14%, 9.20%, 12.27%, 18.41%, and 24.54% of SPC by replacing 0% (FM, control group), 10% (S10), 20% (S20), 30% (S30), 40% (S40), 60% (S60), and 80% (S80) of FM protein, respectively (Table 1). According to the amino acid requirement of *L. vanna*mei (Akiyama et al. 1992), L-lysine (98%), DL-methionine (98%), L-threonine (98%), and L-arginine (98%) were added respectively to balance the content of lysine, methionine, threonine, and arginine in each treatment, and to meet the needs of lysine, methionine, arginine, and threonine in the growth for *L. vannamei*. All ingredients for this experiment were obtained from Guangdong Yuehai diet (Group) Co., Ltd.

Table 1 Ingredients and proximate composition of experiment diets (% dry matter)

Ingredient (%)	Experimental diets						
	FM	S10	S20	S30	S40	S60	S80
Fishmeal FM	30.00	27.00	24.00	21.00	18.00	12.00	6.00
SPC	0.00	3.07	6.14	9.20	12.27	18.41	24.54
Others [a]	63.11	63.11	63.11	63.11	63.11	63.11	63.11
Fish oil	2.00	2.26	2.53	2.79	3.05	3.57	4.14
Compound polyamine [b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Compound vitamin [c]	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cr_2O_3	0.50	0.50	0.50	0.50	0.50	0.50	0.5
L-lysine	0.00	0.03	0.05	0.08	0.11	0.17	0.22
DL-methionine t	0.09	0.11	0.13	0.15	0.16	0.21	0.24
L-arginine	0.32	0.28	0.24	0.2	0.14	0.08	0.00
L-threonine	0.00	0.01	0.01	0.02	0.03	0.04	0.05
Cellulose	2.78	2.43	2.09	1.75	1.43	0.71	0
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Main nutritional composition							
Crude protein (%)	40.25	40.36	40.69	40.33	40.51	40.76	40.59
Crude lipid (%)	7.54	7.41	7.49	7.28	7.37	7.77	7.53
Crude ash (%)	11.77	11.48	11.23	10.66	10.34	9.79	9.31
Gross energy (MJ/kg)	19.54	19.56	19.84	19.11	19.29	19.71	19.77

[a] Others: peanut meal 12%, beer yeast 6%, shrimp shell meal 6%, corn protein meal 10%, wheat flour 26.05%, phospholipid 1.5%, calcium dihydrogen phosphate 1.5%, VC phosphate 0.03%, choline 0.03%

[b] Mineral element premix (g/kg premix): 13.71 g of ferric citrate, 28.28 g of zinc sulfate heptahydrate, 0.12 g of magnesium sulfate heptahydrate, 12.43 g of manganese sulfate monohydrate, 19.84 g of copper sulfate pentahydrate, six cobalt chloride hydrate 4.07 g, potassium iodide 0.03 g, potassium chloride 15.325 g, sodium selenite 0.02 g, cellulose 906.18 g

[c] Vitamin premix (g/kg premix): thiamine 25.50 g; riboflavin 25.0 g; vitamin B6 50.0 g; vitamin B12 0.1 g; vitamin K 5.0 g; vitamin E 99.0 g; cellulose A 10.0 g, vitamin D50 g; nicotinic acid 101.0 g; D-calcium pantothenate 61.0 g; biotin 25.0 g; folic acid 6.25 g; inositol 153.06 g; cellulose 383.44 g

Experimental fish and feeding management

The various raw materials were first crushed through a 60-mesh sieve, and the various diets ingredients were accurately weighed according to the formula (Table 1), and then stirred gradually by mixing in a V-mixer type machine (M-256, South China University of Technology, Guangzhou, China). After adding choline chloride, cold water, and various lipids, materials were well mixed again in a Hobart-type mixer. A pellet diet has a particle size of 1.0 and 1.5 mm. After air drying, seal with a zip lock bag and store in a freezer at – 20 ℃ for later use. The test shrimp was from the shrimp seedlings cultivated by Guangdong Yuehai diet (Group) Co., Ltd. Zhanjiang East Island Seed Farm. The test consisted of 7 treatments, each treatment consisted of 3 replicates, and 840 healthy shrimp seedlings with an initial body weight of 0.44 ± 0.002 g were selected, placed in an average of 21 0.5-m^3 fiberglass steel drums, 40 barrels per barrel, respectively, feed 7 test diets. The test was carried out in the indoor breeding system of Zhanjiang East Island Research Base of Guangdong Yuehai diet (Group) Co., Ltd. feeding 8 to 10% of body weight every day, feeding 4 times at 07:00, 11:30, 17:30, and 21:00. The test water was sedimented and filtered seawater. The

shrimps were inspected for feeding, clams, and growth, and the water quality parameters such as feeding amount, water temperature, pH value, salinity, and dissolved oxygen were recorded. The ammonia nitrogen content in the water was measured periodically every week. During the test, the water temperature was 28.0 ~ 30.5 ℃, the seawater salinity was 26.5 ~ 28.0, and the oxygenation was continuous during the test. The dissolved oxygen was higher than 7.0 mg L^{-1}, the pH value was 7.8 ~ 8.2, and the ammonia nitrogen content was less than 0.03 mg L^{-1}. The test period was 56 days.

Feces and sample collection

Feces were collected by reference to the method of Yang et al. (2009). After the fourth week of the test, after each feeding for 1 h, the remaining diet in the bucket was discharged, and then the feces were sucked out of the sieve mesh by siphoning and the water was drained, and then the feces intact and formed were collected in the sealed pocket. Store in a – 20 ℃ freezer. After each barrel of the fecal sample was collected insufficient amount (dry weight was about 10 g), the fecal sample in each repeat was dried, ground and crushed, sealed, and stored in a refrigerator at – 20 ℃ for analysis of Cr_2O_3 and protein in the fecal sample, amino acid, lipid, and energy content. The feeding was stopped 24 h before the end of the test. At the end of the test, weigh and count the mantissa to calculate the WGR and survival rate (SR). Five shrimps were randomly selected from each barrel and stored in a refrigerator at – 20 ℃ for the analysis of whole shrimp body composition. Another 10 shrimps were taken and the liver and pancreas were peeled off in an ice bath. The weight was weighed and quickly placed in liquid nitrogen. Store and store in an ultra-low temperature freezer at – 80 ℃ to calculate the liver-to-body ratio and measure the enzyme activity index of the hepatopancreas; select 10 shrimps per barrel, draw blood from the pericardial cavity with a 1-mL syringe, and place the blood. Place in 1.5-ml Eppendorf, stand in a refrigerator at 4 ℃ overnight, centrifuge, and take it overnight. The remaining part of the hepatopancreas will be removed, the head, tail shank, and outer shell removed, and the muscles will be removed and quickly stored in a – 20 ℃ refrigerator.

Measurement indicators

Body composition analysis

The contents of moisture, crude protein, crude lipid, and ash in whole shrimp samples were determined by AOAC (1998). Among them, the moisture content was determined by using a constant temperature oven at 105 ℃ shrimp, drying to constant weight to determine the moisture content; crude protein content was determined by automatic Kjeldahl analyzer (2300-Auto-analyzer, Foss Tecator, Sweden); crude lipid content was measured by an automatic fat meter (Soxtec System 2050, Foss Tecator, Sweden); the ash content was measured after burning for 12 h in a 550 ℃ muffle furnace.

Determination of digestive enzyme activity in hepatopancreas

Remove the hepatopancreas sample from the – 80 ℃ ultra-low temperature refrigerator and transfer it to the 4 ℃ refrigerator, and wait for it to thaw for use. Accurately weigh 0.5 g of liver and pancreas, then add pre-chilled physiological saline at 1: 5, homogenize with a glass homogenizer under ice bath conditions, and then place the homogenate at 0 ~ 4 ℃,

9000 r min^{-1}. The supernatant obtained by centrifuging for 30 min in a high-speed refrigerated centrifuge was the crude enzyme solution. For the determination of protease, lipase, and amylase, the protease, lipase, and amylase kit and model Uquant full-band microplate reader developed by Nanjing Jiancheng Biological Research Institute were used.

Measurement of gross energy

Total feed energy, also known as gross energy, refers to the heat generated when the feed is completely burned in vitro, that is, the sum of energy contained in crude protein, crude lipid, and carbohydrate in the feed. Determination methods are direct determination with the bullet calorimeter: firstly, accurately weigh the sample to be measured into the steel cylinder of the bullet calorimeter; fill with a certain pressure of oxygen, and electrify it. The heat released by the combustion of the sample can be exported from the wall of the cylinder so that the temperature of the water outside the single-cylinder rises and then according to the difference between the water temperature before and after the combustion of the sample, the energy value of the material sample can be calculated.

Determination of biochemical indicators in serum

According to reference Ray et al. (2020), the serum total protein content was determined by the protein quantitative (Coomassie brilliant blue) kit developed by Nanjing Jiancheng Bioengineering Research Institute, and the bovine serum albumin was used as the standard, and the unit was expressed as mg ml^{-1}. The serum glucose content was determined by the glucose (glucose oxidase method) kit produced by Zhongsheng Beikong Biotechnology Co., Ltd., and the unit was expressed as mg 100 ml^{-1}. The triglyceride content was determined by using a triglyceride (TG) kit produced by Changchun Huili Biotechnology Co., Ltd., and the unit was expressed as mmol L^{-1}. The total cholesterol content was determined by using the total cholesterol (TC) kit produced by Changchun Hui li Biotechnology Co., Ltd., and the unit was expressed as mmol L^{-1}.

Determination of serum immuno-enzymatic activity

For the determination of immune enzyme activity in serum, refer to Ray et al. (2020), which includes acid phosphatase (ACP), alkaline phosphatase (AKP), superoxide dismutase (SOD), and lysozyme (LZM). The analysis was performed using the detection kit produced by Nanjing Jiancheng Bioengineering Research Institute (China). ACP detection kit (Nanjing Jiancheng, Institute of Bioengineering, China) was used to determine ACP and AKP in serum at 530 nm using phenyl disodium phosphate as a substrate.

Formula for calculations

Refer to NRC (2011) to calculate the apparent digestibility of dry matter, crude protein, amino acids, crude lipid, phosphorus, and gross energy in diet:

Apparent digestibility (%) of diet dry matter $= 100 \times [1\text{-} (D_{Cr} / F_{Cr})]$;

Apparent digestibility of nutrient (%) $= 100 \times [1\text{-} (F / D \times D_{Cr} / F_{Cr})]$.

(where F is the crude protein, amino acid, crude lipid, phosphorus, and gross energy content in the feces, D is the crude protein, amino acid, crude lipid, phosphorus, and gross

energy content in the diet, D_{Cr} is the amount of Cr_2O_3 in the diet, and FCr is the amount of Cr_2O_3 in the feces.)

Test data processing and statistical analysis

The results were expressed as mean ± standard deviation (M ± SD). One-way ANOVA was performed on the data using SPSS (13.0) statistical software, and multiple comparisons were performed in combination with Duncan's. The difference between the treatments was significant, and $P < 0.05$ indicates a significant difference.

Results

Effects of replacing FM with SPC on the growth and feed utilization of juvenile L. vannamei

The different proportions of SPC had significant effects on the WGR, FCR, SGR, and SR of juvenile *L. vannamei* (Table 2; $P < 0.05$). The highest WGR was observed in the control group and the lowest in the 80% replacement group. The WGR of the control group was significantly higher than that of the groups with 40%, 60%, and 80% SPC replacement levels ($P < 0.05$). No significant difference was noted in the groups with 10%, 20%, and 30% SPC replacement levels ($P > 0.05$). The highest FCR was recorded in the 80% replacement group, followed by the 60% group, and the lowest in the control group. The results obtained with SPC replacement levels of 60% and 80% were significantly higher than those of the other groups ($P < 0.05$). The difference between the other groups showed no significance ($P > 0.05$). The SGR of the control group was significantly higher than that of the groups with replacement levels of 10%, 20%, and 30% ($P < 0.05$). No significant difference was observed with the 40% group ($P > 0.05$). The SGR of the 40% replacement group was significantly lower than that of the control and 10% replacement groups ($P < 0.05$), and the difference between other groups showed no significance ($P > 0.05$). The lowest SR was observed in the 80% replacement group, followed by the 60% replacement group; both groups showed no significant difference ($P > 0.05$), but their SGRs were significantly lower than those of the other groups ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$).

Effects of replacing FM with SPC on the hepatosomatic index and protein utilization for juvenile L. vannamei

Table 3 shows that the different proportions of SPC had significant effects on protein efficiency and protein deposition rate of juvenile *L. vannamei* ($P < 0.05$) but no significant effect on the hepatosomatic index ($P > 0.05$). The lowest protein efficiency was observed 80% replacement level group, followed by the 60% replacement group. No significant difference was recorded between the 80 and 60% SPC replacement groups ($P > 0.05$), but their results were significantly lower than those of the other groups ($P < 0.05$). The difference between the remaining groups showed no significance ($P > 0.05$). The protein deposition rate was the highest in the 20% replacement group, followed by the control group, and the lowest was noted in the 80% replacement group. The replacement level was 60% and

Table 2 Effects of replacing fishmeal with soybean protein concentrate (SPC) on growth, diet utilization for juvenile *L. vannamei*

Experimental diets	IBW (g)	TBW (g)	WGR (%)	FCR	SGR (%/d)	SR (%)	Feed intake (g)
FM	0.44 ± 0.005	11.60 ± 0.24 ^{a}	2530.00 ± 23.78 a	1.16 ± 0.01 ^{a}	5.83 ± 0.016 ^{a}	100.00 ± 0.00 ^{a}	517.82 ± 9.10
S10	0.44 ± 0.002	11.32 ± 0.08 ^{ab}	2474.84 ± 35.00 a	1.20 ± 0.04 ^{a}	5.80 ± 0.024 ^{a}	99.16 ± 1.44 ^{a}	522.24 ± 3.20
S20	0.44 ± 0.003	11.20 ± 0.29 ^{ab}	2434.23 ± 50.64 a	1.17 ± 0.03 ^{a}	5.77 ± 0.035 ^{ab}	100.00 ± 0.00 ^{a}	503.57 ± 11.60
S30	0.44 ± 0.002	11.26 ± 0.52 ^{ab}	2460.42 ± 131.56 ^{a}	1.24 ± 0.06 ^{a}	5.78 ± 0.093 ^{ab}	98.33 ± 2.88 ^{a}	525.93 ± 20.80
S40	0.44 ± 0.002	10.75 ± 0.51 ^{bc}	2323.07 ± 124.38^{b}	1.21 ± 0.03 ^{a}	5.69 ± 0.092 ^{bc}	100.00 ± 0.00 ^{a}	499.00 ± 20.40
S60	0.44 ± 0.001	10.47 ± 0.04 ^{c}	2243.65 ± 32.55^{b}	1.41 ± 0.10^{b}	5.64 ± 0.003 ^{c}	93.33 ± 5.77 ^{ab}	526.09 ± 1.60
S80	0.44 ± 0.001	10.46 ± 0.35 ^{c}	2238.94 ± 79.58^{b}	1.85 ± 0.21^{b}	5.62 ± 0.060^{c}	84.16 ± 18.76 ^{b}	483.43 ± 24.00

Values in the table are mean ± S.D ($n = 3$); Different superscripts in the same row represent significant differences ($P < 0.05$)

IBW, initial body weight; *TBW*, terminal body weight; *WGR*, weight gain ratio; *SGR*, specific growth rate; *SR*, survival rate; *FCR*, feed conversion ratio

Table 3 Effects of replacing fishmeal with soybean protein concentrate (SPC) on hepatopancreas body index and protein utilization of juvenile *L. vannamei*

Experimental diets	HBI	PER (%)	PDR (%)
FM	3.27 ± 0.44	1.93 ± 0.019[a]	32.36 ± 0.97 [ab]
S10	3.60 ± 0.62	1.86 ± 0.062[a]	31.28 ± 1.95 [ab]
S20	3.59 ± 0.01	1.91 ± 0.057[a]	33.98 ± 3.64 [a]
S30	3.70 ± 0.07	1.81 ± 0.102[a]	31.94 ± 2.82 [ab]
S40	3.29 ± 0.10	1.87 ± 0.047[a]	31.83 ± 0.76 [ab]
S60	3.61 ± 0.05	1.67 ± 0.085[ab]	27.94 ± 1.72[b]
S80	3.65 ± 0.36	1.51 ± 0.367[b]	25.84 ± 5.38 [b]

Values in the table are mean ± S.D ($n = 3$); Different superscripts in the same row represent significant differences ($P < 0.05$)

HBI, hepatopancreas body index; *PER*, protein efficiency ratio; *PDR*, protein deposition rate

80%, which was significantly lower than the replacement level of 20% ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$). The hepatosomatic index of each group was between 3.27 and 3.70, and the difference between groups showed no significance ($P > 0.05$).

Effects of replacing FM with SPC on whole shrimp composition for juvenile L. vannamei

Table 4 shows that the different proportions of SPC had significant effects on the crude lipid and ash content of whole shrimp ($P < 0.05$) but had no significant effect on the contents of moisture, crude protein, and phosphorus ($P > 0.05$). The lowest content of whole shrimp crude lipid was observed in the 80% replacement group, whereas the highest was noted in the control group. The whole shrimp crude lipid in the 80% replacement group was significantly lower than that of the other groups ($P < 0.05$); the control group exhibited significantly higher whole shrimp crude lipid content than the other groups ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$). The whole shrimp

Table 4 Effects of replacing fishmeal with soybean protein concentrate (SPC) on whole body composition of juvenile *L. vannamei*

Experimental diets	Moisture (%)	Crude protein (%)	Crude lipid (%)	Crude ash (%)	Phosphorus (%)
FM	78.24 ± 0.56	73.93 ± 1.48	9.77 ± 0.33 [a]	13.86 ± 0.10 [a]	7.36 ± 0.24
S10	78.25 ± 1.10	74.21 ± 1.70	8.66 ± 0.28 [b]	12.25 ± 0.90 [b]	7.55 ± 0.38
S20	77.40 ± 2.85	75.74 ± 0.86	8.90 ± 0.39 [b]	12.38 ± 0.05 [b]	7.19 ± 0.51
S30	77.66 ± 0.89	75.47 ± 0.79	8.69 ± 0.57 [b]	12.94 ± 0.47 [b]	7.31 ± 0.72
S40	78.28 ± 0.76	75.22 ± 0.89	8.88 ± 0.77 [b]	12.85 ± 0.19 [b]	7.24 ± 0.26
S60	78.69 ± 0.05	75.29 ± 0.79	8.56 ± 0.19 [b]	12.35 ± 0.20 [b]	7.36 ± 0.43
S80	78.09 ± 0.72	74.08 ± 0.57	8.24 ± 0.17 [c]	12.67 ± 0.51 [b]	7.39 ± 0.30

Values in the table are mean ± S.D ($n = 3$); Different superscripts in the same row represent significant differences ($P < 0.05$)

ash content of the control group was significantly higher than that of the other groups ($P<0.05$); the difference between the other groups was not significant ($P>0.05$). The moisture, crude protein, and phosphorus contents of whole shrimp showed no significant difference among the groups ($P>0.05$).

Effects of replacing FM with SPC on serum biochemical indices for juvenile L. vannamei

Table 5 reveals that different proportions of SPC had significant effects on serum total protein, glucose, total cholesterol (TC), and triglyceride (TG) levels ($P<0.05$). Serum total protein was the highest in the 20% FM replacement group, followed by the control group, whereas the 80% replacement group had the lowest serum total protein. The serum total protein of the 20% replacement group was significantly higher than that of the 40%, 60%, and 80% replacement groups ($P<0.05$). The 30% replacement group exhibited the highest serum glucose content, followed by the 40% group. The serum glucose content of the 30% replacement group was significantly higher than that of the control group ($P<0.05$), and the 10%, 30%, 60%, and 80% replacement groups ($P<0.05$) showed no significant difference with the 40% replacement group ($P>0.05$). The serum glucose content of the 60% and 80% replacement groups was significantly lower than that of the 20%, 30%, and 40% replacement groups ($P<0.05$). No significant difference was observed between the control and 10% replacement groups ($P>0.05$); the difference between the other groups was not significant ($P>0.05$). The highest TC was observed in the control group, followed by the 10% replacement group, and the lowest in the 80%replacement group. The TC of the control group was significantly higher than that of the 40%, 60%, and 80% replacement groups ($P<0.05$). The difference between groups was not significant ($P>0.05$); the difference between the other groups was not significant ($P>0.05$). The 80% replacement group showed the highest TG content, followed by the 60% replacement group, and the 10% replacement group with the lowest value. The TG contents of groups with 60% and 80% replacement levels were significantly higher than those of the control and 10%, 20%, and 30% replacement groups ($P<0.05$) and showed no significant difference from the 40%

Table 5 Effects of replacing fishmeal with soybean protein concentrate (SPC) on biochemical indexes in serum for juvenile *L. vannamei*

Experimental diets	TP (g L^{-1})	GLC (mmol L^{-1})	T-CHO (mmol L^{-1})	TG (mmol L^{-1})
FM	65.56 ± 4.67^{ab}	1.86 ± 0.16^{bc}	0.80 ± 0.05^{a}	0.40 ± 0.04^{b}
S10	64.50 ± 3.81^{ab}	1.92 ± 0.15^{bc}	0.78 ± 0.03^{a}	0.39 ± 0.07^{b}
S20	77.03 ± 9.99^{a}	2.25 ± 0.52^{ab}	0.73 ± 0.07^{ab}	0.40 ± 0.04^{b}
S30	63.23 ± 8.47^{ab}	2.82 ± 0.41^{a}	0.71 ± 0.06^{ab}	0.43 ± 0.03^{b}
S40	60.30 ± 9.73^{b}	2.46 ± 1.12^{ab}	0.67 ± 0.04^{b}	0.51 ± 0.04^{ab}
S60	59.60 ± 7.90^{b}	1.17 ± 0.27^{c}	0.64 ± 0.12^{b}	0.59 ± 0.02^{a}
S80	61.90 ± 9.03^{b}	1.17 ± 0.02^{c}	0.62 ± 0.05^{b}	0.58 ± 0.08^{a}

Values in the table are mean ± S.D ($n=3$); Different superscripts in the same row represent significant differences ($P<0.05$)

TP, total protein; *GLC*, glucose; *T-CHO*, total cholesterol; *TG*, triglyceride

replacement group ($P > 0.05$). The difference between the other groups was not significant ($P > 0.05$).

Effects of replacing FM with SPC on serum non-specific immune enzyme activity for juvenile L. vannamei

The SPC replacement of FM showed a significant effect on the activities of serum acid phosphatase (ACP), alkaline phosphatase (AKP), total superoxide dismutase (T-SOD), and nitric oxide synthase (NOS) in juvenile *L. vannamei* (Table 6; $P < 0.05$). The lowest ACP activity was observed at the 80% replacement level, followed by that observed at 60%, and the highest at 10%. The ACP activity in the 60% and 80% replacement groups was significantly lower than that of the other groups ($P < 0.05$). No significant difference was observed between the control, 10%, and 20% replacement groups ($P > 0.05$), and the value was significantly higher than that of the other groups ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$). The 40%, 60%, and 80% replacement groups showed no significant difference in AKP activity ($P > 0.05$), whose value was significantly lower than that of the other groups ($P < 0.05$). The control, 10%, and 20% replacement groups showed no significant difference with the 30% replacement group ($P > 0.05$), which showed a significantly higher AKP activity than the 40%, 60%, and 80% replacement groups ($P < 0.05$). The 30% replacement and control groups showed no significant difference with the 10% and 40% replacement groups ($P > 0.05$) but significantly higher than the 80% replacement groups ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$). The group with a 10% replacement level showed the highest T-SOD activity, and that with the 80% replacement presented the lowest. The T-SOD activities of the control, 10%, and 20% replacement groups were significantly higher than those of the other groups ($P < 0.05$). The value for the 80% replacement group was significantly lower than those of the remaining groups ($P < 0.05$); the differences between the remaining groups were not significant ($P > 0.05$). The 80% replacement group showed the lowest NOS activity, followed by the 60% replacement group; the values for both groups were significantly lower than those of the other groups ($P < 0.05$). Meanwhile, the values for the control and 30% replacement groups were significantly higher than those of the 40%, 60%,

Table 6 Effects of replacing fishmeal with soybean protein concentrate (SPC) on non-specific immune enzyme activity in serum for juvenile *L. vannamei*

Experimental diets	ACP (U 100 ml^{-1})	AKP (U 100 ml^{-1})	SOD (U 100 ml^{-1})	NOS (U 100 ml^{-1})
FM	9.50 ± 1.30 ^{a}	3.08 ± 0.52 ^{ab}	261.55 ± 7.03 ^{a}	21.11 ± 0.47 ^{a}
S10	10.47 ± .36 ^{a}	3.27 ± 0.22 ^{a}	285.01 ± 14.87 ^{a}	22.46 ± 1.95 ^{ab}
S20	8.45 ± 0.34 ^{b}	3.26 ± 0.09 ^{a}	266.31 ± 27.83^{a}	22.72 ± 0.21 ^{ab}
S30	8.32 ± 0.30 ^{b}	2.69 ± 0.21 ^{bc}	222.13 ± 16.87 ^{b}	21.66 ± 1.39 ^{a}
S40	7.69 ± 0.12 ^{bc}	2.48 ± 0.02 ^{c}	228.25 ± 22.77 ^{b}	19.37 ± 2.68^{b}
S60	7.22 ± 0.17 ^{c}	2.49 ± 0.10 ^{c}	215.43 ± 5.77 ^{b}	15.35 ± 1.10 ^{c}
S80	7.29 ± 0.53 ^{c}	2.63 ± 0.04 ^{c}	171.08 ± 11.97^{c}	16.08 ± 2.27 ^{c}

Values in the table are mean ± S.D ($n = 3$); Different superscripts in the same row represent significant differences ($P < 0.05$)

ACP, acid phosphatase; *AKP*, alkaline phosphatase; *SOD*, superoxide dismutase; *NOS*, nitric oxide synthase

and 80% replacement groups ($P<0.05$). The NOS activity of the 40% replacement group was significantly lower than that of the 10%, 20%, and 30% replacement groups ($P<0.05$) and significantly higher than that of the 60% and 80% replacement groups ($P<0.05$); the remaining groups showed no significant difference ($P>0.05$).

Effects of replacing FM with SPC on apparent digestibility of dry matter, crude protein, crude lipid, and gross energy in the diet

The results in Table 7 show that the digestibility of dry matter, crude protein, crude lipid, gross energy, and phosphorus in the diet showed a significant effect ($P<0.05$) with the changes in the proportion of SPC. The group with a 30% replacement level exhibited the highest apparent digestibility of dry matter, followed by the control group, and the lowest in the 80% replacement group. The values for the 60% and 80% replacement groups were significantly lower than those of the other groups ($P<0.05$), whereas the difference between the other groups was not significant ($P>0.05$). The 10% replacement group presented the highest apparent digestibility of crude protein, followed by the control group. No significant difference was observed between the control and 10%, 20%, and 30% replacement groups ($P>0.05$), whose apparent digestibility of crude protein was significantly higher than that of the other groups ($P<0.05$). The values for the 60% and 80% replacement groups were significantly lower than those of the other groups ($P<0.05$), and the differences between the other groups were not significant ($P>0.05$). The control group exhibited the highest apparent digestibility of crude lipid, followed by the group with 10% replacement. The value for the control group was significantly higher than those of 30 to 80% replacement groups ($P<0.05$) and showed no significant difference between the 10 and 20% replacement groups ($P>0.05$). The apparent digestibility of crude lipid of the 30% replacement group was significantly lower than that of the control group ($P<0.05$) and higher than that of the 40–60% replacement groups ($P<0.05$). The difference between the 10 and 20% substitution groups was not significant ($P>0.05$), whereas that between other groups showed significance ($P<0.05$). The highest gross energy was recorded in the control group and the lowest in the 80% substitution group. The gross energy of the 60% substitution group was significantly higher than that of the 80% substitution group

Table 7 Apparent digestibility coefficients of dry matter, crude protein, crude lipid, and gross energy in the diets for juvenile *L. vannamei* (%)

Experimental diets	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Gross energy (%)
FM	86.33 ± 1.32^{a}	90.15 ± 0.70^{a}	94.75 ± 0.63^{a}	83.91 ± 2.19^{a}
S10	85.69 ± 0.24^{a}	91.39 ± 0.56^{a}	94.09 ± 0.54^{ab}	82.33 ± 1.07^{a}
S20	86.14 ± 1.92^{a}	$90.11 \pm 0.70^{a\,b}$	94.45 ± 0.40^{ab}	82.77 ± 2.28^{a}
S30	86.58 ± 1.36^{a}	89.79 ± 1.21^{b}	93.55 ± 0.56^{b}	83.39 ± 1.48^{a}
S40	86.21 ± 0.36^{a}	87.78 ± 0.56^{b}	92.23 ± 1.07^{c}	81.63 ± 0.60^{a}
S60	84.62 ± 0.26^{b}	84.37 ± 0.62^{c}	90.56 ± 0.58^{d}	78.41 ± 0.89^{b}
S80	83.37 ± 0.48^{b}	81.83 ± 1.50^{c}	87.83 ± 0.69^{e}	75.04 ± 1.26^{c}

Values in the table are mean ± S.D ($n=3$); Different superscripts in the same row represent significant differences ($P<0.05$)

($P < 0.05$) and significantly lower than that of the other groups ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$).

Effects of replacing FM with SPC on apparent digestibility of amino acids in the diet

The results in Table 8 show that the apparent digestibility of amino acids in the treatment showed a significant effect on the changes in the SPC to FM ratio ($P < 0.05$).

Except for leucine, the apparent digestibility of the remaining eight essential amino acids was the lowest in the 80% substitution group, followed by the 60% group. The apparent digestibility of various essential amino acids in the 80% substitution group was significantly lower than that of the remaining groups ($P < 0.05$). The apparent digestibility of leucine was the lowest in the 80% substitution group, followed by the 60% group, and no significant difference was observed between both substitution groups ($P > 0.05$), whose values were significantly lower than those of the others ($P < 0.05$). The difference between the 60 and 40% substitution groups was not significant ($P > 0.05$) and was significantly lower than that of the other groups ($P < 0.05$). The difference between the 40 and 20% substitution groups was not significant ($P > 0.05$) and was significantly lower than that of the other groups ($P < 0.05$); the difference between the other groups was not significant ($P > 0.05$).

The apparent digestibility of eight non-essential amino acids was the lowest in the group with 80% replacement, followed by the 60% replacement group. Except for serine, tyrosine, and alanine, the amino acids in the group with 80% replacement level were significantly lower than that of the control and 10–40% replacement groups ($P < 0.05$). The apparent digestibility of serine was the lowest in the 80% replacement group, and the difference showed no significance with the group with a 10% replacement level ($P > 0.05$) and was lower than that of the other groups ($P < 0.05$). The differences between the other groups were not significant ($P > 0.05$). The apparent digestibility of tyrosine was the highest in the control group, followed by the 10% replacement group. The difference between the control and 10% replacement groups exhibited no significance ($P > 0.05$) but was significantly higher than that of the other groups ($P < 0.05$). The apparent digestibility of tyrosine in the 60% replacement group was significantly lower than that in the other groups ($P < 0.05$). The difference between the other groups was not significant ($P > 0.05$). The highest apparent digestibility of alanine was observed in the 10% replacement group and the lowest in the 80% replacement group. The apparent digestibility of alanine in the groups with 80% and 60% replacement levels was significantly lower than those of the 10% and 30% replacement groups ($P < 0.05$). No significant difference was observed with the 40% replacement group ($P > 0.05$). The 40% replacement group showed a significantly lower apparent digestibility of alanine than the 10% replacement group ($P < 0.05$), whereas the other groups showed no significant difference ($P > 0.05$).

Discussion

Effects of replacing FM with SPC on growth, feed utilization, and whole shrimp composition of juvenile L. vannamei

The effects of replacing FM with SPC have been studied using juvenile *L. vannamei* (Chen et al. 2017; Ray et al. 2020). Studies on other aquatic animals showed that SPC replaces less than 50% of FM, and the WGR, feed utilization, and food intake of *P. monodon*

Table 8 Apparent digestibility of amino acids in the practical diets for juvenile *L. vannamei* (%)

Amino acids composition	Experimental diets						
	FM	S11	S22	S33	S44	S55	S66
Essential amino acids							
Lysine	91.47 ± 0.62 ^{a}	91.55 ± 1.29 ^{a}	90.80 ± 1.70 ^{ab}	90.07 ± 0.43 ^{ab}	88.81 ± 0.63 ^{b}	88.88 ± 1.18 ^{b}	85.30 ± 2.16^{c}
Methionine	74.12 ± 5.24^{ab}	72.74 ± 1.71^{b}	74.96 ± 4.19^{ab}	77.96 ± 2.69^{ab}	79.30 ± 1.31^{a}	73.68 ± 2.73^{b}	62.95 ± 1.27^{c}
Isoleucine	84.92 ± 1.79 ^{ab}	85.89 ± 2.20 ^{a}	85.34 ± 0.13 ^{ab}	82.16 ± 1.64 ^{b}	82.12 ± 1.20 ^{b}	86.85 ± 2.29 ^{c}	76.86 ± 3.48 ^{c}
Leucine	83.64 ± 1.89 ^{ab}	86.08 ± 2.30 ^{a}	84.93 ± 2.07 ^{ab}	85.09 ± 1.28 ^{a}	81.25 ± 2.56 ^{bc}	79.60 ± 1.50 cd	75.43 ± 4.36 ^{d}
Phenylalanine	83.43 ± 1.82 ^{ab}	81.20 ± 0.97 ^{bc}	85.73 ± 2.57 ^{a}	85.73 ± 2.57 ^{a}	85.79 ± 0.55 ^{a}	83.92 ± 1.44 ^{ab}	78.73 ± 4.16 ^{c}
Valine	79.92 ± 3.79 ^{ab}	77.32 ± 1.18^{b}	79.03 ± 1.61 ^{ab}	81.79 ± 2.46^{a}	81.57 ± 0.12^{a}	78.50 ± 2.07 ^{ab}	71.63 ± .58 ^{c}
Histidine	89.76 ± 0.73 ^{ab}	87.68 ± 1.17 ^{b}	90.65 ± 1.53 ^{a}	90.95 ± 1.27 ^{a}	90.06 ± 0.63 ^{ab}	88.85 ± 1.11 ^{ab}	82.93 ± 3.35^{c}
Arginine	91.65 ± 1.41 ^{a}	90.57 ± 0.77 ^{a}	91.93 ± 0.79 ^{a}	92.47 ± 1.01 ^{a}	91.86 ± 0.61 ^{a}	90.86 ± 1.06 ^{a}	87.85 ± 1.92 ^{b}
Threonine	83.32 ± 2.53 ^{a}	81.26 ± 1.19 ^{a}	84.40 ± 1.93 ^{a}	84.08 ± 2.41 ^{a}	83.37 ± 0.46 ^{a}	81.42 ± 1.90 ^{a}	75.22 ± 2.23^{b}
Non-essential amino acids							
Aspartic acid	85.37 ± 2.11 ^{a}	85.39 ± 1.45 ^{ab}	86.55 ± 2.20 ^{a}	86.77 ± 2.23 ^{a}	86.73 ± 0.43 ^{a}	83.26 ± 0.92 ^{bc}	80.66 ± 1.96 ^{c}
Tyrosine	81.41 ± 2.13 ^{ab}	83.68 ± 3.04 ^{a}	83.70 ± 2.60 ^{a}	83.13 ± 0.50 ^{ab}	79.97 ± 1.96 ^{ab}	78.89 ± 0.78 ^{b}	73.85 ± 4.56^{c}
Serine	82.70 ± 2.72 ^{a}	81.40 ± 0.81 ^{ab}	84.01 ± 2.06 ^{a}	84.83 ± 2.03 ^{a}	84.83 ± 0.67 ^{a}	83.37 ± 1.86 ^{a}	77.90 ± 2.80 ^{b}
Glutamic acid	86.59 ± 1.98 ^{ab}	86.77 ± 1.84 ^{ab}	88.44 ± 1.47 ^{a}	88.31 ± 1.77 ^{a}	88.50 ± 0.65 ^{a}	84.28 ± 1.13 ^{b}	82.58 ± 2.30 ^{c}
Proline	83.44 ± 2.42 ^{ab}	86.43 ± 1.30 ^{a}	84.94 ± 2.95 ^{ab}	86.14 ± 0.95 ^{a}	83.00 ± 4.39 ^{ab}	80.73 ± 1.45^{bc}	76.60 ± 3.73^{c}
Glycine	83.85 ± 2.04 ^{ab}	82.49 ± 0.95 ^{a}	84.20 ± 1.60 ^{a}	84.23 ± 2.43 ^{a}	82.78 ± 0.40 ^{ab}	81.40 ± 1.12^{b}	76.48 ± 1.39 ^{c}
Alanine	81.66 ± 2.33 ^{ab}	83.11 ± 3.32 ^{a}	81.80 ± 3.22 ^{ab}	81.84 ± 1.03 ^{ab}	78.38 ± 2.24 ^{bc}	76.92 ± 1.59^{c}	73.15 ± 3.21^{c}

Values in the table are mean ± S.D ($n = 3$); Different superscripts in the same row represent significant differences ($P < 0.05$)

showed no significant effect (Paripatananont et al. 2001). The results of this experiment were close to those obtained on *P. monodon*. SPC can replace 25% of FM in the gingival diet without negatively affecting the growth and feed utilization (Day and González 2000). Aragao et al. (2003) showed that 30% replacement of FM with SPC significantly affected growth. However, other studies reported that SPC alteration of FM hurts fish growth. FM with 75% SPC replacement reduced the GR of *Atlantic salmon* (Storebakken et al. 2000). In *Dentex dentex*, when SPC replaced more than 25% of FM, its growth and diet conversion efficiency were significantly reduced (Chatzifotis et al. 2008). FM with 30% SPC replacement significantly reduced the growth of true carp (Kissil et al. 2000). Deng et al. (2006) showed that 25% SPC replacement of FM in the Japanese flounder (*Paralichthys olivaceus*) diet significantly reduced the diet intake, SGR, diet efficiency, and protein efficiency. When FM was replaced with 100% SPC, the rainbow trout growth and food intake were significantly reduced (Mambrini et al. 1999). Different scholars have varying views on the causes of decreased fish growth performance and feed utilization caused by the proportion of SPC. Anderson and Wolf (1995) suggested that the difference in test results may be due to differences in SPC quality, and the different processing methods resulted in varied contents of antitrypsin and isoflavones (Anderson and Wolf 1995). Francis et al. believed that the removal of specific carbohydrates in SPC during processing may also lead to the decreased palatability of fish (Francis et al. 2001); it may be due to the high content of soluble and insoluble non-starch polysaccharides in the SPC that caused the feed conversion rate to decrease (Knudsen 1997), whereas Mambrini et al. pointed out that the addition of SPC to the diet reduces food intake and thus inhibits growth (Mambrini et al. 1999). Deng Jun Ming also believed that the addition of SPC causes a decrease in gum intake and diet digestibility, leading to a decline in growth and feed utilization (Deng et al. 2006). However, Mambrini et al. (1999) showed that the titer of amino acids is the main factor affecting diet protein intake. Studies on *P. monodon* showed that SPC substitution for 100% fishmeal had no significant effect on survival, which is not consistent with this experiment (Paripatananont et al. 2001). The possible reasons are as follows: The difference in the object may be due to the different tolerance of the protein composition of *P. monodon* and *L. vannamei* to the composition of diet proteins. Second, the composition of the basic diet formula differed in the two trials. Studies on the effect of SPC substitution of fishmeal on the body composition have been conducted, and studies in *P. monodon* showed that the substitution of 0–100% fishmeal with SPC had no significant effect on whole shrimp moisture, crude protein, and crude ash, but crude lipid content decreased significantly with increasing substitution levels, which is consistent with the results of this experiment. (Paripatananont et al. 2001). The research on *Eriocheir sinensis* showed that when SPC replaced 77.19% of FM protein, the crude protein and crude lipid contents of *Eriocheir sinensis* decreased, but had no significant effect on moisture and ash content (Li et al. 2006). Paripatananont et al. (2001) pointed out that the fat content of whole shrimp increased with the increase in SPC level, which may be caused by the difference in fat utilization caused by the imbalance of fatty acids. Various studies focused on the effects of SPC and FM groups on body composition. The replacement of 40% FM by SPC in diet had no significant effect on the moisture, crude protein, and crude lipid content of the whole fish (Berge et al. 1999). A study on golden carp also showed that the whole-body crude lipid content disagrees with the changes in SPC replacement (Kissil et al. 2000). Studies on Senegal have shown that FM with 30% SPC replacement had no significant effect on whole fish crude protein and crude lipid content (Day and González 2000). For African catfish, studies have shown that crude lipid content was unaffected by FM with 0–100% SPC replacement (Francis et al. 2001). However, the proportion of SPC has a significant

effect on the body composition of fish. With the increase in the proportion of SPC instead of FM, the crude lipid content of whole fish is reduced. When replacing FM with 100% SPC, the whole fish showed the lowest fat content (Kim et al. 1998). When the SPC substitution level in FM was increased to 100%, the crude lipid content of whole rainbow trout was reduced (Mambrini et al. 1999). Deng et al. (2006) showed that SPC replaced FM protein, significantly increasing the ash content, whereas crude lipid content was significantly reduced. Studies on Atlantic salmon have shown that as the proportion of SPC gradually replaced the FM in the diet, the ash content of the whole fish was significantly reduced (Aragão et al. 2003), whereas the crude lipid content was increased (Li and Huang 2018). Studies on African catfish showed that when FM was replaced with 100% SPC, the whole fish crude protein content decreased from 17.1 to 16% in the control group, whereas the ash content increased from 2.9 to 4.4% in the control group (Francis et al. 2001). The different proportions of SPC have varied effects on body composition.

Effects of replacing FM with SPC on diet nutrient digestibility for juvenile L. vannamei

The results in Table 7 show that the different proportions of SPC had a significant effect on the dry matter, crude protein, crude lipid, and gross energy apparent digestibility of the diet ($P<0.05$) with the increase in SPC content. The FM content was reduced, and the apparent digestibility of dry matter, crude protein, crude lipid, and gross energy in the diet was significantly low ($P<0.05$). This finding is the same as that observed by Chen et al. (2017). Studies on the apparent digestibility of dry matter in the diet showed that when FM was replaced with more than 75% SPC, the apparent digestibility of dry matter of rainbow trout was significantly reduced from 78.5 to 70.3% in the control group (Stickney et al. 2007). When SPC replaced 50% of FM, the apparent digestibility of dry matter decreased from 67 to 64.8% in the control group (Zhang et al. 2014). Studies on the apparent digestibility of fat showed that when SPC replaced FM by more than 75%, the rainbow trout showed significantly reduced diet crude lipid digestibility from 90.3 to 70.2% in the control group (Mambrini et al. 1999). When SPC in the flounder diet replaced 50% of the fish meal protein, the apparent digestibility of crude lipid also decreased significantly as the level of substitution increases. When the replacement level was 87.5%, the apparent digestibility of crude lipid decreased from 91.3 to 85.8% in the control group (Zhang et al. 2014). Non-starch polysaccharides in soy products can affect the digestibility of crude lipid (Ståle et al. 1999; Storebakken et al. 1998). Studies on the apparent digestibility of gross energy showed that when SPC replaced more than 75% of FM, the gross energy digestibility of rainbow trout was significantly reduced from 87 to 77.2% in the control group (Stickney et al. 2007). When SPC replaced 25% FM, the apparent digestibility of crude protein decreased from 87.9 to 80.4% in the control group (Lin et al. 2004). The results of this test are consistent with those of the study on gums. Berge et al. showed that when SPC replaced 44% of the FM protein, the apparent digestibility of the diet crude protein had no significant effect. However, after SPC was used to replace FM at the 100% level, the apparent digestibility of the diet crude protein was significantly reduced (Berge et al. 1999). Mambrini et al. showed that rainbow trout revealed no significant difference in the apparent digestibility for FM diet crude protein with 75% SPC replacement (Mambrini et al. 1999). Berge et al. pointed out that the reason for the reduction of apparent digestibility of diet crude protein by SPC instead of FM is mainly due to the imbalance of amino acids (Berge et al. 1999). However, anti-nutritional factors in SPC also contribute to the digestibility

of diet crude protein (Francis et al. 2001). The addition of SPC to the diet resulted in a decreased diet crude protein digestibility due to phytic acid content (Spinelli et al. 1983). Plant materials contain different levels of non-starch polysaccharides (NSP), and NSP can increase the viscosity of food, thereby reducing the digestibility of nutrients (Boonyaratpalin et al. 1998; Ståle et al. 1999). NSP can also bind bile acids, enabling the villi to block the movement of the digestive tract. Blockage (Storebakken et al. 1998) leads to a decrease in diet nutrient digestibility. Research on the apparent digestibility of SPC substituted fishmeal for amino acids in the diet has been studied in fish. When SPC replaced 25% of FM, the digestibility of essential, non-essential, and total amino acids of the gums was significantly lower than that of the whole FM group. When SPC replaced 50% of FM, the apparent digestibility of essential amino acids was significantly lower than that of the FM group (Zhang et al. 2014). Studies on rainbow trout showed that when SPC replaced FM at levels higher than 75%, the apparent digestibility of phenylalanine decreased significantly from 83.3 to 73.3% in the control and had no significant effect on the apparent digestibility of other essential amino acids. Masumoto et al. also showed that when using SPC instead of 75% FM, the apparent digestibility of amino acids in the diet was significantly lower than that in the whole FM group (Masumoto et al. 1996). Studies on Senegal have shown that when SPC replaces nearly 80% of FM, amino acid metabolism enzyme activity changes (Médale et al. 1998), and further combining with the results of the present study (Tables 7 and 8), as the SPC content increased, and the FM content decreased, the amino acids in the diet were consistent with the change law of apparent digestibility of protein. Deng et al. believe that the effects of replacing FM with SPC on the apparent digestibility of dietary amino acids, in addition to the anti-nutritional factors, such as trypsin inhibitor, NSP, and SPC added to reduce the palatability of aquatic animals, the amino acid of SPC imbalance is also one of the important influencing factors (Zhang et al. 2014). By adding crystalline amino acids, the amino acid utilization rate of the diet can be improved (Deng et al. 2006), but in this test, the total amount of amino acids in each treatment group was the same given the addition of crystalline amino acids, thereby avoiding the decrease in digestibility caused by the different total amino acids in the diet.

Effects of replacing FM with SPC on serum biochemical indicators for juvenile L. vannamei

TC and TG are important biochemical components of the cell wall, and they help in the control of fat content and low cholesterol in the blood, which leads to the growth and survival of shrimp (Hamiltonreeves et al. 2018). In this study, SPC replaced FM, which TC was greatly reduced. The results are similar to those observed by Biswas (Biswas et al. 2019). The effect of SPC substitution for FM on serum biochemical indicators has been studied in aquatic animals. The study showed that the total protein content in the serum of *Eriocheir sinensis* decreased with the increase of SPC content (Coma et al. 1995). SPC in gingival diet replaced 25%, 50%, 75%, and 100% FM, plasma urea nitrogen showed no significant effect between the content and the whole FM control group (Deng et al. 2006), and the level of plasma urea nitrogen could also reflect aquatic animals. In vivo protein synthesis and diet protein utilization (Waibel et al. 1977), and the present experiment was similar to the results of the above researches. Studies on Chinese mitten crab showed that as the SPC content in the feed increased, its serum glucose content first increased and then decreased (Coma et al. 1995), while in this experiment, as the SPC content increased and the serum glucose content decreased, which was close to the results of this experiment.

The effects of replacing FM with SPC on serum cholesterol levels have not been reported in aquatic animals. Studies in mammals have shown that soy products have the effect of lowering blood cholesterol and increasing blood lipids (Anderson and Wolf 1995; Carroll 1991). However, the effect of lowering cholesterol in different soy products may be related to the soy isoflavone saponins, phytic acid, and trypsin inhibitors contained therein. Soy protein products cause the body's low cholesterol effect; the mechanism is that soy protein product stimulates the endocrine system, causing the thyroid hormone content to increase, thereby causing a decrease in blood cholesterol (Rd, 1986), whereas the pancreas secretes the active bile acid esterase, which can improve the digestion and absorption of cholesterol (Howles et al. 1996) and neutral lipids (Freed 1986) in the diet. The results of this experiment showed that the TC content in serum increased with the increase in SPC content, whereas the content of FM was reduced. A decrease in post-elevation occurred when the SPC replacement level was between 0 and 30%, and the TC content increased with the increase in SPC content ($P < 0.05$). When the SPC replacement level was higher than 40%, with the increase in SPC content, the cholesterol content was significantly lower ($P < 0.05$). In the diet of *L. vannamei*, an increase in SPC content can inhibit the synthesis of cholesterol. The results in Table 5 show that the content of TG increased with the increase in SPC content ($P < 0.05$); however, the effects of replacing FM with SPC on the serum TG content of aquatic animals are rarely reported. Chen et al. (2017) showed that the content of TG in *L. vannamei* decreased with the increase in SPC content. However, they only tested the initial weight of shrimp at 1.8 g, and the initial protein content of the diet was 20%. The specific mechanism needs further research.

Effects of replacing FM with SPC on serum non-specific immune enzyme activity for juvenile L. vannamei

NOS and SOD play important roles in aquatic disease resistance and immune regulation mechanisms (Chakravortty and Hensel 2003). The activity of NOS can be used as an effective indicator to reflect the health status and disease resistance of shrimp (Jiang et al. 2004). AKP and ACP are important regulatory enzymes in animal metabolism and are essential for the body to maintain its internal environment and health. AKP and ACP activity is affected by factors such as nutritional status (Mu et al. 1999). The results showed that with the increase in SPC content and the decrease in FM content, the activities of serum ACP and AKP of *L. vannamei* significantly increased ($P < 0.05$), whereas those of T-SOD and NOS significantly decreased ($P < 0.05$). The effects of replacing FM with SPC on serum non-specific immune enzyme activity had not been reported in shrimp but had been studied in other aquatic organisms. The results of the study on *Eriocheir sinensis* showed that as the proportion of SPC substituted FM increased, the activities of AKP, ACP, and SOD decreased (Li et al. 2006). Studies on rainbow trout have revealed that replacing soy protein isolates with FM can increase the activity of AKP, ACP, and lysozyme in liver tissues of rainbow trout. Studies on gingiva have shown that when SPC replaced 25–100% of FM, gingiva and the activity of aspartate aminotransferase (AST) in hepatopancreas was not affected by changes in the level of replacement, whereas the activity of alanine aminotransferase (ALT) was significantly reduced when the proportion of SPC replaced FM was 75–100% (Deng et al. 2006). However, the effects of replacing FM with SPC on serum NOS activity have not been reported in aquatic organisms. The mechanism of action of plant protein instead of FM on its activity needs further study.

Conclusions

The results of this experiment showed that when the level of SPC replacement fish meal protein was higher than 30%, the growth performance and feed utilization of juvenile *L. vannamei* will be decreased, apparent digestibility of nutrients in the diet will be reduced, total protein, glucose, cholesterol content, ACP, and AKP activity in serum will be reduced, and triglyceride content, NOS, and SOD activity in serum will be increased. Therefore, the appropriate level of SPC to replace fish meal protein was 30%.

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Declarations

Ethics approval All procedures involving live animals were approved by the Guangdong Ocean University Institutional Animal Care and Use Committee.

Conflict of interest The authors declare no competing interests.

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