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Effect of replacement of fishmeal by soybean products with attractants supplementation on the growth performance, feed utilization, body composition, plasma physiological responses and hepatic antioxidant abilities of juvenile golden pompano *Trachinotus ovatus*

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Abstract

A feeding experiment was carried out to develop a practical diet with low fishmeal for juvenile golden pompano (*Trachinotus ovatus*) by substituting fishmeal protein with soybean products mixture. Six isocaloric (17.6 KJ g^{-1}) diets were designed by replacing 0 (FM100), 5 (FM95), 10 (FM90), 15 (FM85), 20 (FM80), and 25% (FM75) of the fishmeal with soybean products mixture (SPM) (soybean meal and soybean protein concentrate). The experiment diets were supplemented with 1.5%, 3%, 4.5%, 6% and 7.5% squid paste as attractants or palatability enhancer. The experimental fish (6.9 g) were cultured in sea cages (1.0 m × 1.0 m × 1.5 m) with 25 fish in each cage. Fish were fed the corresponding experimental diets to satiation twice daily for 8 weeks. At the end of the feeding experiment, no differences were found in the final weight and weight gain (WG) of fish fed FM100, FM85, FM80, and FM75 diets ($P > 0.05$). The WG was significantly ($P < 0.05$) better in the fish-fed FM95 diet compared to FM100 and FM75 diets. FCR in diet FM95 and FM90 was significantly ($P < 0.05$) than that of FM80 and FM75, but these values were not significantly different from those in other treatments. Although the condition factor value in diets FM90, FM85, and FM80 was significantly ($P < 0.05$)

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higher than that of FM100, these values were not significantly different from the rest of the other groups. There was no statistical difference in SGR, FR, VSI, and survival rates among different dietary treatments. The plasma AST activity was significantly lower in fish fed FM90 diet compared to fish fed with control, FM85, FM80, and FM75 ($P < 0.05$). There was no significant difference in plasma AST among all dietary treatments except FM95 and FM90. In contrast, the plasma AKP activity showed the opposite trend. Compared with the control, the fish-fed FM80 and FM75 diets had significantly decreased plasma cholesterol, triglyceride total protein, albumin, and globulin level ($P < 0.05$) while significantly increasing plasma COR levels ($P < 0.05$). There was no significant difference in ALT, Urea and glucose values among all treatments. No statistical difference was observed among the FM100, FM80, and FM75 treatments for those of plasma LYZ activity, C3, and C4 levels. The hepatic T-AOC and SOD activities were significantly increased in fish fed the FM90 diet ($P < 0.05$) compared to those in fish fed with FM100 diet. According to the current experiment, it can be concluded that SPM supplemented with SP could partially substitute fishmeal (25%) for juvenile golden pompano without any adverse symptoms.

Introduction

Golden pompano (*Trachinotus ovatus*) belongs to the family Carangidae (Tutman et al., 2004; Zhou et al., 2018). *T. ovatus*, a carnivorous fish, is widely distributed in China, Japan, Australia, and other countries (Zheng et al., 2014). Recently, interest in the commercial culture of golden pompano has been increasing due to its high price in the market and its adaptability to salinity and temperature ranges (Lin et al., 2012; Othman, 2008). To date, studies of the nutritional requirements of golden pompano are limited. Previous studies reported that the optimum protein and lipid requirements of golden pompano ranged from 43% to 49% and from 6% to 6.5%, respectively (Liu et al., 2011; Liu et al, 2011). Du et al. (2011) found that the optimum dietary lysine level for optimal growth of juvenile pompano was 2.94 g kg^{-1} diet, which corresponds to 6.70 g kg^{-1} dietary protein (Du et al., 2011). Niu et al. (2013) reported that the optimum dietary methionine level for optimal growth of juvenile pompano was 10.6–12.7 g kg^{-1} diet, corresponding to 24.6–29.5 g kg^{-1} dietary protein (Niu et al., 2013). In addition, Lin et al. (2012) found that fermented soybean meal could replace white fish meal by up to 100 g kg^{-1} without negative effects on the growth of *T. ovatus* (Lin et al., 2012). Some amino and fatty acids have been studied in our lab (Huang et al., 2015; Huang et al., 2017; Lin et al., 2015; Qi et al., 2016). However, to our knowledge, few studies have been conducted to evaluate the effect of the replacement of fishmeal by soybean products mixture supplementing attractants in diets for juvenile *T. ovatus*.

Fish meal is the main protein source, especially for carnivorous fish (Lin et al., 2012). Finding suitable protein sources as alternatives to the fish meal is critical in the commercial culture of carnivorous fish species (Shapawi et al., 2007). Soybean proteins have a consistent nutritional profile, relatively balanced amino acid composition, availability, and affordable price, thus becoming one of the most suitable alternative protein sources for fishmeal in the aquafeed industry (Kader et al., 2012). However, during the formulation of soy-based diets, the negative effects of antinutritional factors, imbalance of amino acid, and less palatability of soybeans should be reckoned with. Recently, plant protein has been improved by neutralizing antinutritional factors and improving digestibility. The amino acid and palatability problems could also be overcome by supplementing amino acids or by appropriate feed formulation (Kader et al., 2010). Several previous studies have been conducted using soybean proteins to replace fishmeal, and it was shown that 20–50% of the fishmeal protein could be substituted in the diets of red sea bream (*P. major*) (Takagi et al., 1999); Japanese flounder (*P. olivaceus*) (Deng et al., 2006). However, higher dietary soybean protein levels might have detrimental

effects on the growth performance of fish because of its imbalanced amino acids and decreased palatability. Therefore, soybean protein is one of the potential alternative protein sources for carnivorous fish species. Soybean proteins could be formulated to lower the fishmeal diet by satisfying the deficient amino acids and enhancing its palatability (Takagi et al., 2008). The complementary effect of different ingredients is often more effective than CAA supplementation in addressing nutritional deficiencies in formulating alternative protein-based diets. Animal proteins, especially marine by-products, contain more balanced amino acids and many free amino acids, which could supplement the deficiencies of marine carnivorous fish diets based on high plant protein (Kader et al., 2012; Kousoulaki et al., 2009). Recently, Kader et al. (2010) investigated that the addition of a small amount (10%) of crude ingredients (e.g. fish soluble, squid meal, and krill meal) in high SPC diets (without CAA) was effective in achieving similar performances of red sea bream fed with the fishmeal-based control group (Kader et al., 2010). It was reported that the addition of plant proteins such as DSM to the mixture of fish soluble, squid meal, and krill meal could even replace 100% fishmeal in diets of juvenile red sea bream (Kader et al., 2012).

Therefore, the objective of the present study was to investigate the effect of the gradual replacement of fishmeal by soybean products mixture with attractant supplementation (squid paste) in diets for juvenile *T. ovatus*.

Materials and methods

Experimental diets.

Six experimental diets (**Table 1**) were prepared based on nearly isonitrogenous (42.3% crude protein) and isolipidic (11.3% total lipid). The control diet contains 30% fishmeal (FM100). Fishmeal was gradually substituted at 5, 10, 15, 20, and 25% with soybean products mixture (soybean meal and soybean protein concentrate), and designated as FM95, FM90, FM85, FM80, and FM75, respectively. All the replacement diets (except FM100) were added 1.5%, 3%, 4.5%, 6% and 7.5% squid paste (SP) as attractants or palatability enhancer. Fish oil and lecithin served as lipid sources, and wheat flour as carbohydrate sources. The ingredients were all ground through a 60-mesh screen. Vitamins and minerals premix were mixed by the progressive enlargement method (Zhou, Ge, Niu, Lin, Huang, Tan, 2015). Lipid and distilled water (35–40%, v/w) were added to the premixed dry ingredients and thoroughly mixed until homogenous in a Hobart-type mixer. The 1-mm diameter pellets were wet-extruded using a pelletizer (Institute of Chemical Engineering, South China University of Technology, Guangzhou, China) and then air-dried, sealed in plastic bags, and stored at −20 °C in a freezer until use.

Fish and animal husbandry

The feeding trial was carried out at Shenzhen Experimental Station of the South China Sea Fisheries Research Institute of the Chinese Academy of Fishery Sciences, China. Golden pompano juveniles were obtained from a local hatchery in Shenzhen, China. The fish were acclimated to the experimental condition for two weeks. During this period, commercial feed (42% crude protein) was provided to the fish. The feeding experiment was carried out in sea cages (1.0 m × 1.0 m × 1.5 m). In this process, the water quality parameters were monitored: water temperature is between 27.1 and 30.8 °C, salinity from 27 to 30‰, and pH from 7.4 to 8.0. The dissolved oxygen was not less than 6.0 mg $l^{-1,}$ and the ammonia nitrogen was kept lower than 0.03 mg l^{-1}.

Feeding protocol

At the start of the feeding trial, juveniles (initial mean body weight, 6.87 ± 0.03 g) were randomly stocked in eighteen cages at a stocking density of 25 fish in each cage and in triplicate for each dietary treatment. All fish were manually fed to satiation state twice daily

(08:00 and 16:00) for 8 weeks. The experimental diet consumption and water quality parameters were recorded regularly.

Sample collection and chemical analyses.

At the end of the feeding trial, fish were fasted for 24 h to empty the digestive tract contents, then anesthetized with diluted eugenol (1: 10000; Shanghai Reagent Corp., China) and weighed. The blood from three randomly selected fish in each cage was collected by caudal vein using 2 ml heparinized syringes. After collection, a portion of whole blood was used to analyze respiratory burst activity. The remaining blood was centrifuged (3000 × *g* at 4 °C for 10 min) to acquire plasma (stored at -80 °C for further measurement). The intestine and liver were cut off, frozen in liquid nitrogen, and then stored at -80 °C until analyzed.

The chemical composition of diets and fish was determined by standard methods (AOAC, 2005). Moisture was determined by drying in an oven at 105 °C until a constant weight was obtained. Crude protein content (N × 6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030, Auto-analyzer; Tecator, Höganäs, Sweden). Crude lipid was measured by the ether extraction method using a Soxtec extraction System HT (Soxtec System HT6, Tecator). Ash content was determined by placing the sample in a muffle furnace at 550 °C for 4 h.

Blood biochemical parameters measurements

Plasma glutamic-pyruvic transaminase (ALT), glutamic-oxalacetic transaminase (AST), alkaline phosphatase (AKP) activities, Urea, glucose, cholesterol, triglyceride, contents, total protein, albumin were analyzed by ROCHE-P800 automatic biochemical analyzer (Roche, Basel, Switzerland) using a standard kit method for each assay. Plasma globulin was obtained by subtracting plasma albumin from total protein.

Immunological and hormones assays

The LYZ activity was determined by turbidimetric assay according to the previous study (Muona and Soivio, 1992). The plasma C3 and C4 levels were determined by the immune turbidimetric method, as described in the previous study (Zhou et al., 2014). Plasma cortisol (COR) and Norepinephrine (NE) were estimated by validated enzyme-linked immunosorbent assay (ELISA) methods according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, China).

Hepatic antioxidative enzyme activities measurements

The liver samples were homogenized in cold phosphate buffer (diluted at 1:10) (phosphate buffer: 0.064 M at pH 6.4). Then the homogenate was centrifuged for 20 min (4 °C, 3000 × *g*), and the supernatant was used to quantify hepatic T-AOC, SOD, and MDA. T-AOC was measured by the method described in the previous study (Benzie and Strain, 1996), using commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China). SOD activity and MDA content were determined using xanthine oxides (Marklund and Marklund, 1974) and barbituric acid reaction timing (Draper et al., 1993), respectively. We adopted the Folin method to measure hepatic protein content (Lowry et al., 1951), with bovine serum albumin as the standard.

Statistical analysis

Data from each treatment were subjected to a one-way analysis of variance (ANOVA). Duncan's test was employed to compare means between treatments when overall differences were significant. The level of significant difference was $P < 0.05$. Statistical analysis was performed using SPSS19.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA), and the results are expressed as mean ± SEM (standard error of the mean).

Table 1 Composition of the experimental diets

Ingredients	Diet group					
	FM100	FM95	FM90	FM85	FM80	FM75
Fish meal[a]	30	28.5	27	25.5	24	22.5
Soy protein concentrate	19	19.5	20	20.5	21	21.5
Soybean meal	7	8	9	10	11	12
Peanut meal	6	6	6	6	6	6
Wheat flour	25	23.6	22.2	20.8	19.4	18
Fish oil	8	7.9	7.8	7.7	7.6	7.5
Calcium biphosphate	2	2	2	2	2	2
Vitamin mixture[b]	1	1	1	1	1	1
Mineral mixture[c]	1	1	1	1	1	1
Choline chloride (50%)	0.5	0.5	0.5	0.5	0.5	0.5
Lecithin	0.5	0.5	0.5	0.5	0.5	0.5
Squid pastes[d]	0	1.5	3	4.5	6	7.5
Proximate composition (% dry matter)						
Dry matter	92.1	91.9	91.6	91.3	91.0	90.8
Crude protein	42.3	42.3	42.4	42.4	42.5	42.5
Crude lipid	11.3	11.3	11.4	11.4	11.4	11.5
Ash	7.4	7.2	7.1	6.9	6.7	6.6

[a] White fishmeal was north pacific white fishmeal and purchased from American Seafoods Company, Seattle, Washington, USA.
[b] Vitamin premix (mg or g kg-1 diet): thiamin, 25 mg; riboflavin,45 mg; pyridoxine HCl, 20 mg; vitamin B12, 0.1 mg; vitamin K3,10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 1.20 mg; retinal acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 120 mg; ascorbic acid, 2000 mg; choline chloride, 2500 mg; ethoxyquin 150 mg; wheat middling, 14.012 g.
[c] Mineral premix (mg or g kg-1 diet): NaF, 2 mg; KI, 0.8 mg; $CoCl_2 \cdot 6H_2O$ (1%), 50 mg; $CuSO_4 \cdot 5H_2O$, 10 mg; $FeSO_4 \cdot H_2O$, 80 mg; $ZnSO_4 \cdot H_2O$, 50 mg; $MnSO_4 \cdot H_2O$, 60 mg; $MgSO_4 \cdot 7H_2O$, 1200 mg; $Ca(H_2PO_4)_2 \cdot H_2O$, 3000 mg; NaCl, 100 mg; zoelite, 15.447 g.
[d] Squid pastes were purchased from Lufeng Detaifeng Feed Raw Materials Co., Ltd.

Results

Growth. Growth performance, feed utilization, morphometry index, and survival rate of fish are shown in **Table 2**. Compared to the FM75 group, the final weight was remarkably ($P < 0.05$) improved in the FM95 and FM90 groups. The weight gain (WG) was significantly ($P < 0.05$) increased in the FM90 group compared to FM100, FM85, FM80, and FM75 groups. Among the treatments, fish fed the FM75 diet had the lowest growth performance numerically ($P > 0.05$). The FCR of fish-fed FM95 and FM90 diets were significantly lower than those of fish-fed FM80 and FM75 but not remarkably different ($P < 0.05$) from those in other treatments. There was no difference in SGR and FR among all treatment groups. The CF of fish in all the substitution groups was numerically higher compared to the fish fed the control diet ($P > 0.05$). The CF was significantly increased in the fish-fed FM90 diet and then gradually decreased in the FM90, FM85, and FM80 groups. No difference was also observed in other morphometry indices (such as VSI and HSI) among different treatments. Similarly, there was no significant difference in survival rate ($P > 0.05$) at the end of the feeding trial.

Table 2 Growth performance, feed utilization and morphometry index in juvenile *Trachinotus ovatus* fed test diets for 56 days

Ingredients	Diet group					
	FM100	FM95	FM90	FM85	FM80	FM75
Initial weight (g)	6.83±0.03	6.87±0.03	6.87±0.03	6.87±0.03	6.9±0	6.87±0.03
Final weight (g)	$44±0.5^{ab}$	$44.7±0.1^{a}$	$44.8±1^{a}$	$43±0.7^{ab}$	$42.5±1.5^{ab}$	$41.9±0.5^{b}$
WG (%)	$526.3±2.8^{bc}$	$550.5±2.3^{ab}$	$555.5±5.5^{a}$	$524.3±12.8^{bc}$	$519.4±10.5^{c}$	$504.1±12.2^{c}$
SGR (% day^{-1})	3.36±0.04	3.34±0.01	3.27±0.06	3.31±0.12	3.21±0.07	3.25±0.13
FCR	$1.35±0.01^{ab}$	$1.25±0.01^{b}$	$1.21±0.02^{b}$	$1.32±0.01^{ab}$	$1.41±0.05^{a}$	$1.4±0.09^{a}$
FR (% day^{-1})	3.51±0.06	3.4±0.02	3.45±0.07	3.49±0.1	3.6±0.07	3.55±0.16
VSI	5.86±0.15	6.16±0.31	5.48±0.07	5.77±0.27	5.92±0.15	5.99±0.17
HSI	0.77±0.02	0.75±0.04	0.77±0.03	0.72±0.06	0.69±0.01	0.76±0.04
CF	$2.99±0.02^{b}$	$3.12±0.06^{ab}$	$3.19±0.04^{a}$	$3.14±0.01^{a}$	$3.16±0.04^{a}$	$3.06±0.07^{ab}$
Survival rate (%)	96.0±4.0	98.7±1.3	98.7±1.3	97.3±1.3	98.7±1.3	92±4.6

Values are means ± SEM of three replications. Means in the same line with different superscripts are significantly different ($P < 0.05$). WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; FR, feeding rate; VSI, viserosomatic index; HSI, hepatosomatic index; CF, condition factor

Proximate body composition

The proximate whole-body composition of the fish at the end of the feeding experiment is shown in **Table 3**. There was no significant difference ($P > 0.05$) in the final whole-body proximate composition of the fish in all treatment groups.

Table 3 Proximate composition (% wet weight) of whole body in juvenile *Trachinotus ovatus* fed test diets for 56 days

Ingredients	Diet group					
	FM100	FM95	FM90	FM85	FM80	FM75
Moisture	69.61±0.49	68.95±0.66	69.74±0.75	69.85±0.39	69.39±0.53	69.29±0.47
Crude protein	17.15±0.16	17.38±0.12	17.04±0.19	17.36±0.26	17.36±0.54	17.14±0.38
Crude lipid	6.48±0.72	7.09±0.58	6.33±0.69	6.17±0.37	6.96±0.34	7.07±0.71
Crude ash	3.88±0.24	4.10±0.04	4.07±0.07	4.08±0.06	4.09±0.04	4.17±0.1

Values are means ± SEM of three replications. Means in the same line with different superscripts are significantly different ($P < 0.05$).

Blood biochemical measurements

Plasma AST activity decreased with the increase of substitution level up to FM90 and then increased (**Table 4**). Plasma AST activity was lower in fish fed FM90 diet than those in fish fed with control (FM100), FM85, FM80, and FM75, respectively ($P < 0.05$). There was no significant difference in plasma AST between the FM95 and FM90 diets. In contrast, the plasma AKP activity increased with increasing substitution level up to FM90 and then decreased. The plasma AKP activity of fish fed with the FM90 diet was significantly higher than that of the control group ($P < 0.05$). The other treatments had no significant effect on plasma AKP activity compared to the control. Compared with the control, the fish-fed FM80 and FM75 diets had significantly decreased plasma cholesterol levels ($P < 0.05$), while the other treatment groups had a tendency to decrease.

Similarly, compared with the control, the fish-fed FM90, FM85, FM80, and FM75 diets had significantly increased plasma triglyceride levels ($P < 0.05$). There was no significant difference in plasma triglyceride levels between the control and FM95. Compared with the control, plasma total protein levels were significantly higher in the fish-fed FM95 diets and significantly lower in fish-fed FM85, FM80, and FM75 ($P < 0.05$). No significant difference in plasma total protein level was observed between the control (FM100) and FM90. Compared with the control, the fish-fed FM95 and FM90 diets had significantly increased plasma albumin levels ($P < 0.05$), while the fish-fed FM85, FM80, and FM75 diets had significantly decreased plasma albumin levels ($P < 0.05$). Compared with the control, the plasma globulin level was significantly increased in fish-fed FM95 diets while significantly decreased in fish-fed FM80 and FM75 diets ($P < 0.05$). There was no significant difference in plasma globulin levels among control, FM90, and FM85. No significant difference was observed in ALT, Urea, and Glucose values among all treatments.

Table 4 Plasma parameters in juvenile *Trachinotus ovatus* fed test diets for 56 days

Ingredients	Diet group					
	FM100	FM95	FM90	FM85	FM80	FM75
ALT (U/L)	27.7±3.7	21.2±1.2	30±3.8	30.3±4.9	31.9±2.7	28.2±2.9
AST (U/L)	42.2±2.5^{ab}	38.5±3.3^{bc}	32.0±3.2^{c}	45.7±2.0^{ab}	42.3±2.9^{ab}	51.0±4.0^{a}
AKP (U/L)	20.2±0.7^{b}	23.5±0.5^{ab}	26.7±1.2^{a}	24.7±2.2^{ab}	25±2.6^{ab}	22.8±1.7^{ab}
UREA (mmol/L)	0.93±0.07	1.27±0.22	0.93±0.2	0.83±0.15	0.77±0.13	0.77±0.03
Glucose (mmol/L)	5.13±0.42	5.37±0.75	5.43±1.00	4.73±1.23	5.2±0.36	5.2±2.15
Cholesterol (mmol/L)	2.97±0.12^{a}	2.83±0.03^{ab}	2.63±0.43^{ab}	2.3±0.06^{abc}	1.87±0.09^{c}	2.17±0.33^{bc}
Triglyceride (mmol/L)	1.58±0.06^{a}	1.59±0.05^{a}	1.2±0.11^{b}	1.12±0.01^{b}	0.9±0.11^{b}	0.98±0.2^{b}
Total protein (g/L)	38.7±1^{b}	46.6±0.5^{a}	44.5±2.5^{ab}	32±1.8^{c}	27.6±2.3^{c}	27.7±2.7^{c}
Albumin (g/L)	13.9±0.06^{b}	15.67±0.19^{a}	16.37±0.15^{a}	8.63±0.41^{c}	7.93±0.71^{c}	7.5±0.96^{c}
Globulin (g/L)	24.8±0.9^{bc}	30.9±0.5^{a}	28.2±2.6^{ab}	23.4±1.4^{bc}	19.7±1.6^{c}	20.2±1.7^{c}

Values are means ± SEM of three replications. Means in the same line with different superscripts are significantly different ($P < 0.05$).

Non-specific immune response

The plasma LYZ activity increased with the increase of substitution level until FM90 and then declined (**Table 4**). Plasma LYZ activity was significantly higher in the fish-fed FM90 diet compared to the control group ($P < 0.05$). The other treatments had no significant effect on plasma LYZ activity compared to the control. Compared with the FM75 diet group, the fish-fed FM90 diet had significantly increased plasma C3 levels ($P < 0.05$). There was no significant difference in plasma C3 level among other treatments. The fish among all treatments had similar trends in plasma C4. However, compared with the FM75 diet group, the fish-fed FM85 diet had significantly increased plasma C4 levels ($P < 0.05$). The highest plasma C4 level was found in the fish-fed FM85 diet. The content of plasma COR increased with the increase of substitution level until FM90 and then remained almost constant thereafter. Except for the FM95 group, the plasma COR content was significantly increased in FM90, FM85, FM80, and FM75 groups compared to the control group ($P < 0.05$). Compared with the control, the plasma NA content was significantly increased in FM90, FM85, FM80, and FM75 diet groups ($P < 0.05$). No statistical difference in plasma NA content was found between the control (FM100) and FM90 diets.

Table 5 Immunological and hormones indexes in juvenile *Trachinotus ovatus* fed test diets for 56 days

Ingredients	Diet group					
	FM100	FM95	FM90	FM85	FM80	FM75
LYZ (U ml^{-1})	58.9±0.4^{b}	63.4±1.7^{ab}	64.5±1.9^{a}	61.3±1.2^{ab}	60.8±1.1^{ab}	61.6±1.6^{ab}
C3 (g L^{-1})	1.95±0.48^{ab}	2.33±0.18^{ab}	2.95±0.18^{a}	1.75±0.49^{ab}	1.85±0.24^{ab}	1.32±0.44^{b}
C4 (g L^{-1})	3.38±0.33^{ab}	3.86±0.44^{ab}	3.77±0.5^{ab}	4.55±0.23^{a}	3.67±0.59^{ab}	2.39±0.49^{b}
COR (pg ml^{-1})	801±23^{c}	893±20^{c}	997±3^{b}	1043±68^{ab}	1044±20^{ab}	1127±16^{a}

Values are means ± SEM of three replications. Means in the same line with different superscripts are significantly different ($P < 0.05$).

Hepatic antioxidant status

The hepatic T-AOC increased with increasing substitution level up to FM90 and then decreased. As for the FM95 and FM90 groups, hepatic T-AOC levels were remarkably increased compared to the control group ($P < 0.05$). However, there was no significant difference in hepatic T-AOC among the control (FM100), FM85, FM80, and FM75 diet groups. The hepatic SOD activity increased with the increase of substitution level until FM95 and then decreased. Compared with the control, hepatic SOD activity was significantly higher ($P < 0.05$) and tended to increase in the other groups, FM90, FM85, and FM80. At the same time, there was a trend toward lower hepatic SOD activity in the FM75 group. In contrast to hepatic SOD activity, the hepatic MDA content decreased until FM90 and then increased with replacement levels. Compared with the control group, the hepatic MDA content in the FM95, FM90, FM85, and FM80 diet groups showed a decreasing trend, while the FM75 diet group showed an increasing trend.

Table 6 Hepatic antioxidative status in juvenile *Trachinotus ovatus* fed test diets for 56 days

Ingredients	Diet group					
	FM100	FM95	FM90	FM85	FM80	FM75
T-AOC (U mg^{-1} prot)	2.85±0.16^{b}	4.8±0.53^{a}	5.58±0.46^{a}	3.61±0.19^{b}	3.42±0.27^{b}	3.1±0.51^{b}
SOD (U mg^{-1} prot)	327±17^{bc}	505±31^{a}	362±9^{b}	344±13^{bc}	355±5^{b}	298±7^{c}
MDA (nmol mg^{-1} prot)	2.28±0.41^{ab}	1.64±0.17^{b}	1.69±0.32^{b}	1.88±0.41^{ab}	1.93±0.22^{ab}	2.76±0.26^{a}

Values are means ± SEM of three replications. Means in the same line with different superscripts are significantly different ($P < 0.05$).

Discussion

In the present study, the WG ranged from 504 to 556%, relatively higher than the previous study (Lin et al., 2012) and Niu et al. (2016) who reported a WG of 307–455% for juvenile golden pompano (18 g) fed diets replacing fishmeal with soybean meal for 56 days (Niu et al., 2016). Ma et al. (2014) also reported a WG (%) of 204–257% in juvenile golden pompano (16.7 g) after 8 weeks of growth experiment (Ma et al., 2014). Although several alternative protein sources have been reported to replace fishmeal in many fish diets partially, there are few studies on juvenile *T. ovatus*. Riche and Williams (2011) found that in Florida pompano (2.60 g), the substitution level of soybean meal for fishmeal protein appears to be 380 g kg^{-1} protein (Riche and Williams, 2011), while Gao et al. (2013) reported the replacement of fish meal by soybean meal could reach 50% without significant difference in growth and survival in juvenile golden pompano (18.0 g) when enough DL-Met and Lys-HCL (78%) were supplemented in the diet (Wen, Andreas, Silva, Yan, Evonik Industries, 2013). The fish fed the diets containing 37.0 % USBM (usual soybean meal) replaced 45.1 % fish meal protein, and 44.0 % FSBM (fermented defatted soybean meal) replaced 60.8 % fish meal protein had significantly decreased weight gain rate and feed utilization in juvenile golden pompano than the fish fed the other diets (Liu et al., 2010). In contrast, the current result showed that 25% fishmeal could be substituted in the diets of juvenile golden pompano. Thus, it is clearly shown that soybean protein could be utilized more efficiently by supplementing squid paste, which is consistent with previous studies (Kader et al., 2010; Kader et al., 2012). Comparatively higher fishmeal substitution of fishmeal by soybean products has been reported in some marine fish species (Aragão et al., 2003; Salze et al., 2010), and the full substitution of fishmeal was implemented in rainbow trout (Kaushik et al., 1995) and cobia (Salze et al., 2010). However, CAAs (crystalline amino acid) supplementation was needed to meet their requirements in these studies.

The VSI and HSI of the fish in this study were not significantly affected by the gradual substitution of fishmeal with soybean products and the addition of squid paste. Similar results were also reported in other fish species, such as red sea bream (Kader et al., 2012) and sea bass (Tibaldi et al, 2006). Dietary soybean products did not affect whole body composition among the fish fed with the various diets, which were also consistent with previous studies (Kader et al., 2012; Lin et al., 2012; Tibaldi et al., 2006).

Blood parameters are important indicators of physiological stress response and the overall health status of fish (Kader et al., 2012). AST is one of the ubiquitous aminotransferases in the mitochondrion of fish, which is an important parameter for the diagnosis of hepatopancreas function and damage (Zhou et al., 2013). In this study, plasma AST activity decreased with increasing replacement level up to FM90 and thereafter increased to the same level as the control group. This result indicated that higher soybean products (FM75) with supplementation of squid paste might not impair its hepatic functions, which were comparable to those observed by Lin et al. (2012) in juvenile golden pompano ingesting fermented soybean diets (Lin et al., 2012). Similarly, no significant difference was observed for plasma concentrations of AST in Atlantic cod with increased plant-protein inclusions (Olsen et al., 2007). AKP is an important regulatory enzyme involved in many essential functions in all living organisms (Zhou et al., 2014). In *Drosophila virilis*, AKP activity has been shown to decrease upon heat stress (Sukhanova et al., 1996). In this study, in contrast to plasma AST, the plasma AKP activity increased with increasing substitution levels up to FM90. It then decreased to basal levels in the FM75 group, indicating that higher soybean protein levels did not adversely affect the health status of fish. Although cholesterol, triglyceride, and total protein levels in the present study were decreased in fish-fed lower fishmeal diets, these values were similar to those found in the previous study (Lin et al., 2012). However, the previous study on juvenile red sea bream showed a significant increase in plasma total protein and triglyceride in the fish-fed FM0 diet (Kader et al., 2012).

Lysozyme is an important component of fish immune defense and is wildly used as an indicator of humoral immunity in fish species (Zhou et al., 2014). It is responsible for breaking down the polysaccharide wall of bacteria and thus prevents pathologic infection and disease (Haug et al., 2002). There is a correlation between serum lysozyme activity and total protein concentration in *Limanda limanda* (Hutchinson and Manning, 1996). Similar results were observed in the present study. In the study, compared with the control, the fish-fed FM90 diet had significantly increased plasma LYZ activity. The other treatments had no significant effect on plasma LYZ activity compared to the control. Complement is the main humoral component of innate immune responses. It, therefore, plays a vital role in alerting the host immune system to the presence and clearance of potential pathogens (Boshra et al., 2014). Complement is initiated by one or more of three pathways, namely, the classical pathway, the alternative pathway, and the lectin pathway. C3 is involved in all three pathways, which merge and proceed through the final pathway, leading to the formation of a membrane attack complex that directly lyses pathogenic cells (Boshra et al., 2006). C4 is a key component of classical and lectin pathways (Boshra et al., 2006). In the current study, compared with the control, plasma C3 content was increased in the FM90 diet group, while plasma C4 content increased in the FM85 diet group. There was no statistical difference in plasma C3 and C4 levels among other treatments, respectively. These results showed that the treatments (FM85, FM80, FM75) had no significant effect on plasma LYZ, C3, and C4 activities compared to the control, suggesting that the low fishmeal diets did not lower the health status of the fish.

Stress is one of the emerging factors in aquaculture activities that may affect aspects, including hormone secretion rate, intermediary metabolism, immunity, and nutrient utilization (Li et al., 2009; Zhou et al., 2014). Plasma or serum cortisol concentration is a reliable biological indicator of stress response in fish and terrestrial animals (Li et al., 2009). An increase in the blood cortisol level is widely used to indicate that a fish is under stress (Hsieh et al., 2003). A previous study showed that low dietary fishmeal levels did not affect plasma cortisol in red sea bream (Kader et al., 2012). In the present study, dietary intake of low fishmeal diets increased plasma cortisol levels in golden pompano. The response of plasma cortisol to low fishmeal diets is inconsistent, which means that the stress response caused by a low fishmeal diet may vary from species to species.

The body's defense system of the antioxidation ability is closely related to health status (Zhou et al., 2014). In addition, multiple antioxidants (such as T-AOC, SOD, and MDA) are required to maintain the complex immune system of fish (Jia et al., 2012). The antioxidant system can be divided into two systems: the enzymatic antioxidant system and the non-enzymatic antioxidant system. Antioxidant enzymes include T-AOC, SOD, and MDA, which constitute the first line of enzymatic defense mechanism against free radicals in organisms. In general, the antioxidant defenses of fish depend on nutritional factors to some extent (Sheikhzadeh et al., 2012). For fish, the T-AOC level could reflect the antioxidant capacity and is related to health status (Feng et al., 2011). SOD is one of the main antioxidants in the defense mechanism against lipid peroxidation in a biological system and converts active oxygen molecules into non-toxic compounds (Zimmermann et al., 1973). The decrease in SOD is related to the accumulation of high-living free radicals, which leads to impaired cellular function (Okamoto and Colepicolo, 1998). In this study, the hepatic antioxidant capacity, which can be demonstrated by the T-AOC levels and SOD activities. However, there was no significant difference in hepatic T-AOC levels or SOD activities among the control (FM100) and other treatments (FM85, FM80, FM75) groups, indicating that the low fishmeal diets did not lower the hepatic antioxidant status of the fish. MDA production was a notable oxidation process resulting from the peroxidation of membrane polyunsaturated fatty acid, influencing cell membrane fluidity and the integrity of biomolecules and was an important indicator of lipid peroxidation (Almroth et al., 2005). In the present study, although, compared with the control, the FM95, FM90, FM85, and FM80 diet groups had a tendency to decrease, and the FM75 diet group had a tendency to increase in hepatic MDA level, these values were

comparable. The study showed that low fishmeal diets had no detrimental effect on the speed of lipid peroxidation reaction and body damage in this experiment.

Conclusion

In summary, the soybean product mixture is a suitable strategy as a fishmeal replacement for juvenile golden pompano. The results in the current study suggested that the addition of squid pastes with plant protein, such as soybean products mixture, could substitute even 25% of fishmeal for juvenile golden pompano, which may provide a reference for feed companies to produce low-cost and sustainable fish feed using plant proteins more efficiently.

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