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Effects of dietary fish meal replacement with protein mixtures on growth performance, biochemical composition, and physiological metabolism of juvenile swimming crab, *Portunus trituberculatus*

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Abstract
A feeding trial was conducted to investigate the effects of different levels (0%, 16.67%, 33.33%, 50%, and 66.67%) of fish meal replacement by protein mixtures on the growth, body composition, and physiological metabolism of juvenile swimming crabs, *Portunus trituberculatus*. The results showed that the final body weight (FBW), weight gain rate (WGR), specific growth rate (SGR), intermolt duration (ID), and hepatosomatic index (HSI) initially increased and then decreased with increasing dietary fish meal replacement levels, and the highest FBW, WGR, and SGR values were found in crabs fed Diet 3. The crude protein content in the body significantly decreased, whereas the moisture and ash contents increased significantly with increasing fish meal replacement levels. The digestive enzyme activity of crabs was significantly affected by different levels of fish meal replacement with protein mixtures. The highest glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) levels in the hepatopancreas were detected in crabs fed Diet 5. The serum superoxide dismutase (SOD) activity increased significantly with increasing fish meal replacement levels. In conclusion, the results of this study indicated that the appropriate dietary fish meal replacement level is approximately 33% with no significant negative effects on the growth performance of juvenile *P. trituberculatus*.

Keywords Protein mixtures · Fish meal · Growth performance · Enzyme activities · Hepatopancreas · Serum

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Introduction

Fish meal is an important raw material for aquaculture animal feeds (Zhang et al. 2010). It is used as the main protein source because of its abundant essential nutrients and palatability (Xie et al. 2016). However, as a result of increased aquaculture activities and the impact of El Niño recently, unstable production and the high price of fish meal are challenges for the increasing demand in the aquaculture feed industry (FAO 2012). Therefore, finding alternative protein sources to replace fish meal in diets is fundamental for the sustainable development of the aquaculture feed industry (Liu et al. 2014; Zhou et al. 2016).

Presently, many studies have focused on the considerable success of replacing fish meal with animal or plant protein sources in diets for different aquatic animals. On one hand, research has focused on using dehulled soybean meal, soy protein isolate, and fermented soybean meal in rainbow trout, *Oncorhynchus mykiss* (Barnes et al. 2015), and Pacific white shrimp, *Litopenaeus vannamei* (Gamboa-Delgado et al. 2013) diets, respectively. On the other hand, the utilization of the animal proteins has focused on poultry by-product meal, bone meal, and meat (Millamena 2002; Webster et al. 2000). Although alternative protein sources showed considerable potential in fish meal replacement, they also had some negative effects, such an amino acid imbalance, low protein content, the presence of antinutritional factors (ANFs), and a high fiber or ash content (Francis et al. 2001). This could slow the growth of aquatic animals. Some research suggested that a combination of plant meal with other protein sources in the diet was more effective than a single protein source (Watanabe et al. 1993). Studies on the replacement of fish meal indicated that the use of various protein sources together has been successful (Watanabe et al. 1993). Therefore, compared with the single protein source replacement of fish meal, protein mixtures have a higher protein level, higher digestibility, and less anti-nutritional factors, which can ensure effective nutrient absorption and enhanced aquaculture animal feed development.

In China, the swimming crab, *Portunus trituberculatus*, is an important marine aquaculture species, and in 2017, production reached approximately 123,154 tons (China Fishery Statistical Yearbook 2018). Fish meal, comprising > 20% of diets, is an important raw material of *P. trituberculatus* diets, so it is imperative to determine a fish meal replacement. Presently, soybean meal and other plant protein sources are widely used in aquaculture feed to replace fish meal. However, soybean meal contains several ANFs that depress nutrient digestion or absorption. In addition, certain essential amino acid contents are too low to satisfy the nutritional requirements of crabs, leading to lower growth (Chen et al. 1994). Protein mixtures have been prepared by combining plant and animal proteins, which have been effective in the diets of Chinese mitten crab, *Eriocheir sinensis* (Zhu et al. 2019). Therefore, this research was conducted to evaluate the effects of replacing fish meal with protein mixtures in the diet of juvenile *P. trituberculatus*, as assessed by growth performance, body composition, physiological metabolism, and antioxidant enzyme activities in relation to protein metabolism, to provide a theoretical reference for developing commercial feed with less fish meal for juvenile *P. trituberculatus* diets.

Materials and methods

Experimental diets

Protein mixtures were formulated using fermented soybean meal, pork powder, chicken powder, spray dried porcine blood protein powder, and shrimp powder in a ratio of

10:22:30:23:15, with the crude protein and lipid contents equal to that of fish meal. The experimental diets contained the following: soybean meal, rapeseed meal, fish meal, and protein mixtures as protein sources, and fish oil, lard oil, and soybean oil as lipid sources. Five isonitrogenous and isolipidic experimental diets (defined as Diets 1–5) were formulated to replace fish meal by 0%, 16.67%, 33.33%, 50%, and 66.67%, respectively. To make the diets, the dry ingredients were smashed to pass through an 80-mm mesh filter and thoroughly mixed. The diets (4.00–5.00 mm length) were subsequently extruded through a 2.00-mm orifice and air-dried at room temperature. All experimental diets were packed in a sealed black bag and stored at − 20 °C prior to use. The ingredients and proximate composition of the diets are presented in Table 1.

Crab rearing and experimental conditions

Juvenile *P. trituberculatus* individuals were obtained from the Qidong Base of the Shanghai Fisheries Research Institute (Jiangsu, P.R. China). Prior to the start of the experiment, the crabs were acclimated to the culture environment for 5 days and fed a commercial diet. The culture experiment was carried out in a cement pool with a circulating water system, and crabs were

Table 1 Formulations of five experimental diets (%)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Fish meal	24.00	20.00	16.00	12.00	8.00
Soybean meal	24.00	24.00	24.00	24.00	24.00
Rapeseed meal	14.00	14.00	14.00	14.00	14.00
Protein mixtures	0.00	4.00	8.00	12.00	16.00
Squid meal	4.00	4.00	4.00	4.00	4.00
Fish slurry	5.00	5.00	5.00	5.00	5.00
Brewer's yeast	2.00	2.00	2.00	2.00	2.00
Spirulina powder	0.50	0.50	0.50	0.50	0.50
Wheat flour	17.48	17.48	17.48	17.48	17.48
Vitamin premix[1]	0.20	0.20	0.20	0.20	0.20
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50
Choline chloride (60%)	0.50	0.50	0.50	0.50	0.50
Betaine	0.15	0.15	0.15	0.15	0.15
Taurine	0.30	0.30	0.30	0.30	0.30
Vitamin C (35%)	0.10	0.10	0.10	0.10	0.10
Canthaxanthin (10%)	0.02	0.02	0.02	0.02	0.02
Soy lecithin	2.00	2.00	2.00	2.00	2.00
Fish oil	1.50	1.50	1.50	1.50	1.50
Pork lard	1.50	1.50	1.50	1.50	1.50
Soybean oil	1.00	1.00	1.00	1.00	1.00
Proximate composition (%)					
Moisture	8.71	9.38	9.08	9.17	9.35
Crude protein	37.47	37.02	37.11	37.46	37.26
Crude lipid	10.99	11.01	11.22	11.24	11.60
Ash	8.77	8.63	8.49	8.19	7.94

[1] Vitamin mixture (mg kg^{-1} diet): retinol acetate 125; cholecalciferol 30; alpha-tocopherol 1050; menadione 35.4; thiamine 100; riboflavin 150; Vitamin B_6 150; vitamin B_{12} 0.2; ascorbic acid 700; biotin 4; *D* – calcium pantothenate 250; folic acid 25; nicotinamide 300

[2] Mineral mixture (mg kg^{-1} diet): $FeSO_4 \cdot H_2O$ 200; $CuSO_4 \cdot 5H_2O$ 96; $ZnSO_4 \cdot H_2O$ 360; $MnSO_4 \cdot H_2O$ 120; $MgSO_4 \cdot H_2O$ 240; KH_2PO_4 4200; NaH_2PO_4 500; KI 5.4; $CoCl_2 \cdot 6H_2O$ 2.1; Na_2SeO_3 3

cultured in a plastic basket. Eight plastic baskets (length × width × height = 27 × 19.5 × 11.5 cm; eight crabs) were used as replicates. Three separate replicates of eight baskets for each dietary treatment were randomly distributed in different areas of the cement pool to reduce the influence of external environmental effects. Only juvenile crabs in the intermolt stage that had both chela and all walking legs were chosen for the experiment. The experimental crabs were randomly divided into 5 experimental treatments, 3 replicate units in each treatment, and each replicate included 8 crabs. The crabs were fed once daily with 5% body weight per day at 17:00 h. Uneaten feed and feces were removed 3 h after feeding. The weight and carapace width of crabs were measured 3 to 5 days after molting.

During the experimental period, the water depth in the plastic baskets was kept at 10 cm. All rearing baskets were provided with continuous aeration and maintained under fluorescent lighting (12 h light: 12 h dark). During the experimental period, the water temperature, salinity, pH, ammonia nitrogen, nitrite, and dissolved oxygen ranged from 20.0 to 26.0 °C, 25 ppt, 7.0–9.0, < 0.5 mg L^{-1}, < 0.15 mg L^{-1}, and > 4 mg L^{-1}, respectively.

Sample collection techniques and chemical analyses

Crabs that completed the third molt in 6~10 days were selected for the experimental samples. After removing the water from the body surface, the wet weights of juvenile crabs were measured, and then 0.2 ml of hemolymph was exsanguinated from the base of the third walking limb of each crab and stored in a 1.5-ml centrifuge tube at − 40 °C. The hepatopancreas from each crab was dissected, weighed separately, and then stored at − 40 °C. The weight gain rate (WGR), specific growth rate (SGR), intermolt duration (ID), and hepatosomatic index (HSI) of the juvenile crabs were calculated using the following formulas:

$$\text{Weight gain ratio (WGR, \%)} = 100 \times (W_t - W_0) / W_0;$$
$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100 \times [\ln W_t - \ln W_0] / t;$$
ID (days) was the interval between the second molting date and the first molting date;
$$\text{HSI (\%)} = 100 \times M_H \times M_C^{-1}.$$

In these equations, W_t and W_0 represent the final and initial weights, respectively; t denotes the rearing days; M_H and M_C denote the hepatopancreas wet weight and body wet weight, respectively, of the swimming crabs.

Sample preparation and determination of physiological metabolism indices

The hemolymph was homogenized for 30 s with an IKA Ultra-Turrax homogenizer (T10B, IKA Co., Germany), and the homogenate was centrifuged (12,000 rpm, 20 min) at 4 °C. Then, the supernatant was collected and stored at − 40 °C until the enzymatic activity analyses. For the hepatopancreas sample preparation, approximately 0.2 g of wet hepatopancreas and 1 ml of precooling crustacean physiological saline solution were homogenized with an IKA homogenizer, and similar procedures were performed for the hepatopancreas homogenate. Thereafter, the middle phase of clear liquid in the centrifuge tube was collected for the following determination.

Total protein (TP), glucose (GLU) content, glutamic-oxaloacetic transaminase (GOT) activity, glutamic-pyruvic transaminase (GPT) activity, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content in the serum and hepatopancreas were determined following the instructions of commercial assay kits (Nanjing Jiancheng Bioengineering

Institute, China). Total amino acid (TAA), urea (UREA), uric acid (UA), and total cholesterol (TC) contents in the serum were determined following the instructions of commercial assay kits (Nanjing Jiancheng Institute, Nanjing, China). Digestive enzyme (trypsin, lipase, and amylase) activities in the hepatopancreas were also measured following the instructions of the detection kit (Nanjing Jiancheng Institute, Nanjing, China).

Biochemical composition analysis

The crude protein, crude lipid, moisture, and ash contents in whole crabs were determined according to the methods of the Association of Official Analytical Chemists (AOAC 1995). The moisture content was determined by drying the samples to a constant weight at 105 °C. The crude protein content was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method. The crude lipid content was determined using the ether extraction method with the Soxtec System HT (Soxtec System HT6, Tecator, Sweden). The ash content was determined using a muffle furnace at 550 °C for 8 h.

Statistical analysis

All of the statistical analyses were performed using SPSS 18.0 for Windows. The data were first tested for homogeneity; if the data had similar variances, then, a one-way ANOVA was used to test the main effect of the dietary treatment. Differences among means were tested using Duncan's multiple range tests. The level of significance chosen was $P < 0.05$.

Results

Growth performance of juvenile *P. trituberculatus*

The FBW, WGR, SGR, ID, and HSI are presented in Table 2. The FBW ranged from (11.12 ± 1.08) to (41.21 ± 6.31) g and was significantly affected by the different dietary fish meal/protein mixture ratio levels. The WGR of crabs fed Diets 1–4 were between (239.20 ± 41.90) and (441.34 ± 206.10), higher than that of crabs fed Diet 5 (130.00 ± 96.35), but there were no significant differences in the WGR of crabs between the Diets 1–4. Crabs fed Diet 3 had the highest FBW and WGR among all treatments. SGR of crabs fed Diets 1–5 initially increased and then decreased ($P < 0.05$). The ID of crabs fed Diet 3 was significantly lower than that of crabs fed on other diets ($P < 0.05$). However, the HSI of crabs fed Diet 3 was significantly lower than that of crabs fed Diet 4 ($P < 0.05$).

Body composition of juvenile *P. trituberculatus*

The proximate body compositions of *P. trituberculatus* fed different diets are presented in Table 3. Moisture contents were significantly affected by the different diets ($P < 0.05$). The moisture content of the whole body was significantly lower for crabs fed Diet 1 than those fed Diet 2 ($P < 0.05$), and there was no significant difference among the other three groups ($P > 0.05$). The crude protein contents in the whole body significantly decreased with increasing fish meal replacement levels and ranged from (12.42 ± 0.23) to (7.92 ± 0.74)%. Whole-body lipid contents (ranged from 0.79 to 0.22%) showed no significant differences among all the treatments. The ash contents of

Table 2 Effects of fish meal replacement by protein mixtures on growth, molt, and hepatosomatic index of juvenile *P. trituberculatus*

Items	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Initial body weight (g)	7.40 ± 1.19	8.97 ± 1.42	8.25 ± 2.51	8.31 ± 4.78	5.37 ± 2.03
Final body weight (g)	35.55 ± 1.74^{b}	30.35 ± 5.48^{b}	41.21 ± 6.31^{b}	27.64 ± 13.60^{b}	11.12 ± 1.08^{a}
Weight gain ratio (%)	387.15 ± 40.55^{b}	239.20 ± 24.19^{ab}	441.34 ± 119.00^{b}	248.46 ± 25.15^{ab}	130.00 ± 55.63^{a}
Specific growth rate (%·days^{-1})	1.65 ± 0.42^{b}	2.05 ± 0.23^{b}	2.93 ± 0.40^{a}	1.84 ± 0.28^{b}	1.69 ± 0.39^{b}
Survival rate (%)	42.00 ± 7.21^{b}	46.00 ± 7.22^{b}	88.00 ± 12.5^{c}	21.00 ± 7.22^{a}	13.00 ± 3.25^{a}
Intermolt duration (days)	43.83 ± 18.13^{b}	27.88 ± 7.24^{a}	24.56 ± 6.27^{a}	29.50 ± 15.46^{a}	20.80 ± 12.99^{a}
Hepatosomatic index (%)	6.17 ± 0.40^{ab}	6.82 ± 0.52^{ab}	7.31 ± 0.31^{b}	5.76 ± 0.69^{ab}	5.54 ± 0.48^{a}

Values are expressed as the means ± SD. Values with different letters within the same line are significantly different ($P < 0.05$)

crabs fed Diet 5 were significantly higher than those of crabs fed Diet 1 ($P < 0.05$), and there was no significant difference among the other three treatments ($P > 0.05$).

Serum and hepatopancreas physiological indices of juvenile *P. trituberculatus*

The serum physiological indices from crabs fed different diets are shown in Table 4. The total serum protein in crabs fed Diet 1 was significantly higher than those fed Diet 2 or 5 ($P < 0.05$), but there was no significant difference among Diets 3, 4, and 5 ($P > 0.05$). There were no significant differences among different diets regarding TAA and TC ($P > 0.05$). The serum urea content of crabs fed Diet 2 was significantly lower than those fed Diets 3 and 5 ($P < 0.05$), and there was no significant difference in serum urea content between those fed Diets 1 and 4 ($P > 0.05$). The serum UA content of crabs fed Diet 4 was significantly lower than that in those fed Diets 3 and 5 ($P < 0.05$), and no significant differences were observed among other diets ($P > 0.05$). The serum glucose concentration was significantly affected by the dietary protein mixtures levels, and that in crabs fed Diet 4 was significantly lower than that in those fed the other four diets ($P < 0.05$), but there was no significant difference among the other four treatments ($P > 0.05$).

The hepatopancreas physiological indices of crab fed different diets are shown in Table 4. Significant differences were observed in the total protein concentration in the hepatopancreas between treatments ($P < 0.05$), and that in crabs fed Diet 3 was significantly higher than that in

Table 3 Effect of fish meal replacement by protein mixtures on whole body composition in the body of juvenile *P. trituberculatus* (% wet weight)

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Moisture	73.15 ± 2.88^{a}	79.24 ± 3.67^{b}	76.90 ± 4.24^{ab}	77.24 ± 2.40^{ab}	77.05 ± 6.17^{ab}
Crude protein	12.42 ± 0.23^{d}	11.31 ± 0.15^{c}	11.04 ± 0.17^{c}	10.05 ± 0.08^{b}	7.92 ± 0.74^{a}
Crude lipid	0.79 ± 0.22	0.52 ± 0.19	0.58 ± 0.17	0.60 ± 0.15	0.62 ± 0.17
Ash	6.11 ± 0.17^{a}	6.56 ± 0.10^{ab}	6.40 ± 0.36^{ab}	6.62 ± 0.07^{ab}	7.22 ± 0.12^{c}

Values are expressed as the means ± SD. Values in the same line with different superscripts are significantly different ($P < 0.05$)

Table 4 Effect of fish meal replacement by protein mixtures on physiological indices of serum and hepatopancreas in juvenile *P. trituberculatus*

		Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Serum	Total protein (mg/ml)	0.18 ± 0.003^{c}	0.12 ± 0.01^{a}	0.16 ± 0.02^{bc}	0.16 ± 0.02^{bc}	0.13 ± 0.01^{ab}
	Total amino acid (μmol/ml)	6.10 ± 0.00	7.73 ± 2.82	11.79 ± 6.14	17.48 ± 11.06	11.79 ± 3.07
	Urea (mmol/l)	0.85 ± 0.08^{ab}	0.69 ± 0.02^{a}	0.88 ± 0.09^{b}	0.83 ± 0.16^{ab}	0.98 ± 0.03^{b}
	Uric acid (μmol/l)	78.04 ± 13.84^{ab}	80.18 ± 11.98^{ab}	90.78 ± 6.92^{b}	64.52 ± 8.30^{a}	92.17 ± 6.46^{b}
	Glucose (mmol/l)	1.27 ± 0.20^{b}	1.70 ± 0.17^{b}	1.87 ± 0.83^{b}	0.44 ± 0.14^{a}	1.71 ± 0.17^{b}
	Total cholesterol (mmol/l)	0.26 ± 0.02	0.28 ± 0.02	0.35 ± 0.04	0.33 ± 0.12	0.37 ± 0.04
Hepatopancreas	Total protein (mg/ml)	3.65 ± 0.07^{a}	3.63 ± 0.66^{a}	5.47 ± 0.02^{b}	3.76 ± 0.20^{a}	3.90 ± 0.87^{a}
	Glucose (mmol/l)	12.90 ± 2.17^{ab}	12.89 ± 1.05^{ab}	11.25 ± 2.49^{ab}	15.31 ± 1.08^{b}	8.07 ± 4.36^{a}

Values are expressed as means ± SD. Values within the same row with different superscripts are significantly different ($P < 0.05$)

those fed on other diets, and there were no significant differences among the other four treatments ($P > 0.05$). The hepatopancreas glucose content in crabs fed Diet 4 was significantly higher than that in those fed Diet 5 ($P < 0.05$), but no significant difference was observed among the other three treatments ($P > 0.05$).

Hepatopancreas digestive enzyme activities of juvenile *P. trituberculatus*

Figure 1 shows the different digestive enzyme activities of juvenile crabs fed different diets. The hepatopancreas trypsin activity in crabs fed Diet 3 was significantly lower than in that in those fed Diet 2 ($P < 0.05$) and lower than that in those fed Diets 1, 4, and 5, but there were no significant differences ($P > 0.05$). The hepatopancreas lipase activity in crabs fed Diet 5 was significantly higher than that in those fed the other four diets ($P < 0.05$), and no difference was observed among the other four treatments ($P > 0.05$). The hepatopancreas amylase activity in crabs fed Diet 3 was significantly lower than that in those fed Diets 1 and 2, and was also significantly lower than that in those fed Diet 4 ($P < 0.05$).

Serum and hepatopancreas protein metabolism enzyme activities in juvenile *P. trituberculatus*

GOT and GPT activities in crab serum fed different diets are shown in Fig. 2. Crabs fed Diet 3 had a significantly higher GOT activity than that in those fed Diets 1 and 5 ($P < 0.05$), and no

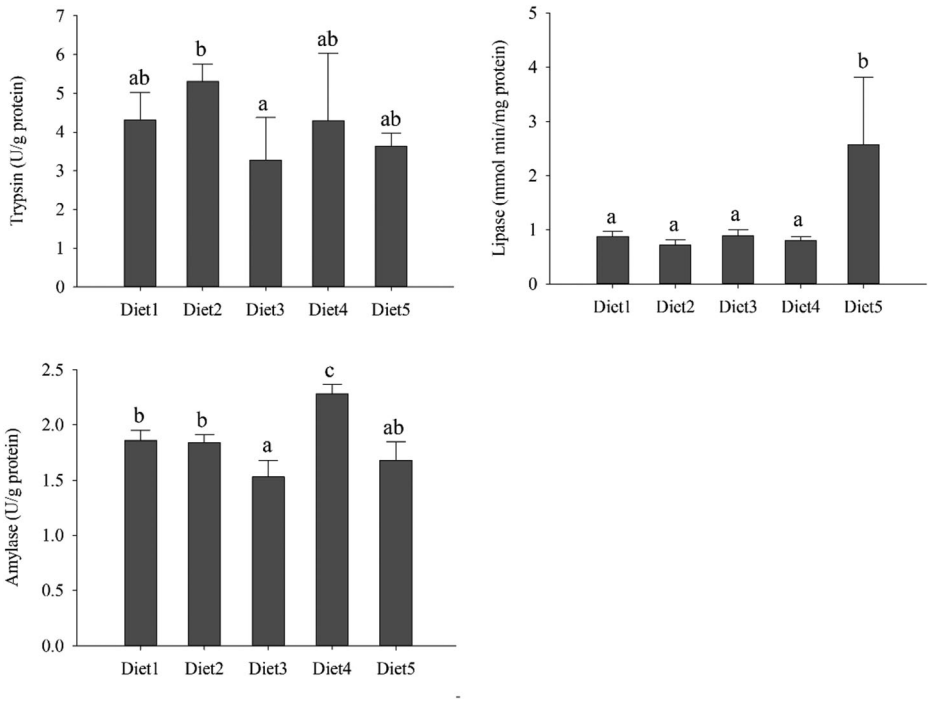

Fig. 1 Effect of fish meal replacement by protein mixtures on digestive enzyme activity of hepatopancreas in juvenile *P. trituberculatus*. Values are means ± SD. The column with different letters is significantly different ($P < 0.05$). The same case in the following figures

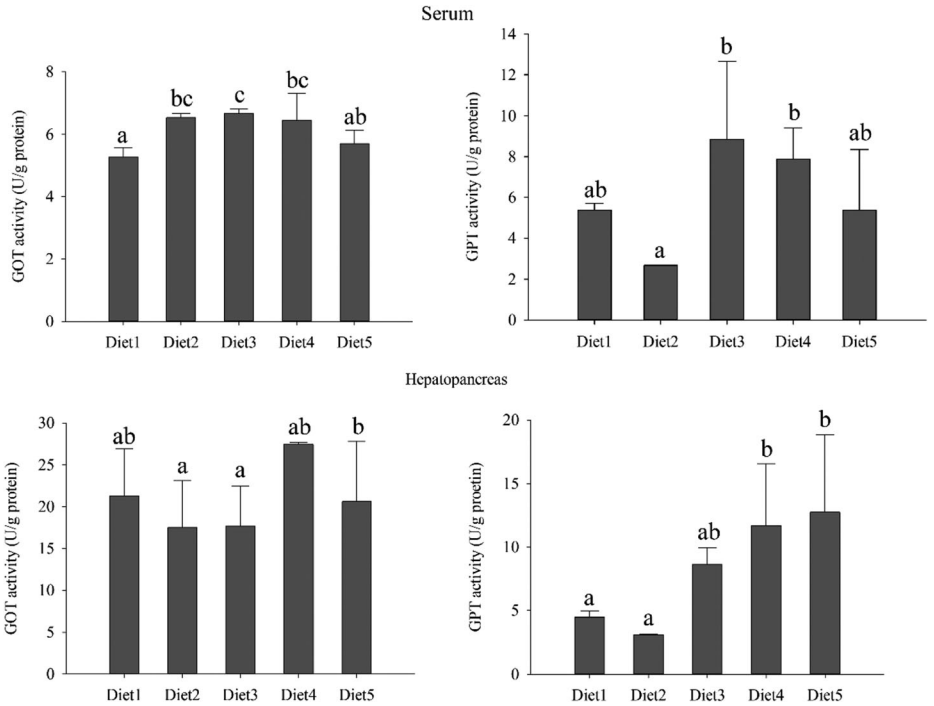

Fig. 2 Effect of fish meal replacement by protein mixtures on protein metabolism enzyme activity of serum and hepatopancreas in juvenile *P. trituberculatus*

significant difference was observed in the serum GOT activity between the other two treatments ($P > 0.05$). The metabolic enzyme GPT activity was significantly elevated ($P < 0.05$) in the serum of crabs fed Diets 3 and 4 than that in those fed Diet 2, and there was no significant difference between the other two treatments.

The hepatopancreas GOT and GPT activities in crabs fed different diets are shown in Fig. 2. Crabs fed Diets 2 and 3 had significantly lower GOT activities than that in those fed Diet 5 ($P < 0.05$), and there was no significant difference in hepatopancreas GOT activities of the among other two treatments ($P > 0.05$). The metabolic enzyme GPT activity was significantly elevated in the hepatopancreas of crabs fed Diet 4 and Diet 5 than that in those fed Diets 1 and 2 ($P < 0.05$).

Serum and hepatopancreas antioxidant enzyme activity in juvenile *P. trituberculatus*

SOD and MDA activities in crab serum fed different diets are shown in Fig. 3. The serum SOD activity significantly increased with increasing fish meal replacement levels and ranged from (521.87 ± 9.31) to (585.99 ± 23.67)%. The crab serum MDA contents were significantly affected by replacing fish meal with protein mixtures. Among these treatments, the serum MDA contents in crabs fed Diets 1 and 3 were significantly higher than those fed Diets 2 and 5, and the serum MDA contents in crabs fed Diet 5 were significantly lower than those fed Diet 4 ($P < 0.05$).

The hepatopancreas SOD and MDA activities in crabs fed different diets are shown in Fig. 3. The crab hepatopancreas SOD activity was not significantly ($P > 0.05$) affected by replacing

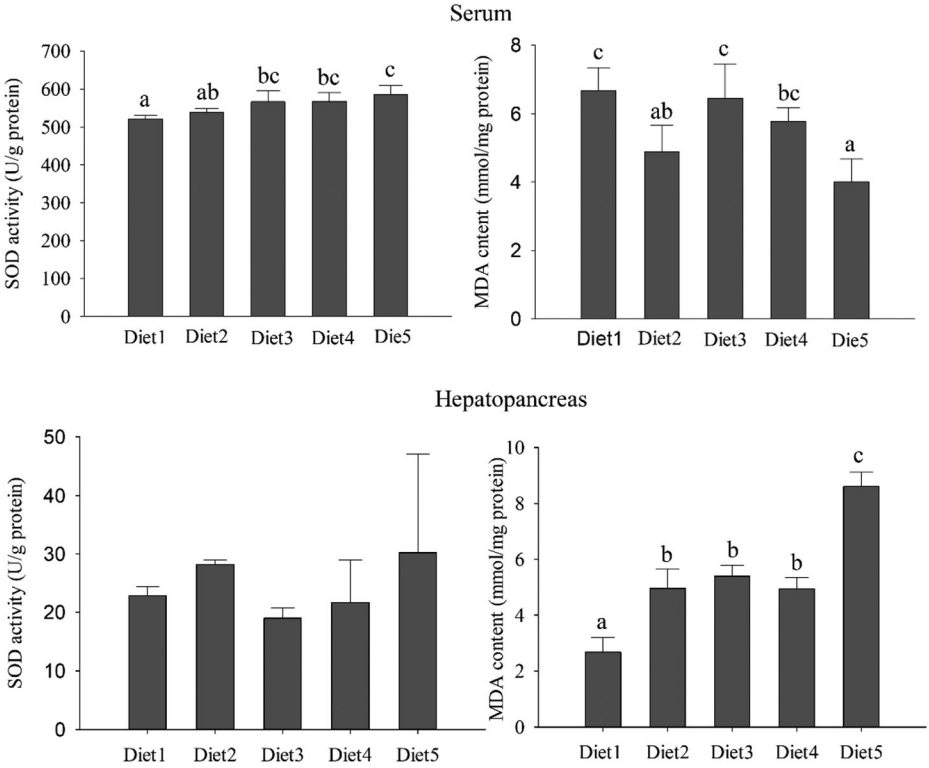

Fig. 3 Effect of fish meal replacement by protein mixtures on antioxidant enzyme activity of serum and hepatopancreas in juvenile *P. trituberculatus*

fish meal with protein mixtures. However, the crab hepatopancreas MDA contents were significantly affected by replacing fish meal with protein mixtures. Among these treatments, the hepatopancreas MDA contents in crabs fed Diets 2, 3, and 4 were significantly higher than that in those fed Diet 1, and were significantly lower than that in those fed Diet 5 ($P < 0.05$), but no significant difference was observed among these three treatments ($P > 0.05$).

Discussion

Effect of fish meal replacement by protein mixtures on growth performance and body composition

The present study revealed significant differences for FBW and WGR between the treatments and control diets (Table 2). These results indicated that dietary fish meal replacement levels significantly affected the growth performance of *P. trituberculatus*. The FBW, WGR, and SGR initially increased and then decreased with increasing dietary fish meal replacement levels, and crabs fed Diet 3 (dietary fish meal replacement of 33%) had the highest FBW, WGR, and SGR among all treatments. In addition, the HSI of crabs fed Diet 4 (fish meal replacement of 50%) was the highest. These results indicated that excessive dietary fish meal replacement (≥ 50%) can reduce the growth performance and nutrient accumulation in juvenile *P. trituberculatus*. A

previous study suggested that the growth performance of crustaceans was affected by the dietary nutritional composition, and the limited nutrition in low fish meal diets may obviously decrease the final body weight (Shen et al. 2012). On one hand, the apparent digestibility of amino acids of fermented soybean, pork meal, chicken meal, and shrimp meal was lower than that of fish meal, so high proportional levels of fish meal replacement in diets would reduce protein and amino acid the utilization, leading to lower growth performance of crustaceans (Zhang et al. 2007). On the other hand, fermented soybean in protein mixtures contained several ANFs, including tannin, phytic acid, saponins, isoflavones, and soybean agglutinin that may affect digestion or nutrient absorption in juvenile crabs (Zhang 2007).

The hepatopancreas of crustaceans has an absorptive and storage role for nutrients, and the HSI represents the storage condition of nutrients in the hepatopancreas (Wu et al. 2010). The protein and fat digestibility of chicken meal and fermented soybean in the protein mixtures was lower than that of fish meal (Zhang et al. 2007; Zhao et al. 2016), which affected the nutrient accumulation in the hepatopancreas, resulting in a decreased HSI. Thus, HSI decreased with increasing fish meal replacement levels. However, the HSI of crabs fed Diets 4 and 5 was higher than that of those fed Diets 1–3. It is possible that the saturated fatty acid content of pork and chicken meals in protein mixtures was higher than that of fish meal (Gao 2017). When the added proportion of pork and chicken meal was too high, the PUFA content in the diet decreased significantly, which led to an increase in HSI (Wu et al. 2010; Chang et al. 2008).

Moisture, crude protein, and ash contents in the whole body of crabs were significantly affected by the mixture protein levels in the diet. Crabs fed diets with the highest fish meal protein level showed a significantly lower moisture content in the whole body, which was in agreement with previous studies in other crustaceans (Catacutan 2002; Jin et al. 2013). However, some studies have indicated that there was no significant difference in the body moisture contents among treatments (Mu et al. 1998; Skalli et al. 2004; Ward et al. 2003). In the present study, the crude protein content in the whole body significantly decreased with an increase in fish meal replacement, which is consistent with the results reported for red claw crayfish *Cherax quadricarinatus* von Martens (Cortés-jacinto et al. 2005) and tongue sole *Cynoglossus semilaevis* Güntuer (Dai et al. 2016). The protein and amino acid digestibility of chicken, pork, and shrimp meal, and fermented soybean in the protein mixture was lower than that of fish meal (Li et al. 2016; Zhang et al. 2007), and high levels of fish meal replacement would reduce the protein and amino acid utilization of juvenile crabs, which would affect the protein and amino acid deposition rate in the body (Zhang et al. 2007). In addition, fermented soybean in protein mixtures contains several ANFs that could affect nutrient digestion and absorption in juvenile crabs. The whole-body ash content significantly increased with increasing levels of fish meal replacement, and similar results were observed for *E. sinensis* (Mu et al. 1998).

Effect of fish meal replacement by protein mixtures on hepatopancreas digestive enzymes

Digestive enzyme activity may have an important function on feed utilization and growth performance, especially protease, amylase, and lipase, which are important for digestive processes (Zhao et al. 2016). The study of digestive enzymes may be helpful for understanding the digestive capacity and nutrient utilization in *P. trituberculatus*. Crustacean digestive enzyme activity indicated the digestive and absorptive capacity is positively correlated with

nutrient content. Lipase activity in the hepatopancreas is related to animal feed, whereas amylase activity is related to plant feed. The results of the present study suggested that enhanced digestive activities in crabs occur at high levels of fish meal replacement. The lipase activity of crabs fed a diet of 67% fish meal protein replaced by protein mixtures was significantly higher than that of other treatments. Furthermore, the amylase activity of crabs fed diets of 50% and 67% fish meal protein replaced by protein mixtures was higher than that of the other treatments, since the protein mixtures contained high proportions of animal feed, including pork and chicken meal, and also had the plant feed, such as soybean. Reports on shrimp *Penaeus monodon* (Anand et al. 2013) and common carp *Cyprinus carpio* (Nandeesha et al. 1994; Nandeesha et al. 1998) showed similar results.

Effect of fish meal substitution by protein mixtures on hepatopancreas protein metabolism enzymes

GOT and GAT are the two most important transaminases in aquatic animals (Wang et al. 2017). They play an important role in the decomposition and synthesis of amino acids, and their activity represents the metabolic intensity of amino acids. These two enzymes are mainly located in the liver and are usually very low in the serum. They are only released into the hemolymph when the cell tissues are damaged. The enzyme activities of the hepatopancreas are normally an indicator of damage to hepatopancreatic cells. The present study found that there were significant differences in GOT and GPT activities in the hepatopancreas among all treatments, and their activities increased with increasing protein mixtures level and compared with that of the control group: crabs fed a diet with 67% fish meal protein replaced by protein mixtures had significantly higher hepatic GOT and GPT activities; this indicated that dietary protein utilization decreased, and the hepatopancreas was damaged to a certain extent. Some researchers observed similar results, where GOT and GPT activities in the liver decreased with increasing dietary soybean meal levels, indicating that dietary protein utilization decreased, followed by liver damage (Lin and Li 2011).

Effect of fish meal replacement by protein mixtures on serum and hepatopancreas antioxidant enzyme activity

SOD is one of the most important antioxidant enzymes in organisms (Li et al. 2008). Animal cells produce excessive oxygen free radicals under some conditions. To cope with oxygen free radical production, SOD eliminates excessive oxygen free radicals, which can produce oxidative stress and cause lipooxidation (Wang et al. 2017; Xie et al. 2016). In the present study, the SOD activity level increased significantly after fish meal was replaced from 0 to 67%. This may be related to the increased lipid content in the hemolymph with increasing fish meal replacement levels. MDA is involved in protective mechanisms within tissue injury following oxidative processes, and its content is affected by the nutritional status of organisms (Winston and Giulio 1991). In the present study, the MDA content of the hepatopancreas in crabs significantly increased with increasing fish meal replacement levels. Furthermore, the MDA content in the hepatopancreas of crabs fed Diet 5 was significantly higher than that of the other treatments. Usually, higher MDA contents indicate more radicals need to be removed (Andersen et al. 1998; Chien et al. 2003; Ross et al. 2001). Therefore, significantly higher MDA activities in the hepatopancreas of crabs at higher fish meal replacement levels might represent that the stress of low fish meal contents resulted in radical accumulation. As fish meal contains antioxidants, including carotenoids and selenium (Li et al. 2017), a high

proportion of substitution will reduce the ability to cope with oxidative stress, resulting in increased MDA contents in the hepatopancreas.

Conclusions

In conclusion, the results of the present study indicated that the appropriate dietary fish meal replacement level is approximately 33% with no significant negative effects on the growth performance of the swimming crab. The results suggested that excessive dietary fish meal replacement (50–67%) can reduce the growth performance and nutrient accumulation in juvenile crabs, and has a negative effect on physiological metabolism.

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Conflict of interest The authors declare that they have no conflict of interest.

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