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Effect of *Schizochytrium limacinum* supplementation to a low fish-meal diet on growth performance, lipid metabolism, apoptosis, autophagy and intestinal histology of *Litopenaeus vannamei*

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In this experiment, we aimed to evaluate the relationship between the addition of *Schizochytrium limacinum* to low fish meal diets on growth performance, apoptosis, autophagy, lipid metabolism, and intestinal health of *Lipenaeus vanamei*. The diet containing 25% fish meal was used as a positive control (FM) and the other three diets contained 15% fish meal and were supplemented with 0, 0.3, and 0.6% *S. Limacinum* (LF, LFLD, LFHD). The shrimp (0.22 ± 0.00 g) were divided into four replicates of 40 shrimp per tank and fed four times daily to apparent satiation for 8 weeks. Results showed that the final weight (FBW) and weight gain rate (WGR) of shrimp fed FM and LFHD diets were significantly increased compared to those fed the LFLD diet ($P < 0.05$), and there was no significant difference in survival rate (SR) and feed conversion rate (FCR) among the groups ($P > 0.05$). Supplementation of *S. Limacinum* in low fish meal diets had no effects on shrimp body composition ($P < 0.05$). There were significant differences ($P < 0.05$) in low-density lipoprotein (LDL-C) glucose (GLU), triglycerides (TG), and total cholesterol (TC) in the hemolymph of shrimp fed the LF diet compared to those fed the LFLD and LFHD diets. HE staining and transmission electron microscopy (TEM) results showed that the microvilli height, mucosal folds height, mucosal folds width and muscle layer thickness in the intestine of shrimp fed the LF diet were significantly reduced compared to those fed the other three diets ($P < 0.05$). Swelling of the endoplasmic reticulum and irregular mitochondria in the gut of shrimp fed the LF diet was also observed by TEM, and the endoplasmic reticulum and mitochondria of shrimp fed the LFHD diet returned to a healthy state. Hepatopancreas genes expression results were showed that the gene expression of 5' -AMP-activated

protein kinase (*ampk*), stearoyl-CoA desaturase (*scd1*), acetyl-CoA carboxylase 1 (*acc1*), and malonyl-CoA decarboxylase (*mcd*) of shrimp fed the LF diet was significantly increased compared to those fed the FM diet ($P < 0.05$). The gene expression of sterol regulatory element-binding protein (*srbeb*) and carnitine palmitoyl transferase 1 (*cpt-1*) of shrimp fed the LFLD diet was significantly increased compared to those fed the LF diet ($P < 0.05$). The gene expression of *acc1*, *mcd* and *scd1* of shrimp fed the LFHD diet was significantly reduced compared to those fed the LF diet ($P < 0.05$). Results of genes expression associated with apoptosis in the hepatopancreas showed that the gene expression of B lymphocytoma-2 (*bcl-2*), BCL2 associated X apoptosis regulator (*bax*) and cysteinyl aspartate specific proteinase 8 (*caspase 8*) of shrimp fed the LF diet was significantly reduced compared to those fed the FM diet ($P < 0.05$). The gene expression of *bcl-2* of shrimp fed the LFHD diet was significantly reduced compared to those fed the LF diet ($P < 0.05$). Genes related to autophagy in the hepatopancreas showed that the expression of autophagy-related protein 12 (*atg 12*), autophagy-related protein 13 (*atg 13*) and beclin1 of shrimp fed LF the diet was significantly reduced compared to those fed the FM diet ($P < 0.05$). The gene expression of *atg 12* and *atg 13* of shrimp fed the LFHD diet was significantly increased compared to those fed the LF diet ($P < 0.05$). In summary, reducing fish meal is detrimental to the growth performance and intestinal health of shrimp, and 0.6% *S. Limacinum* supplementation can improve the growth performance, promotes hepatopancreas lipid metabolism, reduces apoptosis, promotes autophagy and improve intestinal health of *Litopenaeus vannamei*.

KEYWORDS

Litopenaeus vannamei, soy protein concentrate, *Schizochytrium limacinum*, lipid metabolism, apoptosis, autophagy, intestinal health

Introduction

Litopenaeus vannamei has the characteristics of fast growth, strong disease resistance, and delicious taste, which has important economic value, and the annual production has up to 5.8 million tons in 2020 (FAO, 2022; Xu et al., 2022a). Fish meal is rich in amino acids, vitamins, and minerals which are necessary for the growth of fish, shrimp, and crab, and has a special flavor that makes it good palatability (An et al., 2018). Nonetheless, the high cost of fish meal has increased the cost of feed. Previous studies found that fish meal in aquatic animal feed can be substituted by different protein sources, such as soy protein peptide (Lin et al., 2022), soy protein concentrate (Zhu et al., 2020), fermented soy pulp (Kari et al., 2022), hydrolyzed fish protein powder (Hlodzi et al., 2022), bacterial protein meal (Chen et al., 2021b), concentrated dephenolization cottonseed protein (Zhao et al., 2021), blood meal (Kirimi et al., 2016), black soldier fly (Huang et al., 2022), meat and bone meal (Ai et al., 2006), and hydrolyzed feather meal (Campos et al., 2017). Plant proteins are widely available and inexpensive, which were favored by researchers for partial replacement of fish meal in aquatic animal feed (Liao et al., 2022). However, plant protein

generally has the disadvantages of amino acid imbalance and high content of anti-nutritional factors (Jannathulla et al., 2019), and adding excessive plant protein can also affect the growth performance, intestinal microecology, and nutrition metabolism of aquatic animals (Ray et al., 2020). In our previous study, we found that the essential nutrient balance in low fish meal diets improved growth properties and intestinal fitness of shrimp (Xie et al., 2016; Xie et al., 2020c).

Fish meal contains high levels of fish oil, which is rich in n-3 long-chain polyunsaturated fatty acids, especially for docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Cho and Kim, 2011). Consequently, the content of high-unsaturated fatty acids decreases accordingly in the low fish meal diets. Polyunsaturated fatty acids are essential fatty acids for fish and crustaceans, which cannot be synthesized *in vivo* and must be obtained from food (Dyall et al., 2022). DHA is a polyunsaturated fatty acid, which has various functional roles in regulating the physiological health of the body and has important regulatory influences on growth, inflammatory response, development, immune regulation, and antioxidation in aquatic animals (Ruyter et al., 2022). DHA is an essential fatty acid for many fish, shrimp, and shellfish larvae, previous study

proved that dietary supplementation of DHA in the low fish meal diet could improve the immune system response and intestinal fitness of *Penaeus monodon* (Xie et al., 2020b).

Schizochytrium Limacinum (*S. Limacinum*), a type of marine algae, is rich in the highly unsaturated fatty acid DHA (Li et al., 2018). In contrast to other algae, *S. Limacinum* is produced by employing heterotrophic propagation and was regarded as microalgae with the potential to produce DHA in an industrial chain (Sarker et al., 2016; Osmond et al., 2021). Numerous studies have been conducted to demonstrate the improvement of the growth performance of aquatic animals by feeding diets containing *S. Limacinum*, such as *Litopenaeus vannamei* (Wang et al., 2017), *Salmo Salar* (Sprague et al., 2015), *Ictalurus punctatus* (Li et al., 2009), *Danio rerio* (Byreddy et al., 2019), *Trachinotus Ovatus* (Xie et al., 2019), *Epinephelus Lanceolatus* (García-Ortega et al., 2016). In addition, there have been several studies showing that dietary supplementation of *S. Limacinum* be significantly enhanced the non-specific immunity of *Trachinotus Ovatus* (Xie et al., 2019) and improve the intestinal health of *Oreochromis niloticus* (Souza et al., 2020) and *Oncorhynchus mykiss* (Lyons et al., 2016). The results of another study suggest that *S. Limacinum* can be a promising low-level substitute for fish meals, which could improve the fillet texture of largemouth bass (Liao et al., 2022).

Few studies have been reported on the effect of *S. Limacinum* addition in the low fish meal diet of *Litopenaeus vannamei*. Thus, the intention of this experiment was to assess the effects of low fish meal diet supplementation with *S. Limacinum* on growth performance, hemolymph biochemistry, intestinal health, lipid metabolism, apoptosis, and autophagy of *Litopenaeus vannamei*.

Materials and methods

Diet preparation

Two diets with different levels of the fish meal were formulated as the positive control (FM) and negative control (LF), and two levels of *S. Limacinum* were added to the LF, which were labeled as LFLD and LFHD. The nutritional composition of the four diets was shown in Table 1. Ingredients were crushed and passed through an 60 mesh sieve, weighed accurately, and blended well (M-256, South China University of Technology, Guangzhou), next were stirred well with pre-weighed distilled water, fish oil, soybean oil, soy lecithin, and pre-configured coated crystal amino acids as described by Xie et al. (Xie et al., 2020c). The 1.0 mm and 1.5 mm feeds were extruded by a twin-screw extruder (F-26, South China University of Technology, Guangzhou), followed by being heated in an oven at 90°C for 60 min and air-drying at

room temperature, the diets were storage at -20°C until use (Li W. et al., 2022).

Experimental shrimp and management conditions

Juvenile *Litopenaeus Vannamei* were purchased from Zhanjiang Yuehai Seed Co. The shrimp was transferred to a pre-disinfected specimen pond for one month and acclimatized for one week to the experimental conditions before the start of the experiment. Then 640 healthy shrimp with similar body sizes ($0.22 \pm 0.00\text{g}$) were randomly assigned to 16 fiberglass tanks (500 L) with 40 shrimp per tank. The shrimp were periodically fed four times a day (7:00, 11:00, 16:00, 21:00) for 8 weeks. During the time of trial, water temperature and salinity were to be measured daily, with the water temperature at 28.0-30.0°C, salinity at 26-30‰, pH at 7.6-8.1, and ammonia nitrogen level below 0.05 mg/L (He et al., 2017). In addition, each tank was aerated to ensure enough oxygen, and the water was changed by approximately 60% at 2h after the first feeding every day.

Sample collection and analysis

Growth indexes

At the end of 8 weeks of feeding, shrimp were starved and treated for 24 h before being anesthetized using MS-222 (Zhou et al., 2019; Xu et al., 2021). Samples were then collected, and shrimp in each tank was counted and weighed to determine the initial weight (IBW), final weight (FBW), survival rate (SR), feed conversion rate (FCR), and weight gain rate (WGR). Immediately after weighing, 10 shrimp were randomly selected from each tank, and blood was taken using a 1 mL sterile syringe, and placed in a 1.5 mL sterile centrifuge tube. The hemolymph was gathered by centrifugation at 1500 x g for 10 min at 4°C through a benchtop high-speed frozen centrifuge (JIDI-20R, Guangzhou JIDI Instruments Ltd. formula). Six shrimps were randomly detected from each tank, which were stored at -20°C for the shrimp body composition analysis using standard methods (Feldsine et al., 2002). Moisture was determined in a constant-weight oven at 105°C. The content of crude protein and Crude lipid were determined using the Kjeldahl method (Kjeltec™8400, Sweden) and the Soxhlet extraction method (extractant petroleum ether), respectively, according to the description of (Zhang et al., 2018; Liu et al., 2021).

Hemolymph biochemical indexes and enzyme activity analysis

Hemolymph biochemical indicators were measured using kits developed by Nanjing Jiancheng Institute of Biology (China), the content of high-density lipoprotein (HDL-C),

TABLE 1 Formulation and proximate composition of experimental diets (% dry matter).

Ingredient	Treatments			
	FM	LF	LFLD	LFHD
Fish meal	25	15	15	15
Soybean meal	25	25	25	25
Peanut meal	12	12	12	12
Soy protein concentrate	0	10	10	10
Flour	20	20	20	20
Brewer's yeast	2	2	2	2
Shrimp shell powder	2	2	2	2
Chicken Powder	3	3	3	3
Fish Oil	1.5	2.3	2.3	2.3
Soybean Oil	2	1.9	1.9	1.9
Choline	0.2	0.25	0.25	0.25
Soy lecithin	1	1	1	1
Vitamin and mineral premixes ^a	1	1	1	1
Calcium dihydrogen phosphate	1	2	2	2
Vitamin C	0.1	0.1	0.1	0.1
Micro Nutrients Mix	0	0.05665	0.05665	0.05665
Microcrystalline cellulose ^c	3.2	0.65	0.35	0.05
L selenomethionine type II ^b	0	0.007	0.007	0.007
Sodium carboxymethyl cellulose	1	1	1	1
Docosahexaenoic acid	0	0	0.3	0.6
Proximate composition				
Dry matter				
Crude protein	40.02	40.57	40.84	40.62
Crude lipid	7.61	7.55	7.41	7.53

^aVitamin and Mineral Premix ((kg) 1 of diet): thiamine, 5 mg; riboflavin, 10 mg; vitamin A, 5000 IU; vitamin E, 40 mg; vitamin D3, 1000 IU; menadione, 10 mg; pyridoxine, 10 mg; biotin, 0.1 mg; cyanocobalamin, 0.02 mg; calcium pantothenate, 20 mg; folic acid, 1 mg; niacin, 40 mg; vitamin C, 150 mg; iron, 100 mg; iodine, 0.8 mg; copper, 3 mg; zinc, 50 mg; manganese, 12 mg; selenium, 0.3 mg; cobalt, 0.2 mg.

^bL selenomethionine type II Sichuan New Yimei Biotechnology Co., Ltd, Selenium Power II (L-selenomethionine \geq 0.5%, selenium \geq 0.2%)

^cMicro Nutrients Mix (kg⁻¹ of wet weight diet): threonine, 0.5g; glycine, 1g; alanine, 1.5g; methionine, 1.5g; lysine, 2g; γ -aminobutyric acid, 0.2g; taurine, 0.6g; ornithine, 0.12g; phytase, 0.4g; vitamin B2, 1mg; vitamin B12, 10mg; niacin, 10mg; ferrous Glycinate, 60mg; zinc amino acid complexes, 30mg; purchased from Shanghai Aladdin Biochemical Technology Co. and Guangzhou Chengyi Aquaculture Co.

low-density lipoprotein (LDL-C), glucose (GLU), triglyceride (TG), total protein (TP), and total cholesterol (TC), and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured using a full-wavelength enzyme marker (Thermo, Multiskan GO 1510). The commercial kit IDs were as follows: A112-1-1, A113-1-1, A154-1-1, A110-1-1, A045-2, A111-1, C009-2-1, and C009-2-1, respectively. The methods of the kit were tested strictly according to the description of (Gui et al., 2019; Wu et al., 2021).

Quantitative real time PCR analysis

Total RNA was obtained from the hepatopancreas and intestine of *Litopenaeus vannamei* using the TransZol Up Plus RNA kit (Transgen, China), and the extracted RNA was assayed by NanoDrop2000 (Thermo USA), and the massification of RNA was determined by the A260/280 ratio (Zhu et al., 2021;

Zhang et al., 2022a). Reverse transcription was performed using the PrimeScript™ RT kit (Takara, Japan) according to the manufacturer's instructions. Oligo dt (18T) primer random 6 mers, PrimeScript™ RT enzyme mixture I, 5× PrimeScript™ buffer, and RNase-free water were used to reverse transcribe 1000 ng of RNA at 37°C for 15 min, followed by inactivation at 85°C for 5 s (Li Y. et al., 2022; Xu et al., 2022c).

The polymerase chain reaction was performed using SYBR[®] Green Premix Pro Taq HS qPCR Kit II (Accurate Biotechnology (Hunan) Co., Ltd.) and the system was quantified fluorescently on a LightCycler 480 (Roche Applied Science) according to the set procedure for 10. The fluorescence quantification of the samples was performed on a LightCycler 480 (Roche Applied Science) according to a set procedure. The 10 μ l system consisted of 0.5 μ M of forward and reverse specific primers, 5 μ l of 2× SYBR[®] Green Pro Taq HS Premix II, 10 ng of cDNA

template and RNase-free water (Chi et al., 2017). The denaturation step lasted for the 30s at 95°C, followed by 40 amplification cycles, denaturation at 95°C for 5s, and annealing at 60°C for 30s before analysis of the solubility curves. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method using *ef-1 α* as the internal reference gene (Chen et al., 2021a; Wang et al., 2022). (The information of the primers used in this study was shown in Table 2).

Intestinal tissue analysis

Intestine of four shrimp was randomly selected from each tank for hematoxylin-eosin (H&E) stain and transmission electron microscopy (TEM) analysis. The intestine was stored in Bouin's solution for 24 h and then dehydrated in 75% ethanol. Then the tissue was dehydrated and washed with xylene, The samples were cleaned in toluene followed by embedding in paraffin to make solid

wax blocks. A rotary microtome was used to cut the solid wax blocks into transverse section blocks into 5 μ m sections followed by mounting on the slide and staining with hematoxylin-eosin (H&E). The slides were observed under a microscope (Olympus, BX51, Tokyo, Japan) and the built-in software was used to measure the thickness of the muscle layer, the height of the mucosal fold, and the width of the mucosal fold. TEM was performed as the method described before (Xie et al., 2018): intestines were fixed with 2.5% glutaraldehyde for 2h, washed and fixed with osmium acid for 3h, dehydrated and embedded with resin, and ultrathin sections were made of resin blocks, followed by staining with saturated uranyl acetic acid solution for 30 min and distilled water washing followed by lead citrate for 30 min. Finally, the cytoarchitecture was observed by TEM (Hitachi HT7700 TEM, Japan) and the length of microvilli was measured with Image-Pro Plus 6.3 software (Media Cybernetics, Inc., Rockville, USA).

TABLE 2 Primers used for quantitative real-time PCR.

Gene name	Sequence of primer (5' -3')	sources
<i>caspase3 F</i>	ACATTTCTGGGCGGAACACC	AGL61582.1
<i>caspase3 R</i>	GTGACACCCGTGCTTGACACA	
<i>caspase8 F</i>	CACGGAAGCTCTCCCTACAG	(Yin et al., 2021)
<i>caspase8 R</i>	GAAGACCTTGGGTTTCCCCC	
<i>bcl-2 F</i>	CCTTGCTTGACACAGTCGGA	(Yin et al., 2021)
<i>bcl-2 R</i>	CAGACAAGGTCGTGAGGTGG	
<i>bax F</i>	GGTGAATCACAAGAGAGCGA	(Yin et al., 2021)
<i>bax R</i>	TGTTCTCCACGGTGTCTCAC	
<i>atg13 F</i>	GAGACTTTTTACCGCTTCGC	XM_027375959.1
<i>atg13 R</i>	ATCCTGCTGGACCTCTATGG	
<i>atg12 F</i>	GAGAAGGAGAAAACCTGCGAC	XM_027363883.1
<i>atg12 R</i>	CTACCCAACCTACTGGCTTC	
<i>beclin1 F</i>	CTGTCTGAGGTGAGGCTGA	MH797016.1
<i>beclin1 R</i>	ATGTGGAAGGTGGTGTGAA	
<i>ampk F</i>	TCAGAGGAGGAGCAGGAAC	KP272117.1
<i>ampk R</i>	CCCGAGGTCTAATAGGCAC	
<i>srbeF F</i>	ACTGAGCTCAACACCTTCCG	MG770374.1
<i>srbeF R</i>	TGCTGGTGAAGAGCTGTCTG	
<i>acc1 F</i>	TGCATAGAAACGGCATTGCG	XM_027360190.1
<i>acc1 R</i>	TTTGACACCTGAGCCAGACC	
<i>mcd F</i>	AAGACCACAGGAAGGGACCA	XM_027376735.1
<i>mcd R</i>	GACACTTGAGATGCCACCCA	
<i>fas F</i>	CAGGTGGAGATGCTCCTCGTGTT	HM595630.1
<i>fas R</i>	GGTGAAGTACTCGGCTACATGGTT	
<i>cpt-1 F</i>	CAACTTCTACGGCACTGAT	XM_027361886.1
<i>cpt-1 R</i>	GTCGGTCCACCAATCTTC	
<i>scd1 F</i>	TGCTTTACACCTTATCAATGGC	XM_027374708.1
<i>scd1 R</i>	CGTTCGTATGTTCTCTTCGTC	
<i>ef-1α F</i>	GTATTGGAACAGTGCCCGTG	JF288785.1
<i>ef-1α R</i>	ACCAGGGACAGCCTCAGTAAAG	

caspase 3, cysteinyl aspartate specific proteinase 3; *caspase 8*, cysteinyl aspartate specific proteinase 3; *bcl-2*, B lymphocytoma-2; *bax*, BCL2 associated X apoptosis regulator; *Atg13*, autophagy-related protein 13; *atg12*, autophagy related protein 12; *ampk*, 5' -AMP-activated protein kinase; *srbeF*, sterol regulatory element-binding protein; *acc1*, acetyl-CoA carboxylase 1; *mcd*, malonyl-CoA decarboxylase; *fas*, fatty acid synthase; *cpt-1*, carnitine palmitoyl transferase 1; *scd1*, stearoyl-CoA desaturase; *ef-1 α* , elongation factor 1 α .

Calculations and statistical analysis

These statistics are obtained as follows:

$$\text{Survival rate (SR, \%)} = \frac{\text{final number of shrimp}}{\text{initial number of shrimp}} \times 100$$

$$\begin{aligned} \text{Weight gain rate (WGR, \%)} \\ = \frac{\text{final body weight} - \text{initial body weight}}{\text{initial body weight}} \times 100 \end{aligned}$$

$$\begin{aligned} \text{Feed conversion rate (FCR)} \\ = \frac{\text{feed consumed (g)}}{\text{final body weight} - \text{initial body weight}} \end{aligned}$$

The results are expressed as mean \pm SEM. There was a one-way ANOVA for all data and a Duncan's multiple tests was performed using SPSS 21.0 to identify significant between treatment differences. The probability value of $P < 0.05$ is statistically significant and indicates a significant difference in the results.

Result

Growth performance

The results in Table 3 show that the FBW and WGR of shrimp fed the LFLD diet were significantly lower than those fed the FM diet ($P < 0.05$), dietary supplementation of 0.6% *S. Limacinum* significantly increased the growth of shrimp ($P < 0.05$). The SR and FCR of shrimp were similar among the four groups.

TABLE 3 Effect of low fish meal diet supplemented with *Schizochytrium limacinum* on the growth performance of *Litopenaeus vannamei*.

Index	FM	LF	LFLD	LFHD
IBW	0.22 \pm 0.00	0.22 \pm 0.00	0.22 \pm 0.00	0.22 \pm 0.00
FBW	4.67 \pm 0.23 ^b	4.55 \pm 0.04 ^{ab}	4.30 \pm 0.21 ^a	4.59 \pm 0.10 ^b
SR	79.38 \pm 3.75	76.88 \pm 5.15	83.13 \pm 5.15	77.50 \pm 6.77
WGR	2104.77 \pm 9.71 ^c	1986.33 \pm 4.74 ^{ab}	1930.01 \pm 29.19 ^a	2014.02 \pm 21.33 ^b
FCR	1.94 \pm 0.06	1.96 \pm 0.05	2.06 \pm 0.01	2.03 \pm 0.02

Values in the table are the mean of four replicates of treatment and are expressed as mean \pm SEM (n=4). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*; IBW, initial body weight (g); FBW, final body weight (g); SR, survival rate (%); WGR, weight gain rate (%); FCR, Feed conversion rate.

TABLE 4 Effect of low fish meal diet supplemented with *Schizochytrium limacinum* on the body composition of *Litopenaeus vannamei*.

Index	FM	LF	LFLD	LFHD
Moisture (%)	75.28 \pm 0.36	74.90 \pm 0.86	76.63 \pm 2.11	76.27 \pm 0.49
Crude lipid (%)	3.81 \pm 1.39	3.74 \pm 1.13	3.32 \pm 1.66	3.48 \pm 1.45
Crude protein (%)	17.66 \pm 3.07	17.31 \pm 1.45	16.51 \pm 1.67	16.49 \pm 0.90

Values in the table are the mean of four replicates of treatment and are expressed as mean \pm SEM (n=4). Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*.

Analysis of whole shrimp body composition

The results in Table 4 show the whole shrimp body composition.

Hemolymph biochemical indexes

As shown in Table 5, the HDL-C content of shrimp fed the LF diet was significantly increased compared to those fed the FM and LFHD diets ($P < 0.05$). The LDL-C content of shrimp fed the LF and FM diets were significantly increased compared to those fed the LFLD and LFHD diets ($P < 0.05$). The GLU content of shrimp fed the LFLD and LFHD diets were significantly lower than those fed the FM and LF diets ($P < 0.05$). The TG content in the hemolymph of shrimp fed the LF diet was significantly lower than those fed the LFDH diet ($P < 0.05$), and the TG content of shrimp fed the LFLD diet was significantly lower than those fed the other diets ($P < 0.05$). AST and ALT activity in the hemolymph of shrimp fed the LF diet were significantly increased compared to those fed the other diets ($P < 0.05$). TC levels in the hemolymph of shrimp fed the FM diet were significantly increased compared to those fed the other diets ($P < 0.05$).

The expression levels of the lipid metabolism, apoptosis, and autophagy-related genes

Lipid metabolism-related gene expression levels were shown in Figure 1. The gene expression of *ampk* of shrimp fed the LFLD

TABLE 5 Effect of low fish meal diet supplemented with *Schizochytrium limacinum* on hemolymph biochemical parameters of *Litopenaeus vannamei*.

Index	FM	LF	LFLD	LFHD
HDL-C (mmol L ⁻¹)	0.40 ± 0.01 ^{bc}	0.32 ± 0.01 ^a	0.36 ± 0.02 ^{ab}	0.45 ± 0.01 ^c
LDL-C (mmol L ⁻¹)	1.72 ± 0.24 ^c	0.95 ± 0.05 ^b	0.45 ± 0.03 ^a	0.67 ± 0.10 ^a
GLU (mmol L ⁻¹)	1.60 ± 0.07 ^b	1.53 ± 0.03 ^b	1.24 ± 0.09 ^a	1.35 ± 0.06 ^a
TG (mmol L ⁻¹)	2.00 ± 0.22 ^{bc}	1.73 ± 0.11 ^b	1.23 ± 0.12 ^a	2.29 ± 0.24 ^c
AST (U L ⁻¹)	33.23 ± 1.25 ^c	36.96 ± 0.37 ^d	29.65 ± 0.12 ^b	26.92 ± 0.72 ^a
ALT (U L ⁻¹)	36.11 ± 0.11 ^a	44.18 ± 0.55 ^c	39.52 ± 0.41 ^b	35.17 ± 0.55 ^a
TC (mmol L ⁻¹)	1.84 ± 0.06 ^d	1.39 ± 0.13 ^c	0.66 ± 0.05 ^a	0.86 ± 0.14 ^b

Values in the table are the mean of four replicates of treatment and are expressed as mean ± SEM (n=4). Values in the same row with different superscript letters are significantly different (P<0.05).

Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*.

diet was significantly increased compared to those fed the FM and LFHD diets (P<0.05). The gene expression of *srbeq* was significantly higher in shrimp fed the LFLD and LFHD diets than those fed the FM and LF diets, which is decreased with the dietary *S. Limacinum* supplementation (P<0.05). The gene expression of *acc1* and *scd1* of shrimp fed the LFLD and LFHD diets were significantly increased compared to those fed the FM diet, which is decreased with the dietary *S. Limacinum* supplementation (P<0.05). The gene expression of *mcd* and *cpt-1* of shrimp fed the LFLD diet was significantly increased compared to those fed the FM and LFHD diets (P<0.05). The gene expression of *fas* of shrimp fed the LFLD and LFHD diets were significantly reduced compared to those fed the FM and LF diets (P<0.05).

Apoptosis and autophagy-related gene expression levels were shown in Figure 2. The gene expression of *bax* and *caspase 8* of shrimp fed the other three diets were significantly reduced compared to those fed the FM diet (P<0.05). The gene expression of *caspase 3* of shrimp fed the LF diet was significantly reduced compared to those fed the other three diets (P<0.05). The gene expression of *bcl-2* of shrimp fed the LF and LFHD diets were significantly reduced compared to those fed the FM diet (P<0.05). The gene expression of *beclin1* of shrimp fed the other three diets were significantly reduced compared to those fed the FM diet (P<0.05). The gene expression of *atg 12* of shrimp fed the LFLD and LFHD diets were significantly reduced compared to those fed the FM diet, which is decreased with the dietary *S. Limacinum* supplementation (P<0.05). The gene expression of *atg 13* of shrimp fed the LF and LFLD diets was significantly reduced compared to those fed the FM and LFHD diets (P<0.05).

Intestinal histology

The statistical results of the intestinal histology are shown in Table 6. The microvilli height, mucosal fold height, mucosal fold

width, and muscle layer thickness of shrimp fed the LF diet were significantly reduced compared to those fed the other diets (P<0.05). The mucosal fold height, mucosal fold width, and muscle layer thickness of shrimp fed the LFLD diet were higher than those fed the other diets. As shown in Figure 3, TEM results showed that with the decrease in dietary fish meal levels, the endoplasmic reticulum and mitochondria of shrimp fed the LF diet were found to be swollen, in which the mitochondrial matrix became irregularly arranged and tended to dissolve. After the supplementation of 0.3% *S. Limacinum* in the low fish meal diet, the endoplasmic reticulum recovered to its original state, but the mitochondria were irregular and the mitochondrial matrix was marginalized. After supplementation with 0.6% *S. Limacinum*, the mitochondria recovered to the level of fish meal.

Discussion

SPC has been proven as a high-quality protein source to replace fish meal protein in the feed of a wide range of aquatic animal species (Paripatananont et al., 2001; Chen et al., 2019; Zhang et al., 2022b). Several researches have pointed out that satisfactory growth and feed utilization were obtained in juvenile cobia (El-Saidy and Gaber, 2003) and juvenile starry flounder (Li et al., 2015) when dietary SPC inclusion was below 60%, and even further increased SPC content in the diet can cause lower dietary efficiency and higher death rates in fish. However, Zhao et al. showed that the SR and SGR of Nile tilapia were not affected even if the fish meal was totally replaced with SPC (Salze et al., 2010; Zhao et al., 2010). Earlier studies reported that it was possible to reduce the dietary fish meal content from 20% to 5% with SPC without negatively affecting the growth of *Litopenaeus vannamei* (Ray et al., 2020). The results of Paripatananont et al. showed that 50% substitution of fish meal with SPC in the diet of *Penaeus monodon* could support the normal growth of shrimp (Paripatananont et al., 2001), which was similar to the results of the current research. Results of this trial showed that the FBW of *Litopenaeus vannamei* was not influenced when the dietary fish meal content was reduced from 25% to 15%, after

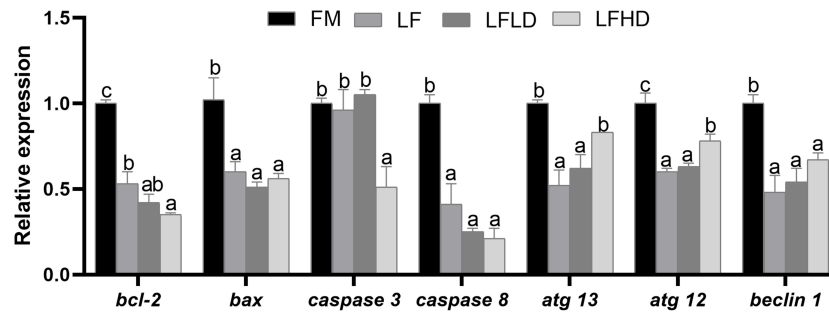


FIGURE 1 Effect of low fish meal diet supplemented with *Schizochytrium Limacinum* on the expression levels of hepatopancreas lipid metabolism-related genes in *Litopenaeus vannamei*. Vertical bars represent the mean \pm SEM (n = 5). Data marked with letters differ significantly (P < 0.05) among groups. Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*.

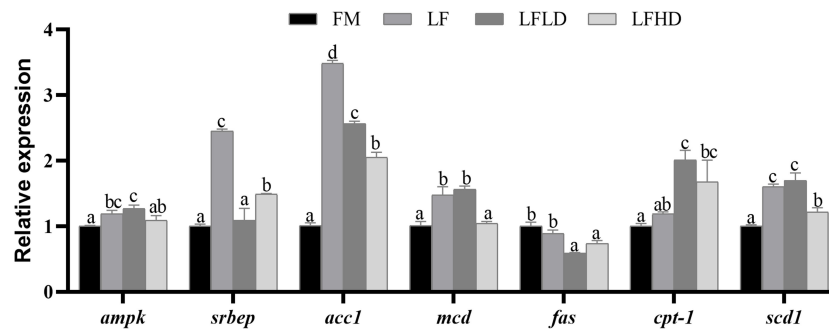


FIGURE 2 Effect of low fish meal diet supplemented with *Schizochytrium Limacinum* on the expression levels of hepatopancreas apoptosis and autophagy-related genes in *Litopenaeus vannamei*. Vertical bars represent the mean \pm SEM (n = 5). Data marked with letters differ significantly (P < 0.05) among groups. Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*.

TABLE 6 Effect of low fish meal diet supplemented with *Schizochytrium limacinum* on the intestinal tissues of *Litopenaeus vannamei*.

Parameters	Diets			
	FM	LF	LFLD	LFHD
Microvilli height (μm)	2.54 \pm 0.01 ^d	1.46 \pm 0.01 ^a	1.67 \pm 0.04 ^b	2.26 \pm 0.08 ^c
Mucosal folds height (μm)	57.45 \pm 3.68 ^b	41.60 \pm 2.73 ^a	89.91 \pm 4.35 ^c	76.01 \pm 6.42 ^c
Mucosal folds width (μm)	49.88 \pm 3.20 ^{bc}	36.18 \pm 1.92 ^a	53.18 \pm 1.06 ^c	45.76 \pm 1.45 ^b
Muscle layer thickness (μm)	56.21 \pm 3.21 ^b	36.80 \pm 2.09 ^a	83.47 \pm 2.86 ^c	78.71 \pm 9.51 ^c

Values in the table are the mean of six replicates of treatment and are expressed as mean \pm SEM (n=6). Values in the same row with different superscript letters are significantly different (P<0.05).

Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*.

amino acids, micronutrients, and fish oil was supplemented in the low fish meal diet to balance the nutritional profile. Several similar studies have also been done on *Epinephelus lanceolatus*, *Salmon*, and *Seriola rivoliana* (Ai et al., 2006; Perez-Velazquez et al., 2018; Katerina et al., 2020). Some studies reported that dietary

supplementation with 4% *S. Limacinum* improved the growth performance of *Litopenaeus vannamei* (Wang et al., 2016), 3% *S. Limacinum* improved the growth performance of *Trachinotus ovatus* (Xie et al., 2019). Interestingly, (Xie et al., 2020b) found that 0.75% *S. Limacinum* supplementation in a low fish meal diet

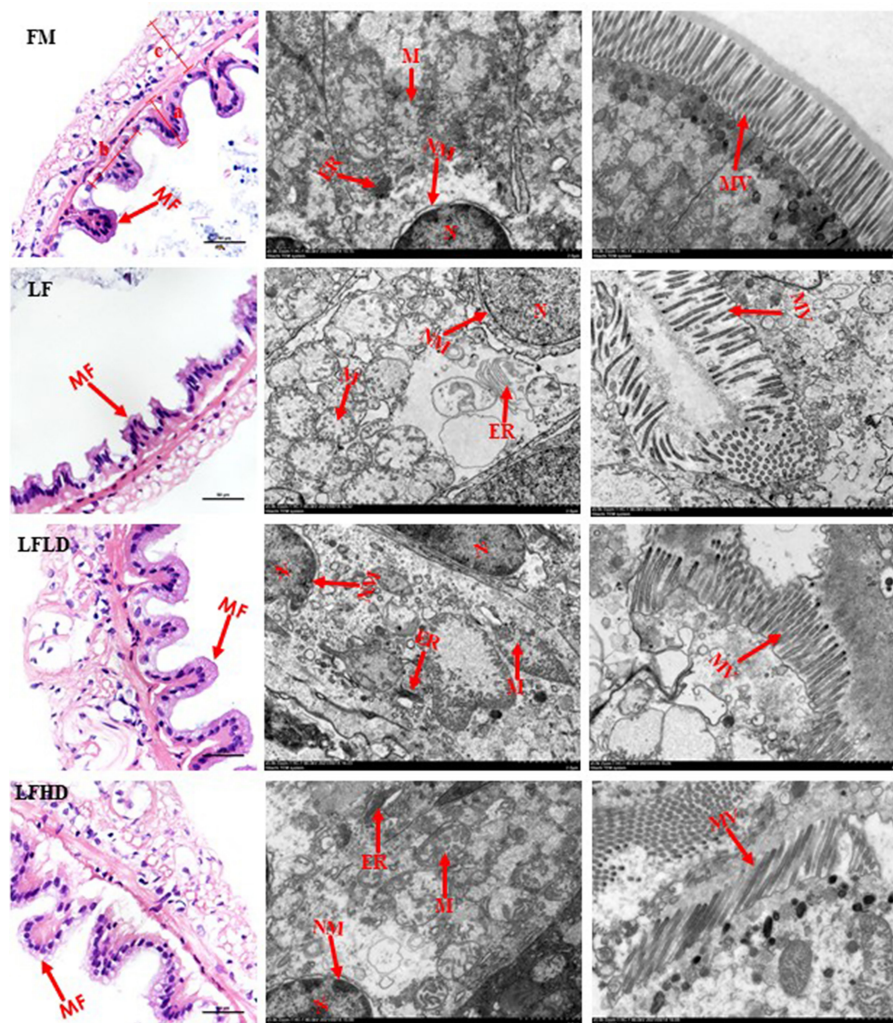


FIGURE 3

Effect of low fish meal diet supplemented with *Schizochytrium limacinum* on the histology of the midgut of *Litopenaeus vannamei*. Where: FM, high fish meal diet; LF, low fishmeal diet; LFLD, LF supplemented with 0.3% *S. Limacinum*; LFHD, LF supplemented with 0.6% *S. Limacinum*. MF, mucosal folds; a, width of mucosal folds; b, height of mucosal folds; c, thickness of the annular sarcolemma; MV, microvilli; ER, endoplasmic reticulum; N, nucleus; NM, nuclear membrane; M, mitochondria.

improved the growth performance of *Penaeus monodon*. High levels of nutrients in *S. Limacinum* such as DHA, docosapentaenoic acid (EPA), and carotenoids may contribute to the growth of animals (Xie et al., 2019). However, some studies have also found that supplementation with high levels of *S. Limacinum* is detrimental to the growth performance of *Litopenaeus vannamei* and blunt snout bream (Wang et al., 2016; Wang C. et al., 2020). Our results showed that the supplementation of 0.6% *S. Limacinum* had a positive effect on the FBW and WGR of shrimp.

Hemolymph is important for fat absorption and transport, and TG is transported as an energy substance between adipose tissue and the liver via hemolymph (Gyan et al., 2021). The fitness of aquatic animals which can be assessed using blood parameters

(Lemaire et al., 1991). Several studies have shown that SPC substitution for fish meal affects blood indicators (Zhang et al., 2019; Wang J. et al., 2020; Zhang Q. et al., 2021). TG and TC are important indexes of lipid sedimentation in animals. LDL-C is responsible for transporting liver cholesterol to tissue cells throughout the body, and HDL-C is responsible for transporting excess cholesterol from blood or tissues to the liver pancreas (Yepiz-Plascencia et al., 2000; Hamilton-Reeves et al., 2007). In the present trial, the levels of TC and TG in the hemolymph of *Litopenaeus vannamei* fed the LFLD diet were significantly reduced, and when the addition level of *S. Limacinum* increased to 0.6%, the levels of TC and TG increased significantly. Studies have shown that unsaturated fatty acids are effective in lowering hemolymph

cholesterol levels (Pentieva et al., 2003) and high levels of dietary unsaturated fatty acids prevent triglycerides from penetrating the lipoprotein particles of the liver, resulting in lower levels of TG secreted by hepatocytes into the hemolymph (Yu et al., 2012). According to (Zhang X. et al., 2021), diets supplemented with 0.5%–2.0% *S. Limacinum* significantly increased the hemolymph TG content of *Litopenaeus vannamei* and had no significant effect on the TC content, which was similar to the present study supplemented with 0.6% *S. Limacinum* but the TC content first decreased significantly and then increased. Hemolymph GLU is the most important energy substance in the hemolymph and is the direct source of energy required for all types of activities in aquatic animals (Boonanuntanasarn et al., 2016). Supplementation of *S. Limacinum* under the present experimental conditions decreased the hemolymph GLU content of shrimp, a result that is inconsistent with the results of studies on *Pelodiscus sinensis* (Zhang X. et al., 2021), possibly because of the species and possibly because of the enhanced energy consumption of the substance metabolism. HDL-C and LDL-C are able to be transported in shrimp for lipids (Yepiz-Plascencia et al., 2000). Whereas HDL-C removes TC from the blood and sedimentation in the liver, which is then excreted from the animal, LDL-C plays an important part in the immune system of shrimp (Yue et al., 2012; Chen et al., 2018). The current research showed a decrease in HDL-C and LDL-C levels and a significant decrease in HDL-C in the hemolymph of *Litopenaeus vannamei* fed the LF diet, which is different from the results hemolymph in *Micropterus salmoides* fed low-level fish meal diet supplemented with 4% *S. Limacinum* (Liao et al., 2022). Reasons for this may be the differences in diet composition, species, and amount of *S. Limacinum* supplementation. AST and ALT activity are important health parameters of liver function in invertebrates (Song et al., 2018). AST and ALT are the two most important transaminases in the body and are generally present in the liver (Zhou et al., 2013; Liu et al., 2019). When the liver is damaged, the AST and ALT stored in the liver will be transferred to the blood (Barcellos et al., 2004). In the current research, the AST and ALT activities in the hemolymph of *Litopenaeus vannamei* fed the LFLD and LFHD diets were significantly reduced, a result consistent with the fact that dietary supplementation with 0.8%–1.2% *S. Limacinum* reduced the blood AST/ALT ratio of *Cyprinus carpio* var. Jian (Liu et al., 2016) and dietary supplementation with 1% *S. Limacinum* reduced the AST and ALT activities in the blood of *Labidochromis caeruleus* (Cui et al., 2018). These results indicated that the supplementation of *S. Limacinum* in the diet can decrease liver injury in aquatic animals.

Due to the altered hemolymph biochemical parameters in shrimp, we further investigated the effect of low fish meal diet supplementation with *S. Limacinum* on the expression of genes related to apoptosis, lipid metabolism, and autophagy in *Litopenaeus vannamei*. Lipid metabolism refers to the process of fat synthesis and catabolism, the digestion of fat, which is subject to the action of a variety of enzymes and bile (Serrano et al., 2021; Su et al., 2022). Lipid metabolism is mainly in the

liver, and research has found that DHA can regulate the molecular mechanism of lipid metabolism and promote hepatocytes to stimulate the synthesis of lipoprotein lipase to further promote lipid metabolism (Morabito et al., 2019; Deragon et al., 2021). *Ampk* is a modulator of energy metabolism in cells. Under low energy regulation, *ampk* inhibits TG synthesis and activates the β -oxidation process of fatty acids to produce more ATP (Gaidhu et al., 2010; Wang et al., 2018; Xu et al., 2022b). The findings of this research revealed that the gene expression of *ampk* and *cpt-1* in shrimp fed the LF diet showed an increasing trend compared to those fed the FM diet but the gene expression of *ampk* and *cpt-1* increased and then decreased after supplementation with *S. Limacinum*, which indicated an increase in energy production from lipolysis. The gene expression of *fas* in shrimp fed the LFLD and LFHD diets was significantly lower than those fed the FM diet, and the gene expression of *acc1* and *cpt-1* in shrimp fed the LFLD and LFHD diets was significantly higher and then significantly lower than those fed the FM diet, suggesting a decrease in lipid synthesis after supplementation with *S. Limacinum*. The combined effect of lipid synthesis genes and lipolysis genes resulted in reduced lipid deposition. *Srebp* is a major regulator of cholesterol and fatty acids and a critical gene in lipid synthesis. (Eberle et al., 2004; Kamisuki et al., 2009). From the experimental results, shrimp fed the high SPC increased the expression of genes related to lipid synthesis, probably due to the ability of soy protein amphiphilic globulin to adsorb lipids (Lusas and Riaz, 1995). It may also be due to DHA's inherent property of lowering lipids (Horrocks and Yeo, 1999). Studies in human cardiovascular disease have found that DHA reduces total blood cholesterol and triglyceride levels (Mozaffarian and Wu, 2011), and in mice, DHA has been found to reduce the size of fat cells and lower body fat levels (Lu et al., 2015). Dietary supplementation with *S. Limacinum* decreased the expression of genes related to lipid synthesis, suggesting that *S. Limacinum* can reduce lipid synthesis. This is similar to the results of Zhu et al. (Zhu et al., 2013) which indicated that supplementation of *S. Limacinum* in the diet promoted lipid metabolism and inhibited fat deposition.

Apoptosis is divided into the endogenous mitochondrial pathway, the endoplasmic reticulum stress pathway, and the exogenous receptor apoptosis pathway (Sitarek et al., 2022). In the endogenous mitochondrial pathway, *bcl-2* family proteins (anti-apoptotic protein *bcl-2* and pro-apoptotic protein *bax*) control outer mitochondrial membrane permeability by regulating mitochondrial membrane potential (Green, 2022). *Bax* is normally found in the cytoplasm and when it receives an endogenous apoptotic signal, it relocates to the surface of mitochondria and constitutes a trans-mitochondrial membrane pore, which releases apoptotic factors. It has been suggested that the opening of the membrane pore causes a decrease in membrane potential and also leads to mitochondrial swelling and deformation (Chipuk et al., 2012).

Caspase 3 is also one of the downstream effectors of the endogenous pathway (Samali et al., 1999). It has been shown that a decrease in mitochondrial membrane potential leads to an upregulation of the *bax/bcl-2* ratio and promotes *caspase 3* activation (Zorova et al., 2018). In addition, *caspase 8* also activates *caspase 3* directly or indirectly in exogenous signal-regulated pathways (Bridgham et al., 2003). The results of the current research showed that supplementation with 0.6% *S. Limacinum* appeared to reduce the expression of *caspase 3* and thus inhibit the apoptosis in the hepatopancreas of shrimp; the expression of *caspase 8* was also significantly reduced and thus inhibit apoptosis. Dietary supplementation with 0.6% *S. Limacinum* activated the expression of *bcl-2* in the hepatopancreas of shrimp to further inhibit apoptosis. It has been suggested that autophagy eliminates damaged proteins and damaged organelles from the body (Tesseraud et al., 2021). Cellular autophagy is a highly conserved metabolic process that degrades its components through lysosomes to maintain cellular homeostasis and plays an important role in degrading damaged organelles, resisting pathogenic infections, and regulating inflammatory responses (Deretic, 2021). The process of cellular autophagy includes the formation of segmented membranes, the formation of autophagosomes, membrane fusion of autophagosomes with lysosomes, and the digestion of inclusions by autophagosomes (Cao et al., 2021). *Atg 13* and *beclin1* are participating in the startup of autophagic bubbles (Kabeya et al., 2005; Hosokawa et al., 2009), and *atg 12* is responsible for the formation of autophagosomes (Radoshevich et al., 2010). In this experiment, dietary supplementation of 0.6% *S. Limacinum* significantly increased hepatopancreas *atg 12* and *atg 13* gene expression in shrimp, and it is hypothesized that *S. Limacinum* has an effect of promoting autophagy. Researches have shown that hepatic autophagy promotes glycolipid metabolism and protein turnover (Song et al., 2010). Several studies have shown that autophagy is involved in intracellular nonspecific immune responses, that an appropriate numbers of autophagy is a self-protective function driving cell survival (Shi and Kehrl, 2008), and *S. Limacinum* could activate autophagy-related gene expression to improve the immune response in shrimp.

Intestinal morphology and structure are important for nutritional intake and sustain normal intestinal function (Gao et al., 2013; Vizcáino et al., 2014), and intestinal morphology is associated with shrimp health (Tang et al., 2009). The height of the mucosal fold and the width of the mucosal folds can be used as a measure of the functional characteristics of the intestinal walls. (Emami et al., 2012). Muscle layer thickness also plays an essential role in the metabolic digestion and absorptivity of the intestine, and increasing the thickness of the muscles can increase the absorptivity and metabolic digestion of nutrients in the intestine (Chen et al., 2021b). The results of this research showed that *S. Limacinum* supplementation in low fish meal diets improved the intestinal morphology and structure of *Litopenaeus vannamei*, with an

increase in mucosal folds length, mucosal folds width, and muscle layer thickness. Meanwhile, the TEM results showed that the intestinal microvilli height of shrimp fed the LFLD and LFHD diets were significantly increased compared to those fed the LF diet, indicating that dietary supplementation of *S. Limacinum* improved the intestinal microvilli structures. However, this result is contrary to previous studies, which showed that dietary supplementation with 1.2% *S. Limacinum* did not affect intestinal microvilli structure in Nile tilapia, possibly due to the different amounts of *S. Limacinum* supplementation and interspecific differences (Souza et al., 2020). The TEM results also showed that shrimp fed a LF diet showed endoplasmic reticulum stress, irregular mitochondrial ridges, and significant swelling in the intestine, which was improved when the diet was supplemented with *S. Limacinum*, and the intestinal mitochondria and endoplasmic reticulum recovered well when supplemented with 0.6% *S. Limacinum*. Earlier researches have demonstrated that reducing fish meal levels can damage the intestinal epithelial structures (Xie et al., 2018) and upregulate the gene expression associated with endoplasmic reticulum stress, which leads to the severity of endoplasmic reticulum stress in the intestine (Xie et al., 2020a). Some studies have reported that dietary supplementation with 3% and 6% defatted *S. Limacinum* can increase intestinal villi height, but excessive levels can impede nutrient absorption (Xiao et al., 2021). Supplementation with 0.75% *S. Limacinum* was found to improve intestinal health and enhance immunity in *Penaeus monodon* (Xie et al., 2020b). The low fish meal diet supplemented with 0.6% *S. Limacinum* in this study not only promoted the early development of the intestinal tract of *Litopenaeus vannamei* but also improved the digestive capacity of the intestine, improved the endoplasmic reticulum and mitochondrial structure, further improving the intestinal health and contributing to the healthy growth of shrimp.

Conclusion

The present study revealed that supplementation with 0.6% *S. Limacinum* in the low fish meal diet levels improved the growth performance, reduced hepatopancreatic cell apoptosis, promoted autophagy, and improved intestinal health in shrimp.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

XY and SX designed the experiments. XY carried out the experiments and drafted the manuscript. BT was accountable for

some aspects (such as ingredients and sites) of the work in ensuring that experiments can be carried out properly. YiL, MS, LC, KQ and YuL were analyzed for data. SX reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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References

- Ai, Q., Mai, K., Tan, B., Xu, W., Duan, Q., Ma, H., et al. (2006). Replacement of fish meal by meat and bone meal in diets for large yellow croaker, *Pseudosciaena crocea*. *Aquaculture* 260, 255–263. doi: 10.1016/j.aquaculture.2006.06.043
- An, W. Q., Li, W. W., Tan, B. P., Yang, Q. H., Dong, X. H., Liu, H. Y., et al. (2018). Optimum calcium and phosphorus supplemental levels in diets of large size *Litopenaeus vannamei*. *J. Guangdong Ocean Univ.* 38, 8–19. doi: 10.3969/j.issn.1673-9159.2018.04.002
- Barcellos, L. J. G., Kreutz, L. C., de Souza, C., Rodrigues, L. B., Fioreze, I., Quevedo, R. M., et al. (2004). Hematological changes in jundiá (*Rhamdia quelen* quoy and gaimard pimelodidae) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. *Aquaculture* 237, 229–236. doi: 10.1016/j.aquaculture.2004.03.026
- Boonantanasarn, S., Wongsasak, U., Pitaksong, T., and Chaijamrus, S. (2016). Effects of dietary supplementation with β -glucan and synbiotics on growth, haemolymph chemistry, and intestinal microbiota and morphology in the pacific white shrimp. *Aquaculture Nutr.* 22, 837–845. doi: 10.1111/anu.12302
- Bridgham, J. T., Wilder, J. A., Hollocher, H., and Johnson, A. L. (2003). All in the family: evolutionary and functional relationships among death receptors. *Cell Death Differ.* 10, 19–25. doi: 10.1038/sj.cdd.4401174
- Byreddy, A. R., Yoganantharajah, P., Gupta, A., Gibert, Y., and Puri, M. (2019). Suitability of novel algal biomass as fish feed: accumulation and distribution of omega-3 long-chain polyunsaturated fatty acid in zebrafish. *Appl. Biochem. Biotechnol.* 188, 112–123. doi: 10.1007/s12010-018-2906-0
- Campos, I., Matos, E., Marques, A., and Valente, L. M. P. (2017). Hydrolyzed feather meal as a partial fishmeal replacement in diets for European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture* 476, 152–159. doi: 10.1016/j.aquaculture.2017.04.024
- Cao, W., Li, J., Yang, K., and Cao, D. (2021). An overview of autophagy: Mechanism, regulation and research progress. *Bull. Cancer* 108, 304–322. doi: 10.1016/j.bulcan.2020.11.004
- Chen, Y., Chi, S., Zhang, S., Dong, X., Yang, Q., Liu, H., et al. (2021a). Effect of black soldier fly (*Hermetia illucens*) larvae meal on lipid and glucose metabolism of pacific white shrimp *Litopenaeus vannamei*. *Br. J. Nutr.* 128, 1–15. doi: 10.1017/S0007114521004670
- Chen, Y., Chi, S., Zhang, S., Dong, X., Yang, Q., Liu, H., et al. (2021b). Replacement of fish meal with methanotroph (*Methylococcus capsulatus*, bath) bacteria meal in the diets of pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture* 541, 736801. doi: 10.1016/j.aquaculture.2021.736801
- Chen, H., Liu, S., Xu, X. R., Diao, Z. H., Sun, K. F., Hao, Q. W., et al. (2018). Tissue distribution, bioaccumulation characteristics and health risk of antibiotics in cultured fish from a typical aquaculture area. *J. Hazard Mater.* 343, 140–148. doi: 10.1016/j.jhazmat.2017.09.017
- Chen, Y., Ma, J., Huang, H., and Zhong, H. (2019). Effects of the replacement of fishmeal by soy protein concentrate on growth performance, apparent digestibility, and retention of protein and amino acid in juvenile pearl gentian grouper. *PLoS One* 14, e0222780. doi: 10.1371/journal.pone.0222780
- Chipuk, J. E., McStay, G. P., Bharti, A., Kuwana, T., Clarke, C. J., Siskind, L. J., et al. (2012). Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell* 148, 988–1000. doi: 10.1016/j.cell.2012.01.038
- Chi, S., Zhong, J., He, Y., Li, J., and Luo, Y. (2017). Cloning and tissue expression of amino acid transporter B0AT1 gene in *Epinephelus coioides*. *J. Guangdong Ocean Univ.* 37, 1–6. doi: 10.3969/j.issn.1673-9159.2017.06.001
- Cho, J. H., and Kim, I. H. (2011). Fish meal-nutritive value. *J. Anim. Physiol. Anim. Nutr. (Berl)* 95, 685–692. doi: 10.1111/j.1439-0396.2010.01109.x
- Cui, P., Sheng, Y., Yang, Y., and Sun, J. (2018). Effects of dietary algae *Dunaliella salina*, *Schizochytrium limacinum* and *Chlorella vulgaris* on growth performance, body color and partial biochemical indices of *Labidochromis caeruleus*. *J. Dalian Ocean Univ.* 33, 6. doi: 10.16535/j.cnki.dlhyxb.2018.06.006
- Deragon, E., Schuler, M., Aiese Cigliano, R., Dellerio, Y., Si Larbi, G., Falconet, D., et al. (2021). An oil hyper-accumulator mutant highlights peroxisomal ATP import as a regulatory step for fatty acid metabolism in *aurantiochytrium limacinum*. *Cells* 10, 2680. doi: 10.3390/cells10102680
- Deretic, V. (2021). Autophagy in inflammation, infection, and immunometabolism. *Immunity* 54, 437–453. doi: 10.1016/j.immuni.2021.01.018
- Dyall, S. C., Balas, L., Bazan, N. G., Brenna, J. T., Chiang, N., da Costa Souza, F., et al. (2022). Polyunsaturated fatty acids and fatty acid-derived lipid mediators: Recent advances in the understanding of their biosynthesis, structures, and functions. *Prog. Lipid Res.* 86, 101165. doi: 10.1016/j.plipres.2022.101165
- Eberle, D., Hegarty, B., Bossard, P., Ferre, P., and Foulfelle, F. (2004). SREBP transcription factors: master regulators of lipid homeostasis. *Biochimie* 86, 839–848. doi: 10.1016/j.biochi.2004.09.018
- El-Saidy, D. M., and Gaber, M. M. (2003). Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia, *Oreochromis niloticus* (L.) diets. *Aquaculture Res.* 34, 1119–1127. doi: 10.1046/j.1365-2109.2003.00914.x

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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- Emami, N. K., Samie, A., Rahmani, H., and Ruiz-Feria, C. (2012). The effect of peppermint essential oil and fructooligosaccharides, as alternatives to virginiamycin, on growth performance, digestibility, gut morphology and immune response of male broilers. *Anim. Feed Sci. Technol.* 175, 57–64. doi: 10.1016/j.anifeedsci.2012.04.001
- FAO (2022). *The state of world fisheries and aquaculture 2022* (Rome: FAO).
- Feldsine, P., Abeyta, C., and Andrews, W. H. (2002). AOAC international methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. *J. AOAC Int.* 85, 1187–1200. doi: 10.1093/jaoac/85.5.1187
- Gaidhu, M. P., Anthony, N. M., Patel, P., Hawke, T. J., and Ceddia, R. B. (2010). Dysregulation of lipolysis and lipid metabolism in visceral and subcutaneous adipocytes by high-fat diet: role of ATGL, HSL, and AMPK. *Am. J. Physiol. Cell Physiol.* 298, C961–C971. doi: 10.1152/ajpcell.00547.2009
- Gao, Y., Han, F., Huang, X., Rong, Y., Yi, H., and Wang, Y. (2013). Changes in gut microbial populations, intestinal morphology, expression of tight junction proteins, and cytokine production between two pig breeds after challenge with *Escherichia coli* K88: a comparative study. *Journal of animal science* 91, 5614–5625. doi: 10.2527/jas.2013-6528
- García-Ortega, A., Kissinger, K. R., and Trushenski, J. T. (2016). Evaluation of fish meal and fish oil replacement by soybean protein and algal meal from *Schizochytrium limacinum* in diets for giant grouper *Epinephelus lanceolatus*. *Aquaculture* 452, 1–8. doi: 10.1016/j.aquaculture.2015.10.020
- Green, D. R. (2022). The mitochondrial pathway of apoptosis part II: The BCL-2 protein family. *Cold Spring Harb. Perspect. Biol.* 14, a041046. doi: 10.1101/cshperspect.a041046
- Gui, L. C., Mai, H. B., Chi, S. Y., Zhou, W. H., Yong-Juan, L. I., Tan, B. P., et al. (2019). Effects of yeast culture on growth performance, hematological parameters, immunity and disease resistance of *Litopenaeus vannamei*. *J. Guangdong Ocean Univ.* 39, 30–37. doi: 10.3969/j.issn.1673-9159.2019.03.005
- Gyan, W., Yang, Q., Tan, B., Xiaohui, D., Chi, S., Liu, H., et al. (2021). Effects of replacing fishmeal with dietary dried distillers grains with solubles on growth, serum biochemical indices, antioxidative functions, and disease resistance for *Litopenaeus vannamei* juveniles. *Aquaculture Rep.* 21, 100821. doi: 10.1016/j.aqrep.2021.100821
- Hamilton-Reeves, J. M., Rebello, S. A., Thomas, W., Slaton, J. W., and Kurzer, M. S. (2007). Isoflavone-rich soy protein isolate suppresses androgen receptor expression without altering estrogen receptor- β expression or serum hormonal profiles in men at high risk of prostate cancer. *J. Nutr.* 137, 1769–1775. doi: 10.1093/jn/137.7.1769
- He, Y. F., Chi, S. Y., Tan, B. P., Zhang, H. L., Dong, X. H., Yang, Q. H., et al. (2017). Effect of yeast culture on intestinal microbiota of *Litopenaeus vannamei*. *J. Guangdong Ocean Univ.* 37, 21–27. doi: 10.3969/j.issn.1673-9159.2017.04.004
- Hlrdzi, V., Wang, J., Kuebutornye, F. K. A., Yang, X., Tan, B., Li, T., et al. (2022). Hydrolysed fish protein powder is better at the growth performance, hepatopancreas and intestinal development of pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture Rep.* 23, 101025. doi: 10.1016/j.aqrep.2022.101025
- Horrocks, L. A., and Yeo, Y. K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacol. Res.* 40, 211–225. doi: 10.1006/phrs.1999.0495
- Hosokawa, N., Sasaki, T., Iemura, S., Natsume, T., Hara, T., and Mizushima, N. (2009). Atg101, a novel mammalian autophagy protein interacting with Atg13. *Autophagy* 5, 973–979. doi: 10.4161/auto.5.7.9296
- Huang, B., Zhang, S., Dong, X., Chi, S., Yang, Q., Liu, H., et al. (2022). Effects of fishmeal replacement by black soldier fly on growth performance, digestive enzyme activity, intestine morphology, intestinal flora and immune response of pearl gentian grouper (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂). *Fish Shellfish Immunol.* 120, 497–506. doi: 10.1016/j.fsi.2021.12.027
- Jannathulla, R., Rajaram, V., Kalanjiam, R., Ambasankar, K., Muralidhar, M., and Dayal, J. S. (2019). Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. *Aquaculture Res.* 50, 3493–3506. doi: 10.1111/are.14324
- Kabeya, Y., Kamada, Y., Baba, M., Takikawa, H., Sasaki, M., and Ohsumi, Y. (2005). Atg17 functions in cooperation with Atg1 and Atg13 in yeast autophagy. *Mol. Biol. Cell.* 16, 2544–2553. doi: 10.1091/mbc.e04-08-0669
- Kamisuki, S., Mao, Q., Abu-Elheiga, L., Gu, Z., Kugimiya, A., Kwon, Y., et al. (2009). A small molecule that blocks fat synthesis by inhibiting the activation of SREBP. *Chem. Biol.* 16, 882–892. doi: 10.1016/j.chembiol.2009.07.007
- Kari, Z. A., Kabir, M. A., Dawood, M. A. O., Razab, M. K. A. A., Ariff, N. S. N. A., Sarkar, T., et al. (2022). Effect of fish meal substitution with fermented soy pulp on growth performance, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*). *Aquaculture* 546, 737418. doi: 10.1016/j.aquaculture.2021.737418
- Katerina, K., Berge, G. M., Turid, M., Aleksei, K., Grete, B., Trine, Y., et al. (2020). Microalgal *Schizochytrium limacinum* biomass improves growth and filet quality when used long-term as a replacement for fish oil, in modern salmon diets. *frontiers in marine science*. *Frontiers in Marine Science* 7, 57. doi: 10.3389/fmars.2020.00057
- Kirimi, J. G., Musalia, L. M., and Munguti, J. M. (2016). Effect of replacing fish meal with blood meal on chemical composition of supplement for Nile tilapia (*Oreochromis niloticus*). *East Afr. Agric. Forestry J.* 82, 1–9. doi: 10.1080/00128325.2016.1158898
- Lemaire, P., Drai, P., Mathieu, A., Lemaire, S., Carriere, S., Giudicelli, J., et al. (1991). Changes with different diets in plasma enzymes (GOT, GPT, LDH, ALP) and plasma lipids (cholesterol, triglycerides) of sea-bass (*Dicentrarchus labrax*). *Aquaculture* 93, 63–75. doi: 10.1016/0044-8486(91)90205-L
- Liao, H., Liu, P., Deng, Y., Zhang, W., Pan, C., Jia, Y., et al. (2022). Feeding effects of low-level fish meal replacement by algal meals of *Schizochytrium limacinum* and *Nannochloropsis salina* on largemouth bass (*Micropterus salmoides*). *Aquaculture* 557, 738311. doi: 10.1016/j.aquaculture.2022.738311
- Li, Y., Huang, J., Chen, Y., Wen, Z., Ou, G., Huang, J., et al. (2022). Effects of low-temperature stress on serum biochemical, antioxidant enzymes activities and apoptosis-related gene expression in liver of juvenile cobia (*Rachycentron canadum*). *J. Guangdong Ocean Univ.* 42, 18–26. doi: 10.3969/j.issn.1673-9159.2022.05.003
- Li, W., Li, L., Liu, H., Tan, B., Dong, X., Yang, Q., et al. (2022). Effects of clostridium butyricum on growth, antioxidant capacity and non-specific immunology of *Litopenaeus vannamei* fed with concentrated cottonseed protein replacement of fishmeal. *J. Guangdong Ocean Univ.* 42, 29–37. doi: 10.3969/j.issn.1673-9159.2022.02.004
- Li, Z., Meng, T., Ling, X., Li, J., Zheng, C., Shi, Y., et al. (2018). Overexpression of malonyl-CoA:ACP transacylase in *Schizochytrium* sp. to improve polyunsaturated fatty acid production. *J. Agric. Food Chem.* 66, 5382–5391. doi: 10.1021/acs.jafc.8b01026
- Li, M. H., Robinson, E. H., Tucker, C. S., Manning, B. B., and Khoo, L. (2009). Effects of dried algae *Schizochytrium* sp., a rich source of docosahexaenoic acid, on growth, fatty acid composition, and sensory quality of channel catfish *Ictalurus punctatus*. *Aquaculture* 292, 232–236. doi: 10.1016/j.aquaculture.2009.04.033
- Liu, H., Tan, B., Ray, G., Zeng, M., Li, M., Chi, S., et al. (2022). A challenge to conventional fish meal: effects of soy protein peptides on growth, histomorphology, lipid metabolism and intestinal health for juvenile pompano *Trachinotus ovatus*. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.815323
- Liu, H., Cheng, Z., Zhai, S., Lin, C., Wang, J. E., Qiao, X., et al. (2016). Effects of dietary *Schizochytrium* on the growth performance, serum nonspecific immunity indices, and disease resistance of *Cyprinus carpio* var. *Jian*. *Prog. Fishery Sci.* 37, 42–28. doi: 10.11758/ykxjz.20151227001
- Liu, H., Li, L., Stephen, A., Tang, Z., Fan, W., Tan, B., et al. (2021). Effects of dietary yeast culture supplementation on growth, intestinal morphology, immunity, and disease resistance in *Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*. *J. Guangdong Ocean University.* 41, 1–11. doi: 10.3969/j.issn.1673-9159.2021.03.001
- Liu, T., Zhang, G., Feng, Y., Kong, C., Ayisi, C. L., Huang, X., et al. (2019). Dietary soybean antigen impairs growth and health through stress-induced non-specific immune responses in pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 84, 124–129. doi: 10.1016/j.fsi.2018.09.062
- Li, P. Y., Wang, J. Y., Song, Z. D., Zhang, L. M., Zhang, H., Li, X. X., et al. (2015). Evaluation of soy protein concentrate as a substitute for fishmeal in diets for juvenile starry flounder (*Platichthys stellatus*). *Aquaculture* 448, 578–585. doi: 10.1016/j.aquaculture.2015.05.049
- Lusas, E. W., and Riaz, M. N. (1995). Soy protein products: processing and use. *J. Nutr.* 125, 573S–580S. doi: 10.1093/jn/125.suppl_3.573S
- Lu, T., Zheng, Z., Zhang, J., Xue, M., and Ge, Y. (2015). The inhibitory effects of DHA on immune inflammatory obesity acta academiae medicinae QingDao universitatis. *Acta Academiae Medicinae QingDao Universitatis* 51, 445–447. doi: 10.13361/j.qdxy.201504023
- Lyons, P. P., Turnbull, J. F., Dawson, K. A., and Crumlish, M. (2016). Effects of low-level dietary microalgae supplementation on the distal intestinal microbiome of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Res.* 48, 2438–2452. doi: 10.1111/are.13080
- Morabito, C., Bournaud, C., Maes, C., Schuler, M., Aiese Cigliano, R., Dellerio, Y., et al. (2019). The lipid metabolism in thraustochytrids. *Prog. Lipid Res.* 76, 101007. doi: 10.1016/j.plipres.2019.101007
- Mozaffarian, D., and Wu, J. H. (2011). Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J. Am. Coll. Cardiol.* 58, 2047–2067. doi: 10.1016/j.jacc.2011.06.063
- Osmond, A. T. Y., Arts, M. T., Hall, J. R., Rise, M. L., Bazinet, R. P., Armenta, R. E., et al. (2021). *Schizochytrium* sp. (T18) oil as a fish oil replacement in diets for juvenile rainbow trout (*Oncorhynchus mykiss*): Effects on growth performance, tissue fatty acid content, and lipid-related transcript expression. *Anim. (Basel)* 11, 1185. doi: 10.3390/ani11041185

- Paripatanont, T., Boonyaratpalin, M., Pengseng, P., and Chotipuntu, P. (2001). Substitution of soy protein concentrate for fishmeal in diets of tiger shrimp *Penaeus monodon*. *Aquaculture Res.* 32, 369–374. doi: 10.1046/j.1355-557x.2001.00045.x
- Pentieva, K., McKillop, D., Duffy, N., de Deckere, E., Jacobs, R., van der Put, N., et al. (2003). Acute absorption of folic acid from a fortified low-fat spread. *Eur. J. Clin. Nutr.* 57, 1235–1241. doi: 10.1038/sj.ejcn.1601674
- Perez-Velazquez, M., Gatlin, D. M., González-Félix, M. L., and García-Ortega, A. (2018). Partial replacement of fishmeal and fish oil by algal meals in diets of red drum *Sciaenops ocellatus*. *Aquaculture* 487, 41–50. doi: 10.1016/j.aquaculture.2018.01.001
- Radoshevich, L., Murrow, L., Chen, N., Fernandez, E., Roy, S., Fung, C., et al. (2010). ATG12 conjugation to ATG3 regulates mitochondrial homeostasis and cell death. *Cell* 142, 590–600. doi: 10.1016/j.cell.2010.07.018
- Ray, G., Liang, D., Yang, Q., Tan, B., Dong, X., Chi, S., et al. (2020). Effects of replacing fishmeal with dietary soybean protein concentrate (SPC) on growth, serum biochemical indices, and antioxidative functions for juvenile shrimp *Litopenaeus vannamei*. *Aquaculture* 516, 734630. doi: 10.1016/j.aquaculture.2019.734630
- Ruyter, B., Bou, M., Berge, G. M., Mørkøre, T., Sissener, N. H., Sanden, M., et al. (2022). A dose-response study with omega-3 rich canola oil as a novel source of docosahexaenoic acid (DHA) in feed for Atlantic salmon (*Salmo salar*) in seawater; effects on performance, tissue fatty acid composition, and fillet quality. *Aquaculture* 561, 738733. doi: 10.1016/j.aquaculture.2022.738733
- Salze, G., McLean, E., Battle, P. R., Schwarz, M. H., and Craig, S. R. (2010). Use of soy protein concentrate and novel ingredients in the total elimination of fish meal and fish oil in diets for juvenile cobia, *Rachycentron canadum*. *Aquaculture* 298, 294–299. doi: 10.1016/j.aquaculture.2009.11.003
- Samali, A., Cai, J., Zhirovovskiy, B., Jones, D. P., and Orrenius, S. (1999). Presence of a pro-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. *EMBO J.* 18, 2040–2048. doi: 10.1093/emboj/18.8.2040
- Sarker, P. K., Kapuscinski, A. R., Lanois, A. J., Livesey, E. D., Bernhard, K. P., and Coley, M. L. (2016). Towards sustainable aquafeeds: Complete substitution of fish oil with marine microalga schizochytrium sp. improves growth and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). *PLoS One* 11, e0156684. doi: 10.1371/journal.pone.0156684
- Serrano, E., Simpfendorfer, R., Medina, A., Sandoval, C., Martínez, A., Morales, R., et al. (2021). Partially replacing fish oil with microalgae (*Schizochytrium limacinum* and *Nannochloropsis oceanica*) in diets for rainbow trout (*Oncorhynchus mykiss*) reared in saltwater with reference to growth performance, muscle fatty acid composition and liver ultrastructure. *Aquaculture Res.* 52, 4401–4413. doi: 10.1111/are.15279
- Shi, C., and Kehrl, J. H. (2008). MyD88 and trif target beclin 1 to trigger autophagy in macrophages. *J. Biol. Chem.* 283, 33175–33182. doi: 10.1074/jbc.M804478200
- Sitarek, P., Synowicz, E., Kowalczyk, T., Bangay, G., Sliwinski, T., Picot, L., et al. (2022). Anticancer properties of *Plectranthus ornatus*-derived phytochemicals inducing apoptosis via mitochondrial pathway. *Int. J. Mol. Sci.* 23, 11653. doi: 10.3390/ijms231911653
- Song, L., Hu, B., Wang, A., Mao, S., Zhang, Y., and Pan, X. (2010). Effects of antibacterial peptide on growth and immunity of *penaeus vannamei*. *J. Guangdong Ocean University.* 30, 28–32.
- Song, Z., Li, P., Wang, J., Sun, Y., and Wang, C. (2018). Dietary inclusion of hydrolyzed soybean and cottonseed meals influence digestion, metabolic enzymes, and growth-related hormones and growth of juvenile turbot (*Scophthalmus maximus*). *Aquaculture Int.* 26, 1017–1033. doi: 10.1007/s10499-018-0265-z
- Souza, F. P., Lima, E. C. S., Urrea-Rojas, A. M., Suphoronski, S. A., Facimoto, C. T., Bezerra Junior, J. D. S., et al. (2020). Effects of dietary supplementation with a microalga (*Schizochytrium* sp.) on the hemato-immunological, and intestinal histological parameters and gut microbiota of Nile tilapia in net cages. *PLoS One* 15, e0226977. doi: 10.1371/journal.pone.0226977
- Sprague, M., Walton, J., Campbell, P. J., Strachan, F., Dick, J. R., and Bell, J. G. (2015). Replacement of fish oil with a DHA-rich algal meal derived from schizochytrium sp. on the fatty acid and persistent organic pollutant levels in diets and flesh of Atlantic salmon (*Salmo salar*, L) post-smolts. *Food Chem.* 185, 413–421. doi: 10.1016/j.foodchem.2015.03.150
- Su, C., Li, J., Lu, Y., Wang, Y., Ding, Y., Pan, L., et al. (2022). Interactive effects of dietary cholesterol and bile acids on the growth, lipid metabolism, immune response and intestinal microbiota of *Litopenaeus vannamei*: Sparing effect of bile acids on cholesterol in shrimp diets. *Aquaculture*, 547, 737412. doi: 10.1016/j.aquaculture.2021.737412
- Tang, L., Wang, G. X., Jiang, J., Feng, L., Yang, L., Li, S. H., et al. (2009). Effect of methionine on intestinal enzymes activities, microflora and humoral immune of juvenile jian carp (*Cyprinus carpio* var. jian). *Aquaculture Nutr.* 15, 477–483. doi: 10.1111/j.1365-2095.2008.00613.x
- Tesseraud, S., Avril, P., Bonnet, M., Bonnieu, A., Cassar-Malek, I., Chabi, B., et al. (2021). Autophagy in farm animals: Current knowledge and future challenges. *Autophagy* 17, 1809–1827. doi: 10.1080/15548627.2020.1798064
- Vizcaino, A., López, G., Sáez, M., Jiménez, J., Barros, A., Hidalgo, L., et al. (2014). Effects of the microalga *scenedesmus almeriensis* as fishmeal alternative in diets for gilthead sea bream, *Sparus aurata*, juveniles. *Aquaculture* 431, 34–43. doi: 10.1016/j.aquaculture.2014.05.010
- Wang, J., Liang, D., Yang, Q., Tan, B., Dong, X., Chi, S., et al. (2020). The effect of partial replacement of fish meal by soy protein concentrate on growth performance, immune responses, gut morphology and intestinal inflammation for juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ x *Epinephelus lanceolatus* ♂). *Fish Shellfish Immunol.* 98, 619–631. doi: 10.1016/j.fsi.2019.10.025
- Wang, Y., Li, M., Filer, K., Xue, Y., Ai, Q., and Mai, K. (2016). Evaluation of *Schizochytrium* meal in microdiets of pacific white shrimp (*Litopenaeus vannamei*) larvae. *Aquaculture Res.* 48, 2328–2336. doi: 10.1111/are.13068
- Wang, Y., Li, M., Filer, K., Xue, Y., Ai, Q., and Mai, K. (2017). Replacement of fish oil with a DHA-rich *Schizochytrium* meal on growth performance, activities of digestive enzyme and fatty acid profile of pacific white shrimp (*Litopenaeus vannamei*) larvae. *Aquaculture Nutr.* 23, 1113–1120. doi: 10.1111/anu.12479
- Wang, C., Liu, W.-b., Huang, Y., Wang, X., Li, X., Zhang, D., et al. (2020). Dietary DHA affects muscle fiber development by activating AMPK/Sirt1 pathway in blunt snout bream (*Megalobrama amblycephala*). *Aquaculture* 518, 734835. doi: 10.1016/j.aquaculture.2019.734835
- Wang, Q., Liu, S., Zhai, A., Zhang, B., and Tian, G. (2018). AMPK-mediated regulation of lipid metabolism by phosphorylation. *Biol. Pharm. Bull.* 41, 985–993. doi: 10.1248/bpb.17-00724
- Wang, L., Zhou, Q., Ma, Q., Yang, Y., Yang, E., Chen, G., et al. (2022). Effects of salinity acclimation on expression of MMP2 and MMP9 in regenerated scales of young rainbow trout (*Oncorhynchus mykiss*). *J. Guangdong Ocean Univ.* 42, 110–115. doi: 10.3969/j.issn.1673-9159.2022.05.014
- Wu, Y., Li, R., Shen, G., Huang, F., Yang, Q., Tan, B., et al. (2021). Effects of dietary small peptides on growth, antioxidant capacity, nonspecific immunity and ingut microflora structure of *litopenaeus vannamei*. *J. Guangdong Ocean Univ.* 41, 1–9. doi: 10.3969/j.issn.1673-9159.2021.05.001
- Xiao, F., Xing, J., Li, H., Xu, X., Hu, Z., and Ji, H. (2021). Effects of the defatted schizochytrium sp. on growth performance, fatty acid composition, histomorphology and antioxidant status of juvenile mirror carp (*Cyprinus carpio* var. *specularis*). *Aquaculture Res.* 52, 3062–3076. doi: 10.1111/are.15150
- Xie, J., Fang, H., Liao, S., Guo, T., Yin, P., Liu, Y., et al. (2019). Study on schizochytrium sp. improving the growth performance and non-specific immunity of golden pompano (*Trachinotus ovatus*) while not affecting the antioxidant capacity. *Fish Shellfish Immunol.* 95, 617–623. doi: 10.1016/j.fsi.2019.10.028
- Xie, S., Liu, Y., Tian, L., Niu, J., and Tan, B. (2020a). Low dietary fish meal induced endoplasmic reticulum stress and impaired phospholipids metabolism in juvenile pacific white shrimp, *Litopenaeus vannamei*. *Front. Physiol.* 11. doi: 10.3389/fphys.2020.01024
- Xie, S., Liu, Y., Zeng, S., Niu, J., and Tian, L. (2016). Partial replacement of fish-meal by soy protein concentrate and soybean meal based protein blend for juvenile pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 464, 296–302. doi: 10.1016/j.aquaculture.2016.07.002
- Xie, S., Wei, D., Chen, S., Zhuang, Z., Yin, P., Liu, Y., et al. (2020c). Dietary fishmeal levels affect anti-oxidative ability and metabolomics profile of juvenile pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutr.* 26, 978–989. doi: 10.1111/anu.13055
- Xie, S., Wei, D., Tan, B., Liu, Y., Tian, L., and Niu, J. (2020b). Schizochytrium limacinum supplementation in a low fish-meal diet improved immune response and intestinal health of juvenile *Penaeus monodon*. *Front. Physiol.* 11. doi: 10.3389/fphys.2020.00613
- Xie, S., Zheng, L., Wan, M., Niu, J., Liu, Y., and Tian, L. (2018). Effect of deoxyvalenol on growth performance, histological morphology, anti-oxidative ability and immune response of juvenile pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 82, 442–452. doi: 10.1016/j.fsi.2018.08.053
- Xu, D., Wu, J., Sun, L., Qin, X., and Fan, X. (2021). Comparison on anesthetic efficacy of eugenol and MS-222 on shrimp *Litopenaeus vannamei*. *J. Guangdong Ocean Univ.* 41, 44–52. doi: 10.3969/j.issn.1673-9159.2021.05.006
- Xu, D., Wu, J., Sun, L., Qin, X., and Fan, X. (2022a). Physiological response and metabolic regulation of *Litopenaeus vannamei* exposed to combination stress of acute cold exposure and chronic waterless duration. *J. Guangdong Ocean Univ.* 42, 20–28. doi: 10.3969/j.issn.1673-9159.2022.01.004
- Xu, J., Xie, S., Chi, S., Zhang, S., Cao, J., and Tan, B. (2022b). Short-term dietary antibiotics altered the intestinal microbiota and improved the lipid metabolism in hybrid grouper fed medium and high-lipid diets. *Aquaculture*, 547, 737453. doi: 10.1016/j.aquaculture.2021.737453
- Xu, J., Yao, X., Li, X., Xie, S., Chi, S., Zhang, S., et al. (2022c). Bile acids reduced the lipid deposition in fatty degenerated hepatocytes of pearl gentian grouper

(*Epinephelus fuscoguttatus* ♂ × *Epinephelus lanceolatus* ♀) in vitro. *Front. Mar. Science*. 9. doi: 10.3389/fmars.2022.861117

Yepiz-Plascencia, G., Vargas-Albores, F., and Higuera-Ciajara, I. (2000). Penaeid shrimp hemolymph lipoproteins. *Aquaculture* 191, 177–189. doi: 10.1016/S0044-8486(00)00427-0

Yin, X., Wei, W., Zhuang, X., Li, Z., Liu, C., Ou, M., et al. (2021). Determining the function of LvSmad3 on *Litopenaeus vannamei* in response to acute low temperature stress. *Dev. Comp. Immunol.* 125, 104209. doi: 10.1016/j.dci.2021.104209

Yue, Y., Liu, Y., Tian, L., Gan, L., Yang, H., and Liang, G. (2012). Effects of replacing fish meal with soybean meal and peanut meal on growth, feed utilization and haemolymph indexes for juvenile white shrimp *Litopenaeus vannamei*, Boone. *Aquaculture Res.* 43, 1687–1696. doi: 10.1111/j.1365-2109.2011.02976.x

Yu, Y., Jiang, S., Lin, H., Wang, Y., and Wang, A. (2012). Effects of dietary lipid sources on the morphology and biochemical index of carassius auratus gibelio. *J. Human Agric. Univ.* 38, 192–197. doi: 10.3724/SP.J.1238.2012.00192

Zhang, W., Tan, B., Deng, J., Yang, Q., Chi, S., Pang, A., et al. (2022b). Soybean protein concentrate causes enteritis in juvenile pearl gentian groupers (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *Anim. Nutr.* doi: 10.1016/j.aninu.2022.08.006

Zhang, W., Tan, B., Pang, A., Deng, J., Yang, Q., and Zhang, H. (2022a). Screening of potential biomarkers for soybean meal induced enteritis in pearl gentian grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). *J. Guangdong Ocean Univ.* 42, 1–12. doi: 10.3969/j.issn.1673-9159.2022.04.001

Zhang, H. T., Xue-Song, P. U., Yang, Q. H., Tan, B. P., Dong, X. H., Chi, S. Y., et al. (2018). Effects of replacing fish meal with high-protein cottonseed meal on growth performance, non-specific immune index and disease resistance for *litopenaeus vannamei*. *J. Guangdong Ocean Univ.* 38, 20–26. doi: 10.3969/j.issn.1673-9159.2018.04.003

Zhang, Q., Zhang, S., Cong, G., Zhang, Y., Madsen, M. H., Tan, B., et al. (2021). Effects of soy protein concentrate in starter phase diet on growth performance, blood biochemical indices, carcass traits, immune organ indices and meat quality of broilers. *Anim. (Basel)* 11, 281. doi: 10.3390/ani11020281

Zhang, X., Zhao, L., and Hu, X. (2021). Progress on nutritional value of *Schizochytrium limacinum* and its application in animal production. *J. Henan Agric. Sci.* 50, 1–10. doi: 10.15933/j.cnki.1004-3268.2021.03.001

Zhang, J., Zhong, L., Peng, M., Chu, W., Liu, Z., Dai, Z., et al. (2019). Replacement of fish meal with soy protein concentrate in diet of juvenile rice field eel *Monopterus albus*. *Aquaculture Rep.* 15, 100235. doi: 10.1016/j.aqrep.2019.100235

Zhao, H., Jiang, R., Xue, M., Xie, S., Wu, X., and Guo, L. (2010). Fishmeal can be completely replaced by soy protein concentrate by increasing feeding frequency in Nile tilapia (*Oreochromis niloticus* GIFT strain) less than 2 g. *Aquaculture Nutr.* 16, 648–653. doi: 10.1111/j.1365-2095.2009.00708.x

Zhao, W., Liu, Z., and Niu, J. (2021). Growth performance, intestinal histomorphology, body composition, hematological and antioxidant parameters of *Oncorhynchus mykiss* were not detrimentally affected by replacement of fish meal with concentrated dephenolization cottonseed protein. *Aquaculture Rep.* 19, 100557. doi: 10.1016/j.aqrep.2020.100557

Zhou, W., Rahimnejad, S., Lu, K., Wang, L., and Liu, W. (2019). Effects of berberine on growth, liver histology, and expression of lipid-related genes in blunt snout bream (*Megalobrama amblycephala*) fed high-fat diets. *Fish Physiol. Biochem.* 45, 83–91. doi: 10.1007/s10695-018-0536-7

Zhou, Q., Wang, Y., Wang, H., and Tan, B. (2013). Dietary threonine requirements of juvenile pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 392, 142–147. doi: 10.1016/j.aquaculture.2013.01.026

Zhu, X., Deng, Q., Guo, H., Li, G., and Zhu, C. (2021). Effect of hydrolyzable tannins on hemolymph and cellular immunological responses of *Litopenaeus vannamei* challenged by vibrio parahaemolyticus. *J. Guangdong Ocean Univ.* 41, 12–19. doi: 10.3969/j.issn.1673-9159.2021.03.002

Zhu, C., Jiang, B., and Zhou, Q. (2013). The effects of different levels of EPA and DHA on lipids metabolism, growth and reproductive performance of rice field eel (*Monopterus albus*). *Acta Hydrobiologica Sin.* 37, 648–655. doi: 10.7541/2013.76

Zhu, R., Li, L., Li, M., Yu, Z., Wang, H., and Wu, L. (2020). The effects of substituting fish meal with soy protein concentrate on growth performance, antioxidant capacity and intestinal histology in juvenile golden crucian carp, *Cyprinus carpio* × *Carassius auratus*. *Aquaculture Rep.* 18, 100435. doi: 10.1016/j.aqrep.2020.100435

Zorova, L. D., Popkov, V. A., Plotnikov, E. Y., Silachev, D. N., Pevzner, I. B., Jankauskas, S. S., et al. (2018). Mitochondrial membrane potential. *Anal. Biochem.* 552, 50–59. doi: 10.1016/j.ab.2017.07.009