Hindawi
Aquaculture Research
Volume 2023, Article ID 8842781, 9 pages
https://doi.org/10.1155/2023/8842781

Research Article

Effects of Dietary Fishmeal Replacement with Soybean Meal on Growth Performance, Digestion, Hepatic Metabolism, Antioxidant Capacity, and Innate Immunity of Juvenile Large Yellow Croaker (*Larimichthys crocea*)

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Received 6 February 2023; Revised 6 April 2023; Accepted 4 November 2023; Published 28 November 2023

Academic Editor: Zhitao Qi

A 70-day feeding trial was conducted to determine the effects of the replacement of fishmeal (FM) by soybean meal (SM) in diets on growth performance, digestion, hepatic metabolism, antioxidant capacity, and innate immunity of juvenile large yellow croaker (*Larimichthys crocea*). Four iso-lipidic and iso-nitrogenous diets replacing 0, 15, 30, and 45 FM protein by SM protein were formulated, named FM, SM15, SM30, and SM45, respectively. Results indicated that the specific growth rate (FM = 1.55, SM15 = 1.50, SM30 = 1.48, and SM45 = 1.34%) of fish showed a significant linear trend ($P < 0.05$). With increasing dietary SM, the activity of trypsin (262.02, 179.34, 144.41, and 123.92 U/mg·prot) significantly decreased (linear trend, $P < 0.05$). The content of high-density lipoprotein cholesterol showed a significant linear trend and quadratic trend, and the maximum was observed in fish fed the diet with 45% protein from SM ($P < 0.05$). The mRNA expression of the mechanistic target of rapamycin (*m-tor*) and ribosomal protein S6 (*rps6*) showed a significant linear trend ($P < 0.05$). The content of malondialdehyde (81.82, 92.91, 79.64, and 126.45 nmol/mg·prot) showed a significant linear trend and quadratic trend; the minimum was observed in fish fed the diet with 30% protein from SM ($P < 0.05$). The activity of acid phosphatase (328.45, 300.08, 254.03, and 223.51 U/mg·prot) significantly decreased with increasing dietary SM substitution levels (linear trend, $P < 0.05$). A significant quadratic trend was observed in the mRNA expression of interferon-γ and interleukin-1β, while the maximum was observed in fish fed the diet with 45% protein from SM ($P < 0.05$). In conclusion, this study demonstrated that the replacement of FM by SM in diets can decrease the growth performance, digestion, antioxidant capacity, and innate immunity and affect the hepatic metabolism of juvenile large yellow croaker. The minimum negative effect was obtained when 30% of FM was replaced by SM.

1. Introduction

Fishmeal (FM) is the traditional protein source of aquatic feed, which has the advantage of sufficient essential amino acids, easy availability, and high digestibility [1]. However, due to overfishing, environmental pollution, and extreme weather, FM production has declined year by year [2]. Especially with the development of aquaculture, FM resources are becoming increasingly scarce, which has led to the soaring price of FM [3]. Therefore, the search for high-quality alternative protein sources of FM has become a research hotspot [1, 3]. At present, vegetable protein is the best alternative to fish meal [4–6].

In recent years, due to the low price and large supply of plant protein sources, many researches about replacing FM with plant protein sources has been carried out [3, 7]. Among

the protein sources (peanut, sweet potato, and melon seed meal), soybean meal (SM) is the most widely used in the production and application of feed. Compared with other protein sources, SM has the advantages of more affordable price, easier digestion and absorption, and better amino acid composition. It becomes the first choice of FM replacement with protein sources [4–6]. However, many factors restrict the replacement of FM by SM, such as unbalanced amino acid composition and antinutritional factors [8–10]. Therefore, the development and utilization of SM still need to be further studied. At present, there are already some studies on FM replacement with SM, fermented SM, and soybean protein concentrate, including Chinese sucker [11], grouper [7], rainbow trout [12], tilapia [13], and so on. However, as far as we know, there are no reports about dietary FM replacement with SM without additional additives in the treatment group on juvenile large yellow croaker.

As the most cultured marine fish in China, the large yellow croaker is popular with consumers because of its delicious meat, rich nutrition, and affordable price [14–16]. However, the high price of FM seriously restricted the further development of the artificial formula feed industry [17, 18]. Therefore, the purpose of this feeding trial is to assess the effect of FM replacement with SM on growth performance, digestion, hepatic metabolism, antioxidant capacity, and innate immunity of juvenile large yellow croaker, which will provide a theoretical basis for the application of FM replacement with SM in artificial formula feed.

Table 1: The formulation of this feed trial (% dry matter).

Ingredients % dry diet	Experimental diet			
	FM	SM15	SM30	SM45
Whitefish meal[a]	35	29.75	24.5	19.25
Krill meal[a]	3	3	3	3
Soybean meal[a]	0	7.75	15.5	23.2
Casein[a]	10.5	10.5	10.5	10.5
Bread flour strong flour[a]	28.5	28.5	28.5	28.5
α-Starch	11.77	8.67	5.57	2.42
Choline chloride	0.2	0.2	0.2	0.2
Fish oil	5.1	5.7	6.3	7
Ca $(H_2PO4)_2$	2	2	2	2
Soybean lecithin	2	2	2	2
Vitamin premix[b]	0.2	0.2	0.2	0.2
Vc polyphosphate	0.05	0.05	0.05	0.05
Mineral premix[b]	1	1	1	1
Attractant	0.5	0.5	0.5	0.5
Yeast cell wall	0.08	0.08	0.08	0.08
Calcium propionate	0.05	0.05	0.05	0.05
Ethoxy quinoline	0.05	0.05	0.05	0.05
Proximate composition % dry diet				
Crude protein	41.54	42.48	42.29	41.83
Crude lipid	11.93	12.28	12.17	11.89

[a]The nutritional composition of ingredients referred to Yao et al. [20], which is provided by Great Seven Biotechnology (Shandong, China). [b]The detailed composition of vitamin and mineral premix was presented by Liu et al. [21].

2. Materials and Methods

2.1. Feed Ingredients. On the basis of previous experimental diets of Yi et al. [19], four iso-nitrogenous (42.03% crude protein) and iso-lipidic (12.07% crude lipid) trail diets were formulated with 0%, 15%, 30%, and 45% of FM replacement with SM, named FM, SM15, SM30, and SM45, respectively (Table 1). This feeding trial was in strict accordance with the Management Rule of Laboratory Animals (Chinese Order No. 676 of the State Council, revised March 1, 2017).

2.2. Feeding Procedure. All of the large yellow croaker were obtained from NingdeFufa Fishery Co., Ltd (Fujian, China). First, the large yellow croaker was acclimated in a big sea cage and fed with commercial feed for 14 days. Then, after 1-day starvation, 720 fish (11.78 ± 0.03 g) were randomly distributed into 12 sea cages ($1 \times 1 \times 2$ m). Four kinds of diets were randomly allocated to triplicate cages. During the feeding trial, fish were fed at 05:00 and 17:00 daily; the salinity was maintained between 25.4 and 29.8‰, the dissolved oxygen was maintained between 6.1 and 7.1 mg/L, and the water temperature was maintained between 19.4 and 22.8°C.

2.3. Sample Collection. Before the trial, the initial body length and weight of a large yellow croaker were measured by rulers and balances. After the trial, the surviving large yellow croaker in each cage was counted for the calculation of the survival rate (SR). After 24 hr of starvation to empty the intestine, the remaining large yellow croaker was anesthetized with eugenol (1 : 10,000) to sample. The final body weight (FBW) and final body length (FBL) of the large yellow croaker were measured by rulers and balances. Five juvenile large yellow croakers in each cage were collected and frozen for body composition analysis. The 1 mL syringes were used to collect the blood of the juvenile large yellow croaker. The blood was coagulated at 4°C for 4 hr, then centrifuged (4,500 rpm, 10 min) to obtain serum for subsequent analysis. Then, the remaining juvenile large yellow croakers were dissected and separated the head kidney, liver, and intestine on ice. The head kidney, liver, and intestine were put into liquid nitrogen for preservation rapidly, then stored at −80°C until use. Ten livers of juvenile large yellow croaker were soaked in 4% paraformaldehyde for 1 day for hematoxylin–eosin staining.

2.4. Analytical Methods

2.4.1. Fish Body and Diets Composition Analysis. The moisture, crude protein, and crude lipid of juvenile large yellow croaker and diets were analyzed by the standard method of AOAC (2003). All samples were laid inside an oven at 110°C for over 1 day until a constant weight for the calculation of moisture. All samples were measured by the Kjeldahl method (Kjeltec^{TM} 8400, FOSS) for the calculation of crude protein content and measured by Automatic Soxhlet Extractor (Soxtec 2050, FOSS) for the calculation of crude lipid content.

2.4.2. The Intestinal Digestive Enzyme Activities. The α-amylase (no. C016-1-1), lipase (no. A054-2-1), and intestinal trypsin (no. A080-2-2) activities of juvenile large yellow

Table 2: Primer sequences used for real-time quantitative PCR.

Gene	Forward primers	Reverse primers	Accession number
ifn-γ	TCAGACCTCCGCACCATCA	GCAACCATTGTAACGCCACTTA	XM019258900
il-1β	CATAGGGATGGGGACAACGA	AGGGGACGGACACAAGGGTA	XM010736551
il-6	CGACACACCCACTATTTACAAC	TCCCATTTTCTGAACTGCCTCT	XM010734753
il-8	AATCTTCGTCGCCTCCATTGT	GAGGGATGATCTCCACCTTCG	XM010737667.3
il-10	AGTCGGTTACTTTCTGTGGTG	TGTATGACGCAATATGGTCTG	XM010738826
tnf-a	ACACCTCTCAGCCACAGGAT	CCGTGTCCCACTCCATAGTT	NM001303385
m-tor	CACCCACCTTCTTCTTTCAGC	CATTTCCTTGGTTTCCCTCTG	XM027288345.1
rps6	TCAGCGTCCTGAACTTGGTC	CTGAGTGCGCTGTCTCTTGA	XM019267468.2
eif4ebp1	CACGACTACTCCACGACTCC	TGGTCACCCCTGGAATGTTG	XM010732553.3
β-actin	GACCTGACAGACTACCTCATG	AGTTGAAGGTGGTCTCGTGGA	GU584189

ifn-γ, interferon γ; *il-1β*, interleukin-1β; *il-6*, interleukin-6; *il-8*, interleukin-8; *il-10*, interleukin-10; *tnf-a*, tumor necrosis factor *a*; *m-tor*, mechanistic target of rapamycin; *rps6*, ribosomal protein S6; *eif4ebp1*, recombinant eukaryotic translation initiation factor 4e binding protein 1.

croaker were detected by kits purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd. The detailed operations refer to the instructions of kits.

2.4.3. The Serum Innates Immune and Antioxidant Activities. The serum catalase (CAT, no. A007-1-1), superoxide dismutase (SOD, no. A001-3-2), acid phosphatase (ACP, no. A060-2-1), alkaline phosphatase (AKP, no. A059-2-1) activities, total antioxidant capacity (T-AOC, no. A015-2-1), and malondialdehyde (MDA, no. A003-1-2) of juvenile large yellow croaker were detected by kits purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd. The detailed operations refer to the instructions of kits.

2.4.4. The Serum Lipid Profiles and Transaminases. The serum aspartate aminotransferase (AST, no. C010-2-1), alanine aminotransferase (ALT, no. C009-2-1) activities, the content of low-density lipoprotein cholesterol (LDL-C, no. A113-1-1), high-density lipoprotein cholesterol (HDL-C, no. A112-1-1), total cholesterol (TCHO, no. A111-1-1), and total triglyceride (TG, no. A110-1-1) of large yellow croaker were detected by kits purchased from Nanjing Jiancheng Bioengineering Institute Co., Ltd. The detailed operations refer to the instructions of kits.

2.4.5. Liver Morphology Analysis. According to the method of published paper by Mai et al. [22], the livers of fish were washed, dehydrated, embedded, sectioned, stained, and finally observed under a microscope.

2.5. RNA Extraction and RT-qPCR. The total RNA extraction from the liver and head kidney of a large yellow croaker was carried out according to the instructions of the TRIzol Reagent kit (Takara, Japan). The RNA integrity was detected by 1.2% denaturing agarose gel under the manufacturer's protocol. The RNA concentration was measured by Nanodrop®2000 (Thermo Fisher Scientific, USA). The cDNA synthesis was carried out in strict accordance with the instructions of the PrimeScript-RT reagent kit (Takara, Japan). Then, the CFX96TM Real-Time System (BIO-RAD, USA) was used for RT-qPCR. In this study, the *β-actin* was regarded as a reference gene. Based on GenBank and a previous study by Wang et al. [23], primer sequences were designed by Megalign software (Table 2). The threshold of primer amplification efficiencies ranged between 0.95 and 1.05. The 20 μL RT-qPCR amplification system included 7 μL RNase-free water, 10 μL SYBR-Premix Ex TaqII (Takara, Japan), 0.5 μL upstream primers, 0.5 μL downstream primers and 2 μL cDNA. The program of qPCR was as follows: 95°C for 2 min, followed by 39 cycles of 95°C for 10 s, 59°C for 10 s, and 72°C for 20 s. Melting curve analysis was carried out to confirm that a single PCR product was present. The mRNA expression levels were calculated with the $2^{-\Delta\Delta CT}$ methods. [24].

2.6. Calculations and Statistical Analysis

$$\text{SR } (\%) = (\text{N1})/(\text{N2}) \times 100;$$

$$\text{Specific growth rate (SGR, \%/day)} = 100\ (\text{Ln }(\text{W1}) - \text{Ln }(\text{W2})) \times 100/D;$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times (\text{final viscera weight})/(\text{W1});$$

$$\text{Hepatosomatic index (HSI, \%)} = 100 \times (\text{final liver weight})/(\text{W1}).$$

Where N1 is final fish number, N2 is initial fish number, W1 is final fish weight, W2 is initial fish weight, and D is the experimental duration.

Results in this study were expressed as means ± S.E.M. All data were analyzed by SPSS Statistics 19.0 with orthogonal polynomial contrasts to assess if the pattern (or trend) was linear or quadratic. The significant level was set as $P < 0.05$.

3. Results

3.1. Survival, Growth Performance, and Body Composition. With increasing dietary SM, the FBW ($P < 0.05$) and SGR ($P < 0.05$) of large yellow croaker showed a significant linear trend and the minimum was observed in fish fed the diet with 45% protein from SM (Table 3). However, no significant linear or quadratic trend was observed in the FBL, VSI, HSI, and SR ($P > 0.05$). For the body composition of the large yellow croaker, no significant linear or quadratic trend was observed in the moisture, crude protein, and crude lipid with increasing dietary SM replacement levels ($P > 0.05$) (Table 4).

3.2. Digestive Enzyme Activities. With increasing dietary SM replacement levels, the activity of trypsin in a large yellow

Table 3: Effects of FM replacement with SM on survival and growth performance of juvenile large yellow croaker.

Parameters	Experimental diet				Polynomial contrasts	
	FM	SM15	SM30	SM45	Linear	Quadratic
Final body length (cm)	13.71 ± 1.26	11.52 ± 0.51	13.63 ± 0.31	12.52 ± 0.46	0.587	0.235
Final body weight (g)	39.50 ± 0.70	38.40 ± 0.56	38.27 ± 1.64	34.75 ± 0.54	0.001	0.064
Specific growth rate (%/day)	1.55 ± 0.04	1.50 ± 0.02	1.48 ± 0.06	1.34 ± 0.02	0.001	0.058
Viscerosomatic index (%)	5.47 ± 0.46	4.01 ± 0.14	6.72 ± 0.74	5.12 ± 0.84	0.932	0.573
Hepatosomatic index (%)	1.74 ± 0.43	1.45 ± 0.12	2.15 ± 0.40	1.50 ± 0.18	0.193	0.791
Survival rate (%)	81.11 ± 2.55	82.78 ± 1.92	81.67 ± 1.67	81.67 ± 3.33	0.708	0.531

Linear = P value of linear trend analyzed by orthogonal polynomial contrasts; Quadratic = P value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$).

Table 4: Effects of FM replacement with SM on body composition of juvenile large yellow croaker.

Whole-body (%)	Experimental diets				Polynomial contrasts	
	FM	Linear	Quadratic	SM45	Linear	Quadratic
Protein	12.10 ± 1.17	10.79 ± 1.44	11.95 ± 0.86	11.26 ± 0.58	0.536	0.647
Lipid	5.11 ± 0.86	4.96 ± 1.03	4.45 ± 0.51	4.86 ± 0.85	0.497	0.944
Moisture	80.78 ± 0.93	81.12 ± 1.24	81.75 ± 0.35	82.31 ± 1.46	0.561	0.419

Linear = P value of linear trend analyzed by orthogonal polynomial contrasts; Quadratic = P value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$).

Table 5: Effects of FM replacement with SM on activities of digestive enzyme of juvenile large yellow croaker.

Parameters	Experimental diets				Polynomial contrasts	
	FM	SM15	SM30	SM45	Linear	Quadratic
Trypsin (U/mg·prot)	262.02 ± 24.9	179.34 ± 29.65	144.41 ± 12.22	123.92 ± 3.02	0.004	0.219
α-Amylase (U/mg·prot)	0.17 ± 0.01	0.19 ± 0.01[a]	0.18 ± 0.01[a]	0.15 ± 0.01	0.002	0.001
Lipase (U/mg·prot)	20.36 ± 0.67	19.12 ± 0.60	21.87 ± 0.86	23.39 ± 0.76	0.002	0.090

Linear = P value of linear trend analyzed by orthogonal polynomial contrasts; Quadratic = P value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$).

Table 6: Effects of FM replacement with SM on serum lipid profiles and transaminases of juvenile large yellow croaker.

Parameters	Experimental diets				Polynomial contrasts	
	FM	SM15	SM30	SM45	Linear	Quadratic
TG (mmol/L)	3.37 ± 0.76	1.42 ± 0.13	6.11 ± 0.62	2.4 ± 0.09	0.004	0.219
TCHO (mmol/L)	2.49 ± 0.25	3.33 ± 0.33	2.07 ± 0.10	3.23 ± 0.61	0.728	0.459
HDL-C (mmol/L)	1.08 ± 0.10	1.08 ± 0.07	0.92 ± 0.16	1.8 ± 0.08	0.001	0.001
LDL-C (mmol/L)	0.73 ± 0.11	1.44 ± 0.10	0.69 ± 0.09	1.28 ± 0.19	0.146	0.628
ALT (U/L)	30.51 ± 1.63	85.05 ± 22.85	50.19 ± 3.68	49.34 ± 12.62	0.544	0.007
AST (U/L)	34.03 ± 10.68	170.05 ± 20.09	37.76 ± 1.77	38.87 ± 3.40	0.004	0.001

Linear = P value of linear trend analyzed by orthogonal polynomial contrasts; Quadratic = P value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$). TG, total triglyceride; TCHO, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

croaker significantly decreased (linear trend, $P < 0.05$, Table 5). The activity of α-amylase in large yellow croaker showed a significant linear trend and quadratic trend, and the minimum was observed in fish fed the diet with 45% protein from SM ($P < 0.05$). At the same time, a significant linear trend was observed in the activity of lipase with the increase of FM replacement with SM, and the maximum was observed in fish fed the diet with 45% protein from SM ($P < 0.05$).

3.3. Lipid Profiles, Transaminases, and Liver Histology. With the SM substitution increasing from 0% to 45%, a significant linear trend was observed in the content of TG, and the maximum was observed in fish fed the diet with SM30 ($P < 0.05$, Table 6). The content of HDL-C in large yellow croaker showed a significant linear trend and quadratic trend, and the maximum was observed in fish fed the diet with 45% protein from SM ($P < 0.05$). However, there was no

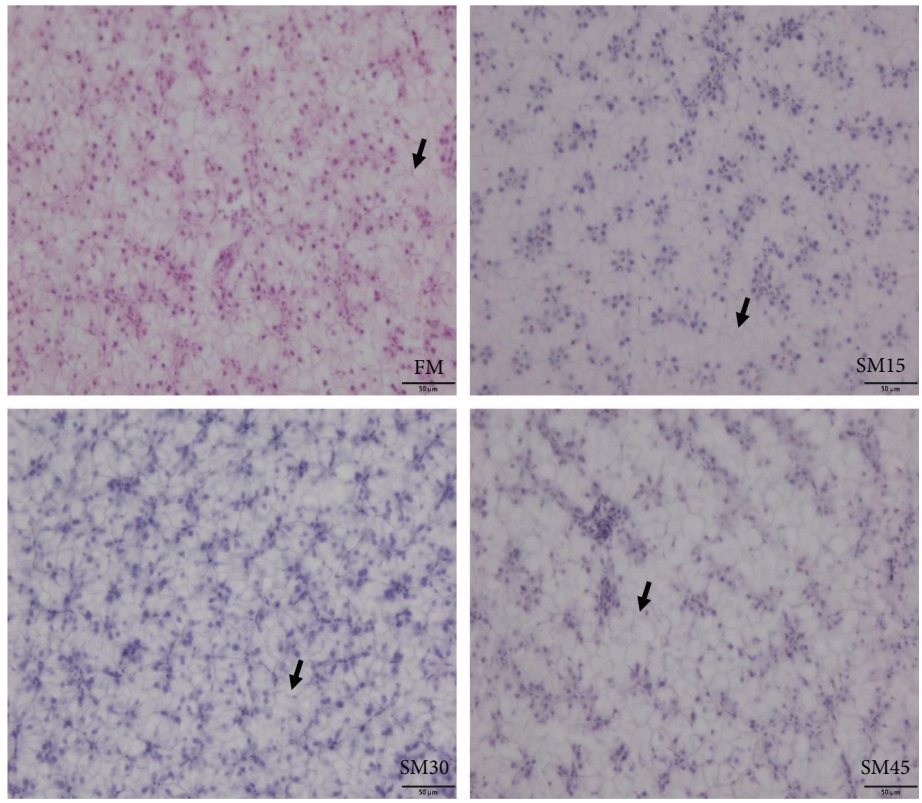

Figure 1: Effects of FM replacement with SM on histopathological changes in the liver of juvenile large yellow croaker, hematoxylin–eosin (H&E).

significant linear or quadratic trend in the content of TCHO and LDL-C ($P > 0.05$). For the transaminases of large yellow croaker, both the activities of ALT and AST showed a significant quadratic trend with the increase of FM replacement with SM; the maximum was observed in fish fed the diet with SM15 ($P < 0.05$). Meanwhile, the activity of AST also showed a significant linear trend ($P < 0.05$). When SM replacement level reached to 15% or 45%, the vacuolation of cells in the liver of a large yellow croaker was more serious than the control, which indicated the degree of liver injury became more serious than the control (Figure 1).

3.4. The Genes Expression of Protein Metabolism-Related. For the protein synthesis genes, the mRNA expression of *m-tor* and *rps6* showed a significant linear trend with the increase of FM replacement with SM ($P < 0.05$, Figure 2). In regard to protein catabolic metabolism genes, no significant linear or quadratic trend was observed in the mRNA expression of *eif4ebp1* among different dietary treatments ($P > 0.05$).

3.5. Antioxidant and Innate Immunity Capacity. As dietary SM replacement levels increased, the content of MDA in large yellow croaker showed a significant linear trend and quadratic trend, and the minimum was observed in fish fed the diet with SM30 ($P < 0.05$, Figure 3). However, there was no significant linear or quadratic trend in the activities of SOD, CAT, and T-AOC ($P > 0.05$, Figure 3(a)–3(c)). For the phosphatase of large yellow croaker, the activity of ACP significantly decreased with the SM substitution increasing from 0% to

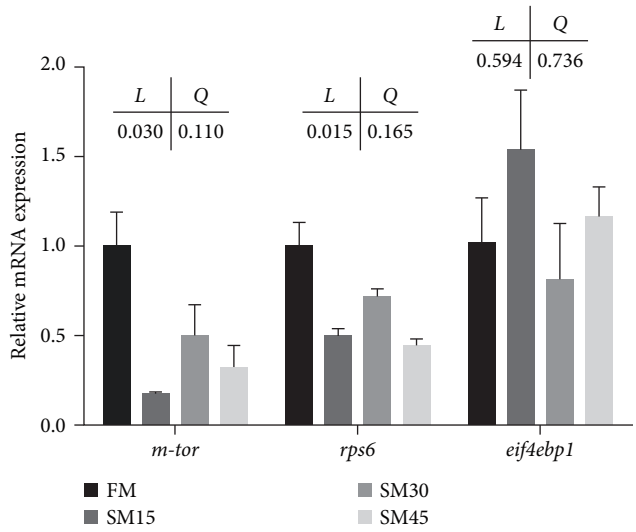

Figure 2: Expression of genes related to protein metabolism in the liver of juvenile large yellow croaker. $L = P$ value of linear trend analyzed by orthogonal polynomial contrasts; $Q = P$ value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$). *m-tor*, mechanistic target of rapamycin; *rps6*, ribosomal protein S6; *eif4ebp1*, recombinant eukaryotic translation initiation factor 4e binding protein 1.

45% (linear trend, $P < 0.05$, Figure 3(d)). No significant linear or quadratic trend was observed in the activity of AKP among different dietary treatments ($P > 0.05$, Figure 3(f)). For the

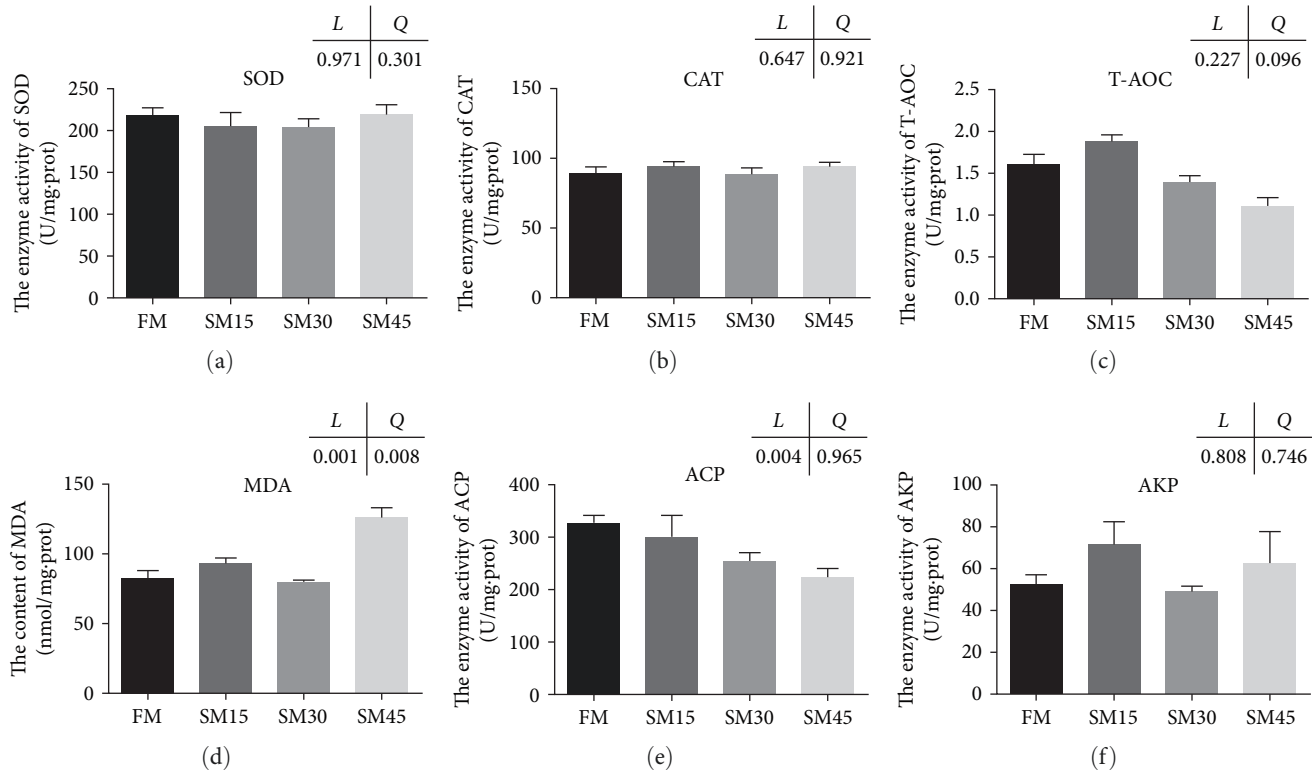

FIGURE 3: Effects of FM replacement with SM on antioxidant and innate immunity capacity of juvenile large yellow croaker. $L = P$ value of linear trend analyzed by orthogonal polynomial contrasts; $Q = P$ value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$). SOD, superoxide dismutase; CAT, catalase; T-AOC, total antioxidant capacity; MDA, malondialdehyde; ACP, acid phosphatase; AKP, alkaline phosphatase.

pro-inflammatory cytokines, the mRNA expression of *tnf-α* and interferon-*γ* (*ifn-γ*) showed a significant linear trend with the increase of FM replacement with SM ($P < 0.05$, Figure 4). Meanwhile, a significant quadratic trend was observed in the mRNA expression of *ifn-γ* and interleukin-1*β* (*il-1β*), while the maximum was observed in fish fed the diet with 45% protein from SM ($P < 0.05$). However, there was no significant linear trend or quadratic trend in the mRNA expression of *il-8* ($P > 0.05$). For the anti-inflammatory cytokines, no significant linear trend or quadratic trend was observed in the mRNA expression of *il-10* ($P > 0.05$).

4. Discussion

Growth performance is a crucial index to measure the applicability of protein alternative feed [25]. The SR of the large yellow croaker was unaffected by the replacement of SM in this study. A similar result also can be found in the data of Chinese sucker [11]. Meanwhile, 45% protein from SM could decrease the FBW and SGR of fish, while 15% protein from SM could decrease the FBL of fish. Similarly, this result was also reported in the study of tilapia [25] and Atlantic salmon [26, 27] treated with different replacement levels of FM by SM. Overall, the replacement of FM by SM in diets could inhibit the growth performance of fish.

Digestive enzymes mainly exist in the intestine tract, affecting the digestion of fish and ultimately affecting the growth performance [28]. This study showed that 30% and 45% protein from SM could decrease the activity of trypsin in the intestine, which reflects the decrease of protein digestibility of large yellow croaker [29]. Meanwhile, 45% protein from SM could decrease the activity of *α*-amylase, which was consistent with the previous research on Atlantic salmon [30], catfish [31], and several aquatic animals [25]. However, the intestinal activity of lipase was increased by 45% protein from SM. This may be due to its lower activity of trypsin and *α*-amylase, which made the large yellow croaker fed the diet with 45% protein from SM have to obtain more energy from fat. In general, improper replacement of FM by SM could reduce the digestive enzyme activities of fish.

Based on some previous studies, the replacement of plant protein for FM will lead to changes in physiological state and blood biochemical indexes [32, 33]. The result indicated that 15% protein from SM could increase the content of serum LDL-C, 30% protein from SM could increase the content of serum TG, while 45% protein from SM could increase the content of serum HDL-C and LDL-C. These data suggested that the replacement of FM by SM in diets could increase the serum lipids level, which was not consistent with the research results of other fish, such as cyprinus carpio-haematopterus [33], Chinese sturgeon [32], and Tiger puffer [34]. This difference may be caused by the different fish species and experimental breeding environment. When the liver is damaged, AST will enter the blood and increase the activity of AST in serum [35]. The result indicated that the AST activity could be increased by 15% protein from SM, which was similar to

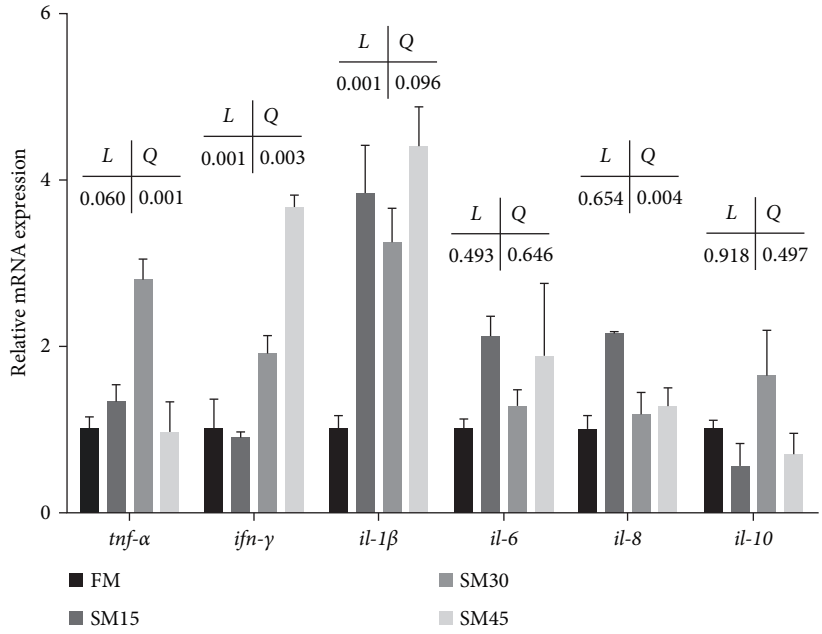

Figure 4: Expression of genes related to inflammation in the head kidney of juvenile large yellow croaker. $L = P$ value of linear trend analyzed by orthogonal polynomial contrasts; $Q = P$ value of quadratic trend analyzed by orthogonal polynomial contrasts (means ± S.E.M, $n = 3$). *tnf-a*, tumor necrosis factor-*α*; *inf-γ*, interferon-*γ*; *il-1β*, interleukin-1*β*; *il-6*, interleukin-6; *il-8*, interleukin-8; *il-10*, interleukin-10.

the research result in starry flounder [36]. Furthermore, this result was also consistent with the results of liver morphology. Combined the result of liver morphology and the result of transaminases in serum, this study demonstrated that inappropriate replacement of FM by SM could lead to liver damage of fish.

Liver protein metabolism of fish could be affected by liver injury [37]. According to previous researches, the replacement of FM by SM in diets can impact the protein composition of several aquatic animals, including juvenile Japanese flounder [32] and *Rhynchocypris lagowskii* Dybowski [38]. For protein synthesis, 15% and 45% protein from SM could decrease the mRNA expression of *m-tor* and rps*6*, which indicated that the replacement of FM by SM might reduce the hepatic metabolic capacity of large yellow croaker.

The severe oxidative stress and severe inflammatory reaction of fish will affect the health [39]. Some studies have proved the replacement of FM by SM decreased the antioxidant and innate immunity capacity of different kinds of fish [40, 41]. The result indicated that 45% protein from SM could decrease the T-AOC of juvenile large yellow croaker, and the content of MDA of large yellow croaker could be increased by 45% protein from SM correspondingly. This result showed that a high proportion replacement of FM by SM could reduce the antioxidant capacity of fish, which was in accordance with the research of *Schizothorax prenanti* [42] and red seabream [43]. Innate immunity can rapidly respond to various invading pathogens and maintain the health of fish [44]. The serum ACP activity could be decreased by 45% protein from SM in the present study, which indicated a decrease in serum immune ability. Previous studies also proved severe inflammatory response could lead to nonspecific immunity reduction, ultimately leading to the reduction of SR [45]. The data showed that 15% protein from SM could increase the mRNA expression of *il-1β*, *il-8*, and *tnf-α*, while 45% protein from SM could increase the mRNA expression of *ifn-γ* and *il-1β*, indicating the inappropriate replacement of FM by SM might induce inflammatory response. Similar results could be found in turbot [46], Atlantic salmon [47], and zebrafish larvae [48]. Overall, the replacement of FM by SM might induce oxidative stress and inflammation, which might affect the health of large yellow croaker.

5. Conclusion

In conclusion, this study assesses the effects of the replacement of FM by SM in diets on growth performance, digestion, hepatic metabolism, antioxidant capacity, and innate immunity of juvenile large yellow croaker. Results indicated improper replacement of FM by SM could decrease the growth performance, digestive enzyme activity, antioxidant, and innate immunity capacity, resulted in morphological changes of the liver and affected hepatic metabolism of juvenile large yellow croaker. The minimum negative effect was obtained when 30% of FM was replaced by SM.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This study was supported by the China Agriculture Research System of MOF and MARA (CARS-47) and the Leading Talent of Technological Innovation of Ten-Thousands Talents Program (grant no. 2018-29).

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