Aquaculture Reports 36 (2024) 102072

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Partial replacement of fish meal by enzymatically hydrolyzed soybean does not adversely impact the growth performance, antioxidant capacity, immunity and intestinal health of the juvenile *Eriocheir sinensis*

Qincheng Huang [a,b,1], Yixin Miao [a,1], Jiadai Liu [a], Han Wang [a], Chuanjie Qin [c], Xiaodan Wang [a], Erchao Li [a,*], Jianguang Qin [d], Liqiao Chen [a,*]

[a] *Laboratory of Aquaculture Nutrition and Environmental Health, School of Life Sciences, East China Normal University, Shanghai 200241, PR China*
[b] *Xianghu Laboratory, Hangzhou 311231, PR China*
[c] *Key Laboratory of Sichuan Province for Fishes Conservation and Utilization in the Upper Reaches of the Yangtze River, Neijiang Normal University, Sichuan 641100, PR China*
[d] *College of Science and Engineering, Flinders University, Adelaide, SA 5001, Australia*

A R T I C L E I N F O

Keywords:
Enzymatic hydrolysis of soybean
Eriocheir sinensis
Antioxidant capacity
Immunity
Intestinal health

A B S T R A C T

An 8-week experiment was conducted to study the effect of dietary enzymatic hydrolysis of full-fat soybean (ESB) in combination with fish meal (FM) on the growth performance, oxidation resistance, immunity and intestinal health of juvenile Chinese mitten crab *Eriocheir sinensis*. The crabs (0.74±0.01 g) were randomly divided into six groups. Six diets were formulated by replacing 0, 15, 30, 45, 60 and 75% of FM (350 g/kg) with ESB (ESB0-control, ESB15, ESB30, ESB45, ESB60 and ESB75, respectively). The weight gain rate and specific gain rate of the crabs were significantly greater in the ESB15 group than in the control group, while they decreased significantly with replacement levels up to 60%. A replacement level of 15% significantly improved the hepatopancreas antioxidant capacity and hemolymph acid phosphatase indices. However, the glutathione peroxidase activity and total antioxidant capacity in the hepatopancreas decreased significantly when the replacement concentration reached 45% and 75%, respectively. The replacement level could increase to 45% without affecting the hepatopancreas malondialdehyde content. Hemolymph acid phosphatase and alkaline phosphatase activities decreased significantly with replacement levels up to 60%. Aspartate aminotransferase activity in the hemolymph increased significantly when the replacement dose reached 75%. Moreover, different replacement levels did not affect the gene expression of the hepatopancreas *ALF1/2*, *crustin*, *relish*, or *LITAF*. The hepatic mRNA levels of *TLR2* and *Myd88* were significantly elevated in the ESB45 and ESB60 groups, respectively. Unlike those in the ESB0 group, intestinal *PM1* and *PM2* expressions were significantly higher in the ESB15 group. In contrast, intestinal PM1, *PM2* and *PT* expressions were significantly lower in the ESB60 and ESB75 groups. The present study suggests that 15% of the optimal replacement level of FM with ESB enhanced growth performance, antioxidation, immunity and intestinal health. To maintain normal growth performance, the replacement level of FM with ESB could not exceed 45%.

1. Introduction

Because of the ideal protein content, balanced amino acids and good palatability, fish meal (FM) is widely used in animal feeds (Tacon, 2004). With the development of the aquaculture industry and the increasing scarcity of marine resources, the FM price has increased annually. Explaining a new protein source is imperative to partially substitute FM in feed (Mugwanya et al., 2023). Compared with FM, vegetable protein sources are widely used to replace FM because of the advantages of high yield, low price, and good sustainability (Nathaly et al., 2019). Soybean protein-related products have been widely used in aquatic animals because of their high protein content and relative balance pattern of amino acids and composition (Gemede and Ratta, 2014; Jing et al., 2021). However, replacing FM with excess soybean protein

* Corresponding authors.
E-mail addresses: ecli@bio.ecnu.edu.cn (E. Li), lqchen@bio.ecnu.edu.cn (L. Chen).
[1] Contributed equally to the work.

https://doi.org/10.1016/j.aqrep.2024.102072
Received 22 January 2024; Received in revised form 11 March 2024; Accepted 4 April 2024
Available online 9 April 2024
2352-5134/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC license (http://creativecommons.org/licenses/by-nc/4.0/).

has negatively affected growth, immune function and intestinal health in aquatic animals (Francis et al., 2001). For instance, high soybean meal levels can reduce the weight gain rate of the cobia *Rachycentron canadum* (Chou et al., 2004) and the intestinal digestive enzyme activities of the Chinese sucker *Myxocyprinus asiaticus* (Yu et al., 2013). Antinutritional factors limit the application of soy protein, as they can reduce the palatability of feed and affect the intestinal health of animals (Krogdahl et al., 2003, 2010). Therefore, it is important to find a way to eliminate the adverse effects of antinutritional factors to improve the utilization of soybean protein.

Proteolysis technology degrades most of the antinutritional factors in soybean protein and has been applied to increase the level of plant protein in aquafeed (Li et al., 2015; Samtiya et al., 2020). Enzyme-treated full-fat soybean (ESB) through enzymatic hydrolysis technology contains various small peptides and emulsified fat and can be easily digested and absorbed (Liu et al., 2021). An in vitro experiment on macrophages indicates that soybean peptides can function as immunomodulatory neutraceuticals (Wen et al., 2021). In aquatic animals, ESB is a potential substitute for FM. For example, the optimal replacement of dietary FM with ESB can improve the serum immune indices and intestinal histomorphology of the largemouth bass *Micropterus salmoides* (Liu et al., 2021). Replacing up to 70% of dietary FM protein with soy protein hydrolysates does not harm growth or feed efficiency in the juvenile starry flounder *Platichthys stellatus* (Song et al., 2014). However, substituting FM with ESB of more than 75% upregulated proinflammatory-related gene expression in the digestive gland and negatively affected the intestinal microbiota in the abalone *Haliotis discus hannai* (Yu et al., 2022). Therefore, it is crucial to determine the proper replacement level of FM by ESB in aquatic animals. To date, little information about the application of ESB in crustaceans is available, except the report that 48% of dietary FM replaced by ECB did not affect the growth or survival rate of the Pacific white shrimp *Litopenaeus vannamei* after pathogen challenge (Fan et al., 2021).

The Chinese mitten crab *Eriocheir sinensis* (*E. sinensis*) represents a crustacean species in aquaculture. Approximately 820,000 tons of *E. sinensis* were produced in China in 2022 (China Fishery Statistical Yearbook, 2023). Generally, the FM level can reach 320 g/kg in feeds for *E. sinensis* (Jiang et al., 2023), and ESB application in aquafeed has great potential. Thus, the present study aimed to evaluate the effects of graded replacement of dietary FM with ESB on the growth, immunity, antioxidant activity, and intestinal health of juvenile *E. sinensis*. These findings could contribute to using ESB in aquafeed to promote the sustainable development of aquaculture.

2. Materials and methods

2.1. Experimental diets

Gelatin, casein, FM and ESB were used as the main protein sources, and mixed oil (fish oil: soybean oil=1:1) cholesterol and phospholipids were used as the main fat sources. The FM concentration in the control group was 35% (i.e., 350 g/kg of diet based on Jiang et al., 2023), and ESB was used to replace 0% (ESB0, control group), 15% (ESB15, 297.5 g/kg), 30% (ESB30, 245.0 g/kg), 45% (ESB45, 192.5 g/kg), 60% (ESB60, 140.0 g/kg) and 75% (ESB75, 87.5 g/kg) of the FM protein based on previous studies by Song et al., (2014) and Yu et al., (2022) (Table 1). All the ingredients were crushed, ground, and filtered through an 80-mesh sieve. The different ingredients were weighed, thoroughly mixed with oil and pure water and finally extruded into long strips with a diameter of 2.5 mm using a double-screw laminator (F-26, SCUT Industrial Factory, Guangzhou, China). The feeds were packed and marked after air-drying to a moisture content <10% (detected through a rapid moisture meter, PRUISTE, DHA-16B120). All the feeds were stored at −20 °C until use.

Table 1
Formulation and proximate composition of the six different experimental diets (on a g/kg dry basis).

Ingredients	Content					
	ESB0	ESB15	ESB30	ESB45	ESB60	ESB75
FM	350.0	297.5	245.0	192.5	140.0	87.5
ESB[a]	0.0	95.0	190.0	285.0	380.5	475.5
Casein	120.0	120.0	120.0	120.0	120.0	120.0
Gelatin	40.0	40.0	40.0	40.0	40.0	40.0
Fish oil: soybean meal (1: 1)	78.0	62.5	47.0	31.5	15.5	0.0
Lecithin[b]	10.0	10.0	10.0	10.0	10.0	10.0
Cholesterol[c]	5.0	5.0	5.0	5.0	5.0	5.0
Choline chloride[c]	5.0	5.0	5.0	5.0	5.0	5.0
Butylated hydroxytoluene[c]	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[d]	30.0	30.0	30.0	30.0	30.0	30.0
Mineral premix[e]	20.0	20.0	20.0	20.0	20.0	20.0
Sodium alginate[c]	20.0	20.0	20.0	20.0	20.0	20.0
cellulose	141.0	114.0	87.0	60.0	33.0	6.0
α- starch	180.0	180.0	180.0	180.0	180.0	180.0
Proximate composition (%)						
Crude protein	40.71	41.22	41.15	41.26	41.23	40.23
Crude lipid	10.03	9.77	10.30	10.21	10.48	10.42
Moisture	8.67	8.52	8.64	8.81	8.83	8.63
Ash	7.56	7.49	7.44	7.55	7.45	7.47

[a] . ESB: Enzymatically hydrolyzed full-fat soybeans (crude protein 37%, crude lipid 20%; Jiangsu Fuhai Biotechnology Co., Ltd.).
[b] . Shanghai Taiwei, Ltd., Shanghai, China.
[c] . Sangon Biotech, Ltd., Shanghai, China.
[d] . the same as Han et al., (2020)
[e] . the same as Huang et al., (2022).

2.2. Animals and feeding management

The experiment was carried out at the Zhejiang Freshwater Fisheries Research Institute (Huzhou, Zhejiang). The crabs were purchased from a local aquafarm in Chongming, Shanghai. Before the formal experiment, the crabs were temporarily cultured in a square plastic pool for two weeks. Then, 960 crabs (initial weight 0.74 ± 0.01 g) were randomly divided into 24 white polyethylene plastic buckets (300 L). Four replicated buckets were used in each treatment, and 40 crabs were placed in each bucket. Each bucket contained the same number of arch tiles and bundles of avoidance tubes to shelter the crabs. The experimental water was filtered through a filtration system and fully aerated before use. The trial lasted eight weeks, and the daily feeding amount was 4% of the body weight. The feed residue and feces were siphoned out every morning, and the amount of water exchanged was 2/3 that of the aquaculture water. During the test, the water temperature ranged from 25 to 27 °C, ammonia nitrogen was lower than 0.05 mg/L, dissolved oxygen exceeded 7.0 mg/L, and pH was between 7.4 and 8.0.

2.3. Sample collection and ethical statement

The management of the crabs in this study was performed under the approval and supervision of the Animal Experimentation Ethics Committee of East China Normal University (f20201001).

At the end of the 8-week experiment, the crabs were starved for 24 h before sampling, and the juvenile crabs in each tank were counted and weighed. Five crabs were randomly selected from each tank and stored in a refrigerator at −20 °C for whole-body composition analysis. The hepatopancreas tissues from four crabs in each tank were sampled to analyze the hepatosomatic indices. The hepatopancreas was collected from four other crabs in each tank to analyse gene expression and enzyme activity. After quick refrigeration in liquid nitrogen, the hepatopancreas tissue was stored in a freezer at −80 °C for subsequent analysis. Hemolymph was collected from eight crabs in each tank through a syringe (1-mL) on the leg joints for biochemical analysis.

2.4. Nutritional components of the diets and whole body

The nutrient components of the diets and crabs were determined according to standard AOAC methods (AOAC, 1995). The moisture content was analyzed by drying the samples to an immobile weight under 105 °C (Standard methods for feeds 930.15 and tissue 950.46). Crude protein was determined by digesting the samples on a digester and using a KjeltecTM 8200 (Kjeltec, Foss, Sweden) by the Kjeldahl method (Standard method 990.03). The total lipids in the sample were determined via the chloroform-methanol method previously used in our laboratory (Huang et al., 2022). First, the lipids were extracted with 0.37 mol/L KCL and chloroform-methanol solution and then dried in a vacuum drying oven (DZF-6050, Jinghong Co., Ltd., Shanghai, China). The ash content of the samples was analyzed by heating them in a carbonization furnace to complete carbonization and then heating them in a muffle furnace (PCD-E3000 series, Japan) at 550 °C for 8 h (Standard method 942.05).

A total of 0.12 g of feed powder was decomposed in a 6 mol/L hydrochloric acid solution (1 mL, 160 °C for 20 min). An amino acid analyzer (S433DS-433, Secam, Germany) was used to estimate the amino acid composition (Table 2). The chromatographic column—LCA K06/Na, detection wavelength—570 nm + 440 nm, temperature—58 ℃ ~74 ℃.

A high-performance liquid chromatograph (Waters 2695, Waters, USA) was used to determine the molecular weight distribution of the peptides in the feeds (Table 3). The chromatographic column was TSKgel 2000 SWXL300 mm×7.8 mm, the detection wavelength 220 nm, the flow rate 0.5 mL/min, and the column temperature 30 ℃. The mobile phase composition was water: acetonitrile: trifluoroacetic acid = 60: 40: 0.1.

2.5. Biochemical indicators

Hemolymph samples were placed in a refrigerator at 4 °C for 24 h and then centrifuged (4500 g, 10 min, 4 °C). The supernatant was collected to measure the hepatopancreas health: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, and immune state: acid phosphatase (ACP) and alkaline phosphatase (AKP) activities. The hepatopancreas samples were homogenized and centrifuged (4 °C, 3500 g, 10 min) in a 1.5-mL centrifuge tube ($W_{tissue}/V_{physiological\ saline}$ = 1:9), after which the malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, and total antioxidant capacity (T-AOC) were measured. All the above kits were purchased from Nanjing Jiancheng Bioengineering Institute, and the operation steps were carried out according to the corresponding kit instructions.

Table 2
Amino acid composition of the experimental diets (%).

Amino acid composition	Diets					
	ESB0	ESB15	ESB30	ESB45	ESB60	ESB75
EAA						
Lys	3.08	2.91	2.83	2.75	2.62	2.41
Met	0.91	0.87	0.82	0.79	0.75	0.71
Leu	3.17	3.02	2.89	2.68	2.48	2.27
Ile	1.70	1.65	1.52	1.54	1.48	1.2
Arg	2.12	2.32	2.48	2.50	2.69	2.81
Phe	1.68	1.66	1.62	1.64	1.48	1.43
Thr	1.70	1.65	1.53	1.54	1.43	1.32
Val	2.08	1.99	1.87	1.76	1.62	1.48
His	1.19	1.13	1.08	1.02	0.93	0.82
NEAA						
Asp	3.47	3.48	3.50	3.51	3.53	3.55
Ser	1.80	1.78	1.76	1.74	1.72	1.68
Glu	6.61	6.54	6.47	6.40	6.32	6.26
Gly	2.58	3.15	3.62	4.13	4.71	5.45
Ala	2.30	2.41	2.52	2.60	2.73	2.81
Cys	0.23	0.22	0.24	0.25	0.25	0.26
Tyr	1.20	1.16	1.09	0.92	0.89	0.77
Pro	2.90	2.80	2.84	2.81	2.73	3.53

EAA: essential amino acid, NEAA: nonessential amino acid.
Lys: lysine, Met: methionine, Leu: leucine, Ile: isoleucine, Arg: arginine, Phe: phenylalanine, Thr: threonine, Val: valine, His: histidine, Asp: aspartic acid, Ser: serine, Glu: glutamic acid, Gly: glycine, Ala: alanine, Cys: cysteine, Tyr: tyrosine, Pro: proline.

Table 3
Peptide molecular weight distribution of the experimental diets (% dry matter).

Molecular weight distribution (kDA)	Diets					
	ESB0	ESB15	ESB30	ESB45	ESB60	ESB75
>10000	48.37	42.02	35.23	29.34	26.60	21.22
5000–10000	6.41	5.84	6.38	5.56	5.60	4.69
1000–5000	7.30	10.72	13.90	16.35	17.58	19.19
<1000	37.92	41.42	44.48	48.76	50.22	54.90

2.6. Gene expression analysis

Total RNA in the hepatopancreas was extracted with RNAisoPlus (Takara, Dalian, China). RNA quality was measured via a NanoDrop 2000 spectrophotometer (Thermo, USA), and only samples with absorbance (260/280 nm) values between 1.8 and 2.0 were used in subsequent analysis. The RNA was inverse transcribed into cDNA with PrimeScriptTM RT Reagent kits (Takara, Dalian, China) for subsequent real-time PCR analysis. The specific primers for related genes were designed by the National Center of Biotechnology Information (NCBI), and the amplification efficiency of the primers was verified before the formal experiment. Real-time PCR was performed using a CFX96 Real-Time RCR system (Bio-Rad, USA). The volume of the qRT—PCR mixture was 10 μL: 0.2 μL of the forward and reverse primers, 1 μL of the diluted cDNA template, 3.6 μL of RNase-free water and 5 μL of 2 × ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China). The qRT—PCR amplification procedure was as follows: 95 °C for 30 s (predenaturation); 95 °C for 10 s (denaturation); and 60 °C for 30 s for 40 cycles (annealing). After the amplification program, a melting curve program (from 60 °C to 95 °C at a rate of 0.1 °C/s) was used to test the specificity of the amplification reaction. The data were calculated and statistically analyzed by the $2^{-\Delta\Delta CT}$ method. Information on primers is referred to in Table 4.

2.7. Calculations and statistical analysis

Survival rate (SR, %) = (number of crabs at the end of the experiment/number of crabs at the beginning of the experiment) ×100;

Weight gain (WG, %) = (average FBW - average IBW) ×100 /IBW;

Specific growth rate (SGR, %day^{-1}) = [ln (average FBW)-ln (average IBW)] ×100 /test days;

Hepatopancreas index (HSI, %) = (wet weight of hepatopancreas /wet weight of crab) ×100;

The feed conversion ratio (FCR) = feed intake/(FBW - IBW + dead crab weight).

FBW = final body weight; IBW: initial body weight.

All the experimental data were analyzed using SPSS 20.0. The data first passed the homogeneity test for variance. Then if one-way ANOVA was significant, Duncan's multiple comparison test was applied to determine the significant differences among treatments ($P < 0.05$). All the results are displayed as the means and corresponding pooled standard errors of the means (SEMs). Orthogonal polynomial contrasts were applied to check whether the trend was quadratic or linear (Wu et al., 2018).

Table 4
Primer pair sequences of the genes used for quantitative real-time PCR.

Gene	Position	Primer Sequence (5'~3')	Length	Access No.
β-actin	Forward	TCGTGCGAGACATCAAGGAAA	21	KM244725.1
	Reverse	AGGAAGGAAGGCTGGAAGAGTG	22	
ALF1	Forward	GCTGGCTGGACCGGATTATT	20	DQ793214.1
	Reverse	ATCACACGGGTGTTGCAGAT	20	
ALF2	Forward	TGTCACCCCGCCTCATTAAG	20	GU014699.1
	Reverse	GTCAGAGACTCCCCCTGGAT	20	
Crustin	Forward	ACCACCCAAAACATGCTCCA	20	FJ974138.1
	Reverse	GGCTTGCAGACATGTTCACC	20	
TLR-2	Forward	CATACCAGGACGACGAAC	18	KC011816.1
	Reverse	AGACATTGAGCGAGGAGA	18	
Myd88	Forward	GCCATCGCAGTCGCCAAGTT	20	KM433864.1
	Reverse	GGCATCCTGTTCATCCAGTTCTGAC	25	
relish	Forward	TCTCCCTACTCTGACCATTCC	21	GQ871279.1
	Reverse	TTCCCACCATCTCACTCTTGT	21	
LITAF	Forward	ATCAGCTCCCCCACCCTATG	20	KF892539.1
	Reverse	GTTGTTGGAGCAGCACCTTG	20	
PT	Forward	GACCTGCACCTTTGACGAGA	20	KM433863.1
	Reverse	GTATTTGGTGCAGTCGAGCG	20	
PM1	Forward	CGCCGAGAAGTGTGACTACA	20	KU041138.1
	Reverse	GAAGTAGCCGTTCTGTCGCA	20	
PM2	Forward	CTCGTCGATGACCAAGGACC	20	KU041139.1
	Reverse	CCTCTGCGGGAAGGAAACTT	20	

ALF1/2: anti-lipopolysaccharide factor 1/2, Myd88: myeloid differentiation factor 88, LITAF: lipopolysaccharide-induced TNF-α factor, TLR-2: toll-like receptor 2.

3. Results

3.1. Growth performance and feed utilization

The FBW, WG, SGR, HSI and FCR changed linearly and quadratically ($P<0.05$; Table 5). Crabs fed ESB15 had the highest values of FBW, WG and SGR. The FBW, WG, and SGR of the crabs fed the ESB15 diet were significantly greater than those of the other groups ($P<0.05$). Moreover, compared with those fed the ESB30 and ESB45 diets, the FBW, WG and SGR of the crabs fed the ESB60 and ESB75 diets significantly decreased ($P<0.05$).

The HSIs of crabs fed ESB15 and ESB30 were significantly greater than those of crabs fed the diets ESB60 and ESB75 ($P<0.05$). Although the FCR changed linearly and quadratically ($P<0.05$), no marked difference was observed among the six groups ($P>0.05$).

Table 5
Growth performance of juvenile crabs fed different experimental diets after 8 weeks.

	SR (%)	FBW (g)	WG (%)	SGR (% day^{-1})	HSI (%)	FCR
ESB0	84.29	2.3^{b}	211.13^{b}	2.03^{b}	8.80^{ab}	1.45
ESB15	80.00	2.53^{c}	244.04^{c}	2.21^{c}	9.70^{b}	1.32
ESB30	79.05	2.32^{b}	214.68^{b}	2.05^{b}	9.46^{b}	1.37
ESB45	78.10	2.20^{b}	198.87^{b}	1.95^{b}	8.38^{ab}	1.37
ESB60	83.81	1.97^{a}	169.55^{a}	1.77^{a}	7.94^{a}	1.52
ESB75	77.14	1.96^{a}	165.60^{a}	1.74^{a}	7.60^{a}	1.58
SEM	1.21	0.05	6.05	0.04	0.23	0.03
P value	0.46	0.00	0.00	0.00	0.04	0.01
Regression analysis						
Linear						
Adj.R^2	-0.016	0.57	0.57	0.58	0.34	0.28
P value	0.40	0.00	0.00	0.00	0.01	0.01
Quadratic						
Adj.R^2	-0.08	0.60	0.60	0.62	0.42	0.51
P value	0.65	0.00	0.00	0.00	0.01	0.00

Adj. R2 = adjusted R square. Values in the same column with different superscripts are significantly different (P < 0.05, n=4) and are the same as follows. SR: survival rate, FBW: final body weight, WG: weight gain, SGR: specific growth rate, HSI: hepatopancreas somatic index, FCR: feed coefficient.

3.2. Body composition

The crude protein, lipids, ash and moisture contents did not significantly differ among the crabs fed the different diets ($P>0.05$; Table 6). However, the total lipids in the whole body showed linear and quadratic trends ($P<0.05$).

3.3. Antioxidant capacity of the hepatopancreas

The SOD and GPx activities and the concentration of MDA in the hepatopancreas of crabs fed different diets changed linearly and quadratically ($P<0.05$; Table 7). Different substitution levels of FM with ESB significantly improved SOD activity ($P<0.05$). There was no significant difference in SOD activity in crabs fed the ESB15–75 diet ($P>0.05$). GPx activity in the hepatopancreas of crabs fed ESB15 was significantly greater than in the other groups ($P<0.05$). ESB45, ESB60 and ESB75 significantly reduced the GPx activity in the hepatopancreas compared with ESB0, ESB15 and ESB30 in crabs fed diets($P<0.05$). The diet containing ESB15 had the lowest MDA content in the hepatopancreas of the crabs. Compared with ESB0 and ESB15, dietary ESB60 and ESB75 significantly increased the content of hepatopancreas MDA ($P<0.05$). The T-AOC in the hepatopancreas changed quadratically ($P<0.05$). The T-AOC values in crabs fed the ESB15 and ESB30 diets

Table 6
Proximate composition of juvenile crabs fed different diets after 8 weeks (%, n=4).

Treatments	Moisture	Crude lipid	Crude Protein	Ash
ESB0	66.96	3.82	12.41	11.47
ESB15	65.48	4.14	12.46	11.62
ESB30	65.46	4.07	12.93	12.23
ESB45	63.97	3.12	12.11	12.01
ESB60	65.50	3.05	12.27	11.92
ESB75	64.57	3.13	12.67	12.32
SEM	0.44	0.16	0.17	0.17
P value	0.65	0.08	0.85	0.69
Regression analysis				
Linear				
Adj.R^2	0.07	0.25	-0.05	0.05
P value	0.17	0.01	0.99	0.15
Quadratic				
Adj.R^2	0.05	0.22	-0.10	0.01
P value	0.30	0.04	0.97	0.34

Table 7
Antioxidant capacity of the hepatopancreas of crabs fed different diets after 8 weeks (n=4).

Treatments	T-AOC (mmol/g)	SOD (U/mg)	GPx (U/mg)	MDA (nmol/mg)
ESB0	0.04^{b}	7.15^{a}	15.62^{b}	2.62^{b}
ESB15	0.06^{c}	10.42^{b}	19.61^{c}	1.45^{a}
ESB30	0.06^{c}	10.97^{b}	15.21^{b}	3.55^{ab}
ESB45	0.05^{b}	11.72^{b}	11.39^{a}	3.07^{ab}
ESB60	0.04^{ab}	11.99^{b}	8.86^{a}	4.19^{c}
ESB75	0.03^{a}	10.57^{b}	9.16^{a}	3.84^{c}
SEM	0.00	0.49	0.91	0.22
P value	0.00	0.03	0.00	0.00
Regression analysis				
Linear				
Adj.R^2	-0.04	0.19	0.58	0.31
P value	0.71	0.02	0.00	0.00
Quadratic				
Adj.R^2	0.30	0.39	0.58	0.29
P value	0.01	0.00	0.00	0.01

T-AOC: total antioxidant capacity, SOD: superoxide dismutase, GPx: glutathione peroxidase, MDA: malondialdehyde.

were significantly greater than those fed the ESB0, ESB45 and ESB75 diets ($P<0.05$).

3.4. Hemolymph biochemical and immune indices

Hemolymph AKP, ACP, AST and ALT levels changed linearly and quadratically ($P<0.05$; Table 8). Hemolymph AKP activity was significantly greater in crabs fed the ESB0–45 diet than in those fed the ESB60 and ESB75 diets ($P<0.05$). The hemolymph activity of ACP in crabs fed the ESB60 and ESB75 diets was significantly lower than in those fed the ESB0–45 diet ($P<0.05$). Crabs fed the ESB15 diet exhibited significantly greater ACP activity than those fed the ESB0 and ESB45 diets ($P<0.05$).

Hemolymph AST levels in crabs fed the ESB15–60 diet were significantly lower than those fed the ESB75 diet ($P<0.05$). Although the variation in ALT activity changed significantly, no remarkable discrepancy was observed among the different groups ($P>0.05$).

3.5. Gene expression related to hepatopancreas immunity, inflammation and intestinal health

The expression of the hepatopancreas genes *ALF1*, *ALF2*, *crustin*, *relish* and *LITAF* was not markedly affected by the substitution of FM with ESB ($P>0.05$; Table 9). The mRNA levels of *TLR2* and *Myd88* changed linearly and quadratically ($P<0.05$). Crabs fed the ESB15 diet presented significantly lower *TLR2* mRNA levels than those fed the ESB45–75 diet ($P<0.05$). There were high mRNA levels of *TLR2* in crabs fed the ESB45 and ESB75 diets relative to those in crabs fed the ESB0 diet ($P<0.05$). The gene expression of *Myd88* in crabs fed the ESB15 diet was significantly lower than in those fed the ESB30–75 diet ($P<0.05$). No prominent difference in the gene expression of *Myd88* was found between crabs fed the ESB30–75 diet ($P>0.05$).

Table 8
Hemolymph biochemical and immune indices of juvenile crabs fed different diets after 8 weeks (n=4).

Treatments	AKP (king unit/ 100 mL)	ACP (king unit/ 100 mL)	AST (U/ L)	ALT (U/ L)
ESB0	9.06^{bc}	2.29^{b}	17.51^{ab}	9.19
ESB15	9.75^{c}	2.70^{c}	11.96^{a}	8.77
ESB30	8.48^{b}	2.51^{bc}	11.95^{a}	7.31
ESB45	8.35^{b}	2.30^{b}	14.11^{a}	9.89
ESB60	5.38^{a}	0.48^{a}	16.32^{a}	13.80
ESB75	4.85^{a}	0.23^{a}	22.03^{b}	14.46
SEM	0.40	0.22	0.87	0.63
P value	0.00	0.00	0.00	0.00
Regression analysis				
Linear				
Adj.R^2	0.77	0.66	0.10	0.58
P value	0.00	0.00	0.03	0.00
Quadratic				
Adj.R^2	0.85	0.84	0.35	0.79
P value	0.00	0.00	0.00	0.00

AST: aspartate aminotransferase, ALT: alanine aminotransferase, ACP: acid phosphatase, AKP: alkaline phosphatase.

The intestinal mRNA levels of *PM1*, *PM2* and *PT* changed linearly and quadratically ($P<0.05$). Crabs fed ESB15 and ESB30 exhibited significantly greater expression of *PM1* than those fed the other diet ($P<0.05$). In contrast to diet ESB45, the dietary ESB60 and ESB75 significantly reduced the expression of *PM1* in the crabs ($P<0.05$). Crabs fed the ESB15 diet presented significantly greater *PM2* levels than those fed the other diet ($P<0.05$). There was a significantly lower expression of *PM2* in crabs fed the ESB60 and ESB75 diets than in those fed the other diets ($P<0.05$). No significant difference was found in the expression of *PT* in crabs fed the ESB0–30 diet ($P>0.05$). Expression of *PT* was significantly greater in crabs fed the ESB15 and ESB30 diets than in those fed the ESB45–75 diet ($P<0.05$).

4. Discussion

Aquatic animals can utilize proper levels of soybean proteins in feeds because of their considerable amino acid composition (Choi et al., 2020; Xie et al., 2016a). Moreover, the ESB contains fewer antinutritional factors and more small peptides than other antimicrobial agents to improve animal feed utilization efficiency and protein digestibility (Dawood and Koshio, 2020; Song et al.,2020; Wen et al., 2021). The present study obtained the crabs' best growth performance when 15% of the FM was replaced with ESB. Moreover, when 8% FM was replaced with ESB, the channel catfish *Ictalurus punctatus* exhibited improved growth performance (Xiao et al., 2022). Largemouth bass exhibited the best growth when 16% of dietary FM was replaced with ESB (Liu et al., 2021). These differences may be partly attributed to animal species and basal FM content in the diets. However, the excessive addition of plant proteins could cause a rapid decline in the growth of aquatic animals (Gemede and Ratta, 2014). The decrease in growth performance of crabs fed ESB60 and ESB75 relative to those fed FM might be due to the unbalanced amino acid composition, superfluous antinutritional factors, poor palatability and oligopeptides (Chen et al., 2013, Krogdahl et al., 2003). In addition, the FCR quadratically changed and was high when the substitution level was greater than 45%. Excessive substitution of FM with ESB may decrease feed utilization by impairing the intestinal health of animals (Liu et al., 2021). Overall, the results showed that a substitution level of <45% did not cause a significantly adverse impact on the growth of the crabs.

The lipid concentration in the crabs' whole body changed quadratically, and a high value was observed at treatments ESB15 and 30. This might be because dietary ESB provided more emulsified lipids, which could promote lipid absorption and digestion (Xiao et al., 2022). An appropriate level of ESB reduced the muscle lipid content in the largemouth bass (Liu et al., 2021). Dietary ESB in place of FM increased the lipid content in channel catfish's whole body and muscle (Xiao et al., 2022) and in the whole body of Pacific white shrimp (Fan et al., 2021). Thus, the relationship between organism lipid content and dietary ESB level remains unclear. It is speculated that dietary ESB affects lipid content through lipid metabolism remodeling (Song et al., 2020). ESB contains abundant vegetable oils (approximately 20%), which can change the composition of dietary fatty acids. However, further investigations into the effect of dietary ESB on lipid metabolism are needed.

The homeostasis of free radicals in the body is crucial for life (Sturve et al., 2008). SOD plays the role of converting O^{2-} into H_2O_2 and O_2 (Han et al., 2006). The T-AOC is a comprehensive indicator of oxidation resistance (Sun et al., 2024). As one of the key antioxidant enzymes in

Table 9
Hepatopancreatic immunity, inflammation, and intestinal peritrophic membrane-related gene expression in juvenile crabs fed different diets after 8 weeks (n=4).

Treatments	*ALF1*	*ALF2*	*Crustin*	*TLR2*	*Myd88*	*relish*	*LITAF*	*PM1*	*PM2*	*PT*
ESB0	1.00	1.00	1.00	1.00^{ab}	1.00^{ab}	1.00	1.00	1.00^{b}	1.00^{b}	1.00^{bc}
ESB15	1.70	1.54	1.36	0.87^{a}	0.82^{a}	0.40	0.88	1.29^{c}	1.80^{c}	1.29^{c}
ESB30	1.38	1.07	1.25	1.26^{abc}	1.20^{bc}	0.66	1.21	1.25^{c}	1.02^{b}	1.32^{c}
ESB45	1.40	1.37	1.27	1.64^{c}	1.35^{bc}	0.90	0.85	0.95^{b}	1.36^{b}	0.94^{b}
ESB60	1.14	0.87	1.36	1.45^{bc}	1.41^{c}	0.80	1.24	0.57^{a}	0.50^{a}	0.57^{a}
ESB75	1.04	0.81	1.39	1.55^{c}	1.39^{c}	0.95	1.73	0.47^{a}	0.34^{a}	0.36^{a}
SEM	0.11	0.09	0.05	0.08	0.06	0.09	0.08	0.08	0.11	0.08
P value	0.47	0.10	0.51	0.02	0.01	0.56	0.08	0.00	0.00	0.00
Regression analysis										
Linear										
Adj.R^2	-0.01	0.06	0.04	0.27	0.39	-0.02	0.05	0.65	0.42	0.41
P value	0.35	0.11	0.12	0.00	0.00	0.56	0.11	0.00	0.00	0.00
Quadratic										
Adj.R^2	0.03	0.08	0.02	0.28	0.36	-0.02	0.08	0.81	0.52	0.67
P value	0.26	0.13	0.28	0.01	0.00	0.54	0.12	0.00	0.00	0.00

ALF1/2: anti-lipopolysaccharide factor 1/2, Myd88: myeloid differentiation factor 88, LITAF: lipopolysaccharide-induced TNF-α factor, PM1/2: peritrophin 1, PT: peritrophin.

organisms, GPx can scavenge free oxygen (Xie et al., 2016b). MDA is a product of cytotoxic lipid peroxidation, which adversely affects the body (Wang et al., 2023). The liver or hepatopancreas is the center of metabolism in aquatic animals. In the present study, appropriately substituting FM with ESB improved the oxidation resistance and remitted lipid peroxidation in the hepatopancreas. Dietary FM replacement with ESB, which enhances the antioxidant system of the liver, was also reported in the largemouth bass (Zhang et al., 2019). The anti-oxidative effect might occur because ESB can produce several anti-oxidative peptides (Kim et al., 2021). In addition, dietary ESB could improve iron absorption, indirectly enhancing organisms' antioxidant capacity (Lv et al., 2009). It can also activate the antioxidant-related signaling pathway (NF-E2-related factor 2), upregulating the expression of antioxidant enzymes (Song et al., 2020). However, excess replacement of FM with ESB reduced the hepatopancreas GPx capacity (45%) and aggravated lipid peroxidation (60 and 75%). Hepatopancreas health was further evaluated through the content of AST and ALT in the hemolymph. The variation trend of AST and ALT indicated that excess replacement of FM with ESB had a negative effect on hepatopancreas health in crabs. Excess replacement of FM with ESB also elevates the hemolymph ALT concentration in the abalone (Yu et al., 2022) and the blood ALT and AST concentrations in juvenile largemouth bass *Micropterus salmoides* (Liu et al., 2021). Thus, the substitution level of FM with ESB could not exceed 45% according to the normal hepatopancreas antioxidant capacity and health in the present study.

Nonspecific immunity is important for invertebrates that lack adaptive immunity (Yu et al., 2022). AKP and ACP are typical hydrolases that eliminate pathogen macromolecules and extracellular invaders (Di et al., 2013). Most phosphatases are located in cellular lysosomes and play important roles in the nonspecific immune response of crustaceans (Xiong et al., 2018). In accordance with the negative effect on growth performance, compared with ESB0, ESB60 and ESB75 significantly reduced the activities of hemolymph ACP and AKP, indicating that these two levels were inadvisable. The nonspecific immune response of crustaceans depends on recognizing immune receptors. Among several different pattern recognition receptors, Toll receptors have been extensively studied (Amarante et al., 2018). Toll receptors that bind to *Myd88* can activate the expression of *NF-κB* in crabs (Constant et al., 2021; Huang et al., 2018). In crustaceans, the *relish* gene is homologous to the *NF-κB* gene (Zheng et al., 2019), and the activated *relish* gene can upregulate the mRNA expression of the downstream proinflammatory transcription factor *LITAF* (Ping et al., 2012). In the present study, most genes, including those associated with antimicrobial peptides (*ALF1*, *ALF2*, *crustin*), *relish* and *LITAF*, were not affected in crabs fed different diets. However, the obvious impact on the expression of *TLR2* and *Myd88* in the hepatopancreas suggests that a substitution level of 45–75% might burden the immune system. An appropriate content of soybean peptides can improve nonspecific immunity and cell viability in immune cells (Wen et al., 2021). The excessive application of ESB can cause amino acid deficiency and import β-conglycinin and glycinin, inducing oxidative damage and inflammation (Yu et al., 2022).

The intestinal tract is the crucial site for the absorption and digestion of organisms (Ma et al., 2021). The peritrophic matrix is a semipermeable noncellular membrane structure in the intestine of crustaceans that can protect the intestine from physical damage and pathogenic bacterial invasion and promote the absorption and utilization of nutrients (Han et al., 2020; Park et al., 2016). In the present study, the expression patterns of *PM1*, *PM2*, and *PT* indicate that these proteins play a positive role in intestinal health due to the proper substitution level of ESB (15 and 30%). This may be because more active peptides are produced by enzymatic hydrolysis, which facilitates the development of intestinal mucosal cells (Xiao et al., 2022; Xu et al., 2012). The positive effect of proper ESB levels on intestinal health has also been demonstrated in juvenile largemouth bass (Liu et al., 2021) and Pacific white shrimp (Fan et al., 2021). Consistent with the growth phenotype, diets containing ESB60 and ESB70 led to lower expression of *PM1*, *PM2*, and *PT*, indicating excessive replacement.

5. Conclusion

Dietary FM could be replaced by up to 45%, i.e. at 192.5 g/kg in the diet, without negatively affecting growth performance. Replacing 15% of dietary FM with ESB at 297.5 g/kg FM enhanced the crabs' growth, anti-oxidative capacity, immunity, and intestinal health. However, excessive substitution of dietary FM with ESB significantly impaired oxidation resistance, immune parameters (≥45%) and intestinal health (≥60%) in crabs. These findings indicate that ESB can potentially substitute FM for *E. sinensis*.

CRediT authorship contribution statement

Qincheng Huang: Writing – original draft, Data curation. **Yixin Miao**: Project administration, Investigation. **Jiadai Liu**: Investigation. **Han Wang**: Investigation. **Chuanjie Qin**: Resources. **Xiaodan Wang**: Writing – review & editing. **Erchao Li**: Writing – review & editing. **Jianguang Qin**: Writing – review & editing. **Liqiao Chen**: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data Availability

The data that has been used is confidential.

Acknowledgment

Supported by: National Key R&D Program of China (2023YFD2402003), the China Agriculture Research System of MOF and MARA, the National Natural Science Foundation of China (32072986), the Agriculture Research System of Shanghai, China (202404), and the National Subsidization Program for Postdoctoral Researcher (GZC20231128).

References

Amarante, M.G.P., Adjemian, S., Branco, L.M., Zanetti, L.C., Weinlich, R., Bortoluci, K.R., 2018. Pattern recognition receptors and the host cell death molecular machinery. Front. Immunol. 9, 2379.

AOAC, 1995. Official Methods of Analysis of Official Chemists International, 16thed. Association of Official Analytical Chemists, Arlington, VA.

Chen, N., Ma, X., Zhao, M., Ji, Z., Tong, C., 2013. Suitable inclusion levels of white fish meal and two kinds of soybean meal in diets for juvenile largemouth bass (*Micropterus salmoides*). J. Fish. China 37 (9), 1389–1400.

China Fishery Statistical Yearbook, 2023. China Agriculture Press, Beijing, China, p. 24.

Choi, D.G., He, M., Fang, H., Wang, X.L., Li, X.Q., Leng, X.J., 2020. Replacement of fish meal with two fermented soybean meals in diets for rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr. 26 (1), 37–46.

Chou, R.L., Her, B.Y., Su, M.S., Hwang, G., Wu, Y.H., Chen, H.Y., 2004. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. Aquaculture 229 (1-4), 325–333.

Constant, D.A., Nice, T.J., Rauch, I., 2021. Innate immune sensing by epithelial barriers. Curr. Opin. Immunol. 73, 1–8.

Dawood, M.A., Koshio, S., 2020. Application of fermentation strategy in aquafeed for sustainable aquaculture. Rev. Aquac. 12 (2), 987–1002.

Di, G.L., Zhang, Z.X., Ke, C.H., 2013. Phagocytosis and respiratory burst activity of haemocytes from the ivory snail, *Babylonia areolata*. Fish. Shellfish Immunol. 35 (2), 366–374.

Fan, Y., Luo, K., Guo, Y., Gao, W., Xu, Q., Zhang, W., et al., 2021. Replacement of fish meal by enzyme-treated soybean on the growth performance, intestine microflora, immune responses and disease resistance of Pacific white shrimp *Litopenaeus vannamei*. Aquac. Res. 52 (10), 4619–4628.

Francis, G., Makkar, H.P., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199 (3-4), 197–227.

Gemede, H.F., Ratta, N., 2014. Antinutritional factors in plant foods: potential health benefits and adverse effects. Int. J. Nutr. Food Sci. 3 (4), 284–289.

Han, H.J., Kim, D.H., Lee, D.C., Kim, S.M., Park, S.I., 2006. Pathogenicity of *Edwardsiella tarda* to olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel). J. Fish. Dis. 29 (10), 601–609.

Han, F.L., Xu, C., Qi, C.L., Lin, Z.D., Li, E.C., Wang, C.L., et al., 2020. Sodium butyrate can improve intestinal integrity and immunity in juvenile Chinese mitten crab (*Eriocheir sinensis*) fed glycinin. Fish. Shellfish Immunol. 102, 400–411.

Huang, Q., Wang, X., Bu, X., Song, Y., You, J., Zhang, C., et al., 2022. Dietary vitamin A affects growth performance, immunity, antioxidant capacity, and lipid metabolism of juvenile Chinese mitten crab *Eriocheir sinensis*. Aquaculture 548, 737556.

Huang, Y., Wen, W., Xu, Z., Pan, J., Zhao, Z., Qian, R., 2018. *Eriocheir sinensis* microRNA-7 targets crab Myd88 to enhance white spot syndrome virus replication. Fish. Shellfish Immunol. 92, 274–283.

Jiang, W., Jia, X., Xie, N., Wen, C., Ma, S., Jiang, G., et al., 2023. Aquafeed fermentation improves dietary nutritional quality and benefits feeding behavior, meat flavor, and intestinal microbiota of Chinese mitten crab (*Eriocheir sinensis*). Anim. Nutr. 14, 1–19.

Jing, T., Zhou, M., Li, Z., Li, Y., Sun, W., Liang, J., et al., 2021. Effects of fish meal replacement with soy peptide protein on the growth performance, digestive enzymes,and antioxidant capacity of juvenile yellow catfish (*Pelteobagrus fulvidraco*). Prog. Fish. Sci. 42 (5), 149–157.

Kim, I.S., Yang, W.S., Kim, C.H., 2021. Beneficial effects of soybean-derived bioactive peptides. Int. J. Mol. Sci. 22 (16), 8570.

Krogdahl, A., Bakke-McKellep, A.M., Baeverfjord, G., 2003. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L. Aquac. Nutr. 9 (6), 361–371.

Krogdahl, A., Penn, M., Thorsen, J., Refstie, S., Bakke, A.M., 2010. Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. Aquac. Res. 41 (3), 333–344.

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.

Liu, X., Chi, S.Y., Li, S., Cheng, X.L., Gao, W.H., Xu, Q.Q., et al., 2021. Substitution of fish meal with enzyme-treated soybean in diets for juvenile largemouth bass (*Micropterus salmoides*). Aquac. Nutr. 27 (5), 1569–1577.

Lv, Y., Liu, Q., Bao, X., Tang, W., Yang, B., Guo, S., 2009. Identification and characteristics of iron-chelating peptides from soybean protein hydrolysates using IMAC-Fe^{3+}. J. Agric. Food Chem. 57 (11), 4593–4597.

Ma, R., Wang, Y., Zhao, S., Ma, Q., Yin, M., Li, X., Fang, W., 2021. Bacterial flora in the gill tissues and intestinal tracts of male and female Chinese mitten crabs (*Eriocheir sinensis*) with different diets in a mud pond. Curr. Microbiol 78, 2291–2297.

Mugwanya, M., Dawood, M.A., Kimera, F., Sewilam, H., 2023. Replacement of fish meal with fermented plant proteins in the aquafeed industry: a systematic review and meta-analysis. Rev. Aquacult 15 (1), 62–88.

Nathaly, M.C., Enrique, M.R., Francisco, J.C., José, L.C., José, A.L., Saúl, R.C., et al., 2019. Advances in the use of alternative protein sources for tilapia feeding. Rev. Aquac. 11 (3), 515–526.

Park, M.J., Kim, B.Y., Jin, B.R., 2016. Molecular characterization of a chitin-binding protein with the peritrophin-a domain from the Asiatic honeybee *Apis cerana*. J. Asia-Pac. Entomol. 19 (4), 963–968.

Ping, J., Jing, H., Qian, J., Chen, L., Xu, X., Fei, Ma, 2012. Identification and characterization of a putative lipopolysaccharide-induced TNF-α factor (LITAF) gene from Amphioxus (*Branchiostoma belcheri*): an insight into the innate immunity of *Amphioxus* and the evolution of LITAF. Fish. Shellfish Immunol. 32 (6), 1223–1228.

Samtiya, M., Aluko, R.E., Dhewa, T., 2020. Plant food anti-nutritional factors and their reduction strategies: an overview. Food Prod., Process. Nutr. 2 (1), 14.

Song, Z.D., Li, H.Y., Wang, J.Y., Li, P.Y., Sun, Y.Z., Zhang, L.M., 2014. Effects of fishmeal replacement with soy protein hydrolysates on growth performance, blood biochemistry, gastrointestinal digestion and muscle composition of juvenile starry flounder (*Platichthys stellatus*). Aquaculture 426 96–104.

Song, Y., Yan, L., Jiang, W., Xiao, W., Feng, L., Wu, P., Liu, Y., Kuang, S., Tang, L., Zhou, X., 2020. Enzyme-treated soy protein supplementation in low protein diet improved flesh tenderness, juiciness, flavor, healthiness, and antioxidant capacity in on-growing grass carp (*Ctenopharyngodon idella*). Fish. Physiol. Biochem 46, 213–230.

Sturve, J., Almroth, B.C., Forlin, L., 2008. Oxidative stress in rainbow trout (*Oncorhynchus mykiss*) exposed to sewage treatment plant effluent. Ecotoxicol. Environ. Saf. 70 (3), 446–452.

Sun, X., Fang, Z., Yu, H., Zhao, H., Wang, Y., Zhou, F., Zhao, L., Sun, J., Tian, Y., 2024. Effects of Enterococcus faecium (R8a) on nonspecific immune gene expression, immunity and intestinal flora of gi*ant tiger shrimp (Penaeus monodon*). Sci. Rep. 14 (1), 1823.

Tacon, A.G., 2004. Use of fish meal and fish oil in aquaculture: a global perspective. Aquat. Resour., Cult. Dev. 2004, 12.

Wang, L., Zang, M., Zhao, X., Cheng, X., Li, X., Bai, J., 2023. Lipid oxidation and free radical formation of shrimp (*Penaeus vannamei*) during hot air drying. J. Food Meas. Charact. 17 (4), 3493–3504.

Wen, L.R., Jiang, Y.M., Zhou, X.S., Bi, H.M., Yang, B., 2021. Structure identification of soybean peptides and their immunomodulatory activity. Food Chem. 359, 8.

Wu, M., Wu, X., Lu, S., Gao, Y., Yao, W., Li, X., et al., 2018. Dietary arginine affects growth, gut morphology, oxidation resistance and immunity of hybrid grouper (*Epinephelus fuscoguttatus*♀×*Epinephelus lanceolatus*♂) juveniles. Br. J. Nutr. 120 (3), 269–282.

Xiao, X., Zhang, W., Du, R., Ma, J., Wang, Z., Lv, B., et al., 2022. Study of enzyme-hydrolyzed soybean replacing fish meal and/or chicken meal on the growth of channel catfish (*Ictalurus punctatus*). Aquacu. Rep. 27, 101344.

Xie, S., Liu, Y., Zeng, S., Niu, J., Tian, L., 2016a. 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.

Xie, S., Zhou, W., Tian, L., Niu, J., Liu, Y.J.F., Immunology, S., 2016b. Effect of N-acetyl cysteine and glycine supplementation on growth performance, glutathione synthesis, anti-oxidative and immune ability of Nile tilapia, *Oreochromis niloticus*. Fish. Shellfish Immunol. 55, 233–241.

Xiong, J., Jin, M., Yuan, Y., Luo, J.X., Lu, Y., Zhou, Q.C., et al., 2018. Dietary nucleotide-rich yeast supplementation improves growth, innate immunity and intestinal morphology of Pacific white shrimp (*Litopenaeus vannamei*). Aquac. Nutr. 24 (5), 1425–1435.

Xu, F.Z., Li, L.M., Liu, H.J., Zhan, K., Ding, X.L., Advances, V., 2012. Effects of fermented soybean meal on performance, serum biochemical parameters and intestinal morphology of laying hens. J. Anim. Vet. Adv. 11 (5), 649–654.

Yu, D.H., Gong, S.Y., Yuan, Y.C., Lin, Y.C., 2013. Effects of replacing fish meal with soybean meal on growth, body composition and digestive enzyme activities of juvenile Chinese sucker, *Myxocyprinus asiaticus*. Aquac. Nutr. 19 (1), 84–90.

Yu, X., Luo, K., Rao, W., Chen, P., Lei, K., Liu, C., et al., 2022. Effects of replacing dietary fish meal with enzyme-treated soybean meal on growth performance, intestinal microbiota, immunity and mTOR pathway in abalone *Haliotis discus hannai*. Fish. Shellfish Immunol. 130, 9–21.

Zhang, G.G., Xiang, L.I., Cai, X.B., Zhang, S.X., Hua, X.M., Huang, Z.Y., et al., 2019. Effects of enzymatic hydrolyzed soybean meal on growth performance, liver function and metabolism of largemouth bass (*Micropterus salmoides*). Acta Hydrobiol. Sin. 43 (5), 1001–1012.

Zheng, X., Chi, C., Xu, C., Liu, J., Liu, W.J.F., Immunology, S., 2019. Effects of dietary supplementation with icariin on growth performance, antioxidant capacity and non-specific immunity of Chinese mitten crab (*Eriocheir sinensis*). Fish. Shellfish Immunol. 90, 264–273.