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Original Research Article

Dietary black soldier fly oil enhances growth performance, flesh quality, and health status of largemouth bass (*Micropterus salmoides*)

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ABSTRACT

The study aimed to assess the effects of dietary black soldier fly oil (BSFO) on the growth performance, flesh quality, and health status of largemouth bass (*Micropterus salmoides*). Six iso-nitrogenous and isolipid diets were formulated by substituting fish oil and soybean oil (1/2, wt/wt) with BSFO in percentages of 0%, 20%, 40%, 60%, 80%, and 100%, respectively. The diets were fed to 960 fish (initial body weight = 16.5 g) in four replicates for 8 weeks. Indicators related to growth performance, body composition, hematology, flesh quality, expression of genes related to inflammatory cytokines and apoptosis, and the response of fish to *Aeromonas veronii* challenge were analyzed. The results showed that the weight gain rate was numerically improved in all BSFO substitution groups, ranging from 9.3% to 44.0% compared to the control group. The highest survival rate and the lowest hepatosomatic index and condition factor were observed in the BFSO20 group. In terms of flesh quality, the water-holding capacity of the dorsal muscle was elevated with higher levels of dietary BSFO. However, significant changes in texture properties (cohesiveness, gluing, and chewiness) were observed in the BSFO20 group ($P < 0.05$). Six hematological parameters related to glycolipid and liver function were optimized in most of the BFSO substitution groups. Furthermore, the expressions of six inflammation- and apoptosis-related genes (*IL-1β*, *Bcl-xl*, *BAX*, *caspase8*, *TNF-α*, and *IL-10*) were significantly affected by dietary BSFO ($P < 0.05$). Following bacterial challenge, the seven-day cumulative survival rates of fish were considerably increased from 10.0% in the control group to 60.0% and 66.7% in the BSFO80 and BSFO100 groups, respectively. One-variable linear regression analysis revealed that various parameters related to fish growth, flesh quality, and health status were significantly influenced by dietary BSFO substitution levels in a dose-dependent manner ($P < 0.05$). In conclusion, substituting around 20% of dietary fish oil and soybean oil with BSFO is promising in improving the growth performance and flesh quality of *M. salmoides*. However, to enhance immunity and disease resistance, it is recommended to further increase the inclusion of BSFO in the diet.

1. Introduction

Fish oil has long been used as a source of essential oil sources in aquatic feed due to its availability, palatability, and appropriate fatty acid profiles for aquatic animals. However, the global environmental and economic impacts have resulted in a shortage of fish oil supplies. The annual world production of fish oil is only 1.1 million tonnes, with 75% of it being used in aquaculture. China, in

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particular, heavily relies on fish oil imports, accounting for about 60% of its fish oil consumption (Li et al., 2023). According to the statistical data of General Administration of Customs of the People's Republic of China (http://www.customs.gov.cn/), the average import price of the feed grade refined fish oil was increased by 49.5% in the past five years (2009–2023). Consequently, efforts have been made to find alternative sources of oil that are readily available, sustainable, and nutritionally beneficial.

Black soldier fly (BSFL) *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae are considered the new "superstar" producers of sustainable feed or food products for their high-quality protein (18% to 55%) and oil (15% to 49%) inclusion (Muller et al., 2017). According to a recent commercial research report, the global black soldier fly industry is expected to be worth 3882.4 million USD by 2028, and the market is anticipated to grow at a substantial compound annual growth rate of 32.5% in the upcoming years, based on the world's expanding population and the quickly rising demand for meat products (Credence Research, 2023). The nutritional value assessment and application technology of black soldier fly protein fraction have been thoroughly studied, but the remaining oil extract has remained largely unexploited. The black soldier fly oil (BSFO) has a unique profile comprising 40% to 50% saturated medium-chain fatty acids (MCFA), which have distinct immunomodulatory properties. In addition, lauric acid, a member of MCFA, has been found to possess broad-spectrum antibacterial and antiviral effects (do Couto et al., 2021; Li et al., 2022a; Nitbani et al., 2022; Rabani et al., 2019; Ushakova et al., 2016; Wang and Shelomi, 2017). Recent studies have highlighted the potential of BSFO as a high-quality dietary fat source for several fish species, including *Salmo salar*, *Oncorhynchus mykiss*, *Cyprinus carpiovar Jian*, *Totoaba macdonaldi* (Belghit et al., 2018; Dumas et al., 2018; Kumar et al., 2021; Maldonado-Othón et al., 2022), for its good palatability, growth enhancement, and strong resistance against various pathogens in vivo and in vitro (Fawole et al., 2021; Mohamed et al., 2021; Wu et al., 2021).

Largemouth bass (*Micropterus salmoides*) belongs to the family Centrarchidae, and the genus *Micropterus*. It is originally native to Canada and the United States and has now become a major cultured fish in China, with a national production of over 700,000 tonnes in 2022 (Fisheries Bureau of the Ministry of Agriculture and Rural Affairs, 2022). Previous studies indicate this species may possess the ability to synthesize eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from linolenic acid, which is essential for their physiological needs and consumers (Yadav et al., 2020). Another finding suggested that the growth performance of this fish was not significantly affected when the dietary n-3/n-6 fatty acid ratio ranged from 0.1 to 1.0 (Subhadra et al., 2006). Similarly, the growth indicators, feed utilization, and survival of *M. salmoides* were unaffected by different dietary lipid sources, including coconut oil, rapeseed oil, linseed oil, and fish oil (Liang et al., 2022). These results imply that various non-fish oils could be both cost- and function-effective to be incorporated into the diets of *M. salmoides*. Accordingly, more candidate dietary oil sources, such as BSFO, and its potential nutritional value and application technology for *M. salmoides* require comprehensive examination.

The objective of this study was to investigate the feasibility of using BSFO as a partial or complete replacement for fish oil and soy oil in the diet of *M. salmoides* based on the assessment of various parameters related to growth performance, flesh quality, and health status.

2. Materials and methods

2.1. Animal ethics statement

The animal management procedures followed the guidelines of the Animal Care and Use Committee of Zhongkai Agricultural Engineering University (approval number: ZHKUMO-2022-055). The animal experiments comply with the ARRIVE guidelines.

2.2. Experimental diets

Six iso-nitrogenous (46% crude protein) and isolipidic (10% crude lipid) diets were formulated by using fish meal and soybean meal as the main protein sources and fish oil, soy oil, and BSFO as the main lipid sources. The mixture of fish oil and soybean oil (1/2, wt/wt) was gradually replaced by BSFO at ratios of 0%, 20%, 40%, 60%, 80%, and 100% in the six experimental diets, labeled as Control, BSFO20, BSFO40, BSFO60, BSFO80, and BSFO100, respectively. All the solid ingredients were finely smashed, and passed through a 60-mesh sieve, and then thoroughly mixed with liquid ingredients before being extruded into pellets with a diameter of 2 mm. The pellets were dried at 50 °C and stored at −20 °C until use. The formulation, measured value of the proximate composition, and fatty acid profile of the experimental diets are presented in Table 1.

2.3. Experimental fish and the feeding trial

The larvae of *M. salmoides* were obtained from Yangshan County Liyang Aquatic Technology Co. Ltd., Qingyuan, Guangdong Province. The fish were maintained in square net cages (2.0 m × 2.0 m × 2.0 m) installed in a freshwater pond covering an area of 3333 m^2. The fish were fed with the commercial diet (crude protein 45%, crude lipid 8%) to apparent satiation for 2 weeks for acclimation. A total of 960 apparent healthy fish (initial mean weight of 16.5 g) were selected and randomly divided into 6 groups. The fish were assigned to 24 cages (1.5 m × 1.5 m × 1.5 m), that is 40 fish per cage. Six experimental diets were fed to fish in groups twice daily (06:30 and 17:00) to apparent satiation. The feeding trial lasted for eight weeks. During this period, water temperature varied from 32 to 36 °C, and dissolved oxygen and ammonia levels were monitored daily and maintained at 5.5 ± 0.3 mg/L and 0.02 ± 0.01 mg/L, respectively, by continuous aeration into each cage through an air compressor.

2.4. Sample collection

At the end of the 8-week feeding trial, the experimental fish were fasted for 24 h. All of the fish from each cage were counted and weighed for weight gain rate (WGR) and survival rate (SR) determination. Twelve fish from each cage were randomly selected, with three fish stored at −40 °C for approximate nutrient composition analysis, and another three fish individually weighed, measured, and dissected to calculate the condition factor (CF), viscera index (VSI) and hepatosomatic index (HSI) calculation. The liver of these fish was immediately removed and stored in liquid nitrogen for gene expression analysis. The left dorsal muscle was completely removed for immediate texture profile analysis. The remaining six fish were anaesthetized with MS-222 and blood samples (0.2 mL per fish) were collected from their tail veins for hematological indicator analysis. To obtain serum, the blood sample was cooled at 4 °C for 2 h before being centrifuged at 1800 × *g* for 15 min. The serum was then removed and stored at −80 °C for further analysis.

2.5. The proximate composition and fatty acid profile analysis

The proximate composition, including the content of dry matter, crude protein, crude lipid, crude ash and crude fiber in both the diets and fish sample, were analyzed in triplicate using the methods 930.15, 984.13, 954.02, 942.05, and 962.09, respectively

Table 1
Formulation and nutritional composition of the experimental diets.

Ingredients, g/kg DM basis	Control	BSFO20	BSFO40	BSFO60	BSFO80	BSFO100
Steamed fish meal[1]	420	420	420	420	420	420
Soybean meal[2]	180	180	180	180	180	180
Soybean protein concentrate[3]	100	100	100	100	100	100
Wheat flour[4]	180	180	180	180	180	180
Soybean oil	40	32	24	16	8	0
Fish oil	20	16	12	8	4	0
BSFO[5]	0	12	24	36	48	60
Monocalcium phosphate	20	20	20	20	20	20
Premix[6]	40	40	40	40	40	40
Proximate composition, % air dry basis						
Dry matter	93.3	92.7	93.5	93.8	93.0	93.0
Crude protein	46.8	46.8	46.6	46.6	46.6	46.5
Crude fat	10.5	10.5	10.6	10.5	10.3	10.6
Crude ash	13.3	13.5	13.4	13.2	13.2	13.5
Crude fiber	2.5	2.2	2.6	2.6	2.3	2.4
Nitrogen-free extract[7]	20.2	19.7	20.3	20.9	20.6	20.0
Gross energy[8], kJ/g	20.3	20.2	20.3	20.3	20.2	20.2
Fatty acid profile[9], % total fatty acids						
C12:0	0.00	2.56	6.22	8.10	10.84	14.47
C14:0	2.99	3.35	4.76	4.00	4.38	4.85
C16:0	15.00	15.83	18.98	17.42	18.3	19.01
C16:1n-9	2.97	3.13	3.62	3.34	3.51	3.68
C18:0	4.27	4.14	3.46	3.86	3.71	3.45
C18:1n-9	24.65	24.08	20.40	22.43	21.4	20.39
C18:2n-6	30.98	28.66	26.03	24.81	22.66	19.97
C18:3n-3	3.96	3.50	2.84	2.72	2.29	1.84
C20:4n-6	0.31	0.34	0.48	0.40	0.44	0.47
C20:5n-3	7.05	6.92	6.43	6.54	6.40	6.44
C22:6n-3	4.03	3.82	3.51	3.3	3.01	2.89
ΣSFA	24.59	28.12	35.32	35.46	39.22	43.58
ΣMUFA	28.48	28.05	24.66	26.44	25.59	24.7
ΣPUFA	46.71	43.62	39.47	37.96	34.99	31.71
Σn3-PUFA	15.11	14.31	12.78	12.56	11.70	11.17
Σn6-PUFA	31.37	29.08	26.51	25.21	23.10	20.44

BSFO = black soldier fly oil; SFA = saturated fatty acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
[1] Steamed fish meal: crude protein 68.1%, crude lipid 8.0%, crude ash 15.3%.
[2] Soybean meal: crude protein 42.0%, crude lipid 0.7%, crude ash 6.1%.
[3] Soybean protein concentrate: crude protein 64.9%, crude lipid 0.8%, crude ash 6.2%.
[4] Wheat flour: crude protein 13.3%, crude lipid 0.7%, crude ash 1.4%.
[5] Black soldier fly oil (BSFO) was obtained by pressing the dried black soldier fly pre-pupal (oil content 36.1%) raised on kitchen waste (provided by Guangzhou An Rui Jie Environmental Protection Technology Co, Ltd., Guangzhou, China). The fatty acid composition (% total fatty acids) of BSFO is: C12:0, 22.63; C14:0, 2.98; C16:0, 18.55; C16:1n-9, 4.20; C18:0, 0.78; C18:1n-9, 10.80; C18:2n-6, 32.33; C18:3n-3, 2.08; C20:4n-6, 0.17; C20:5n-3, 2.76; C22:6n-3, 0.48.
[6] Premix was designed for carnivorous fish by Evergreen Feed Industry, Co., Ltd., Zhanjiang, China. It contained the following per kilogram of premix: vitamin A 4,000,000 IU, vitamin D_3 2,000,000 IU, vitamin E 30 g, vitamin K_3 10 g, vitamin B_1 5 g, vitamin B_2 15 g, vitamin B_6 8 g, calcium pantothenate 25 g, folic acid 2.5 g, biotin 0.08 g, nicotinic acid 40 g, vitamin B_{12} 0.02 g, inositol 150 g, $MgSO_4 \cdot H_2O$ 12 g, KCl 90 g, Met-Cu 3 g, $FeSO_4 \cdot H_2O$ 1 g, $ZnSO_4 \cdot H_2O$ 10 g, $Ca(IO_3)_2$ 0.06 g, Met-Co 0.16 g, $NaSeO_3$ 0.0036 g.
[7] Nitrogen-free extract (%) = 100 - (moisture + crude protein + crude fat + crude ash + crude fiber).
[8] Gross energy (kJ/g) = [crude protein × 0.056 + crude fat × 0.094 + (100 - crude protein - crude fat - crude ash) × 0.042] × 4.184.
[9] Individual fatty acids with contents below 1.0% are not listed in the Table.

(AOAC, 2000). Dry matter content was determined by drying in an oven at 105 °C for 12 h; nitrogen content was determined by the Kjeldahl method; and the crude protein content (N × 6.25) was calculated by the nitrogen content. Crude fat was analyzed by the Soxtec extractor method with the Soxtec System (Tecator, Hoganas, Sweden). Crude ash content was analyzed by drying samples at 550 °C by using a muffle furnace.

To determine the fatty acid profiles in diet and muscle samples, freeze-dried samples were saponified and esterified to obtain fatty acid methyl esters according to the method described by Ramos-Bueno et al. (2016) and then analyzed using capillary column gas chromatography (7890A gas chromatograph, Agilent). Using a DB-23 column (30 m × 0.25 mm × 0.20 μm; Agilent) under the following conditions: detector, hydrogen flame detector (FID); injection volume, 1 μL; inlet heater, 260 °C; split ratio, 35:1; detector heater, 280 °C; H_2 flow, 40 mL/min; air flow, 400 mL/min; purge flow, 25 mL/min; gas, 99.999% pure; air pressure, 0.4 MPa; N_2 pressure, 0.5 to 0.8 MPa; H_2 pressure, 0.3 to 0.4 MPa. The results were expressed as percentages of total fatty acids calculated by area normalization method.

2.6. Determination of flesh pH value, water holding capacity and texture properties

The pH, drip loss rate, freezing loss rate and steaming loss rate of dorsal muscle were determined following the method described by Zhou et al. (2021). Seven textural parameters including hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness and resilience were measured using a texture analyzer (TA. XT. plus, Stable Micro System, UK). The analyzer was set with the following working parameters: cylindrical stainless-steel probe with a diameter of 5 mm; pre-, mid- and post-test speeds of 1, 2 and 5 mm/s, respectively; the strain mode: downward pressure distance of 75% of the sample thickness, with 2 downward presses for each sample and a time interval of 5 s; the trigger mode was automatic, and the trigger force was set at 5 g.

2.7. Hematological indicators determination

Ten hematological indicators were measured using a fully automatic biochemical analyzer (HITACHI, model 7170, Japan),

following the manufacturer's instructions for the test kits, which were total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bile acids (TBA), alkaline phosphatase (ALP), glucose (GLU), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and free fatty acids (FFA), respectively.

2.8. RT-qPCR analysis

The expression levels of 12 genes related to inflammation (*IL-1β*, *TNF-α*, *IL-15*, *TGF-β*, *IL-10*) and apoptosis (*BAX*, *BAD*, *Bcl-2*, *BAG*, *Bcl-xL*, *caspase3*, *caspase8*, *caspase9*) were determined using qPCR analysis. Total RNA was extracted from liver samples using the TransZol Up Plus RNA Kit (TransGen Biotech), and its concentration was determined with the micro-UV-Vis spectrophotometer NanoDrop One (Thermo Scientific). The RNA was then reversed to cDNA using the TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix kit (TransGen Biotech, Beijing, China). The relative expression of target genes was detected by RT-qPCR following the instructions of the PrefectStart Green qPCR SuperMix kit (TransGen Biotech, Beijing, China). With the β-actin gene used as a reference for relative gene expression, the $2^{-\Delta\Delta Ct}$ technique was utilized to calculate the relative mRNA expression levels of the target genes. The sequence, fragment size, and annealing temperature of various primers are listed in Table 2.

2.9. Bacterial challenge test

At the end of the feeding trial, 30 fish from each group were randomly selected and divided into three replicates, with 10 fish in each PVC tank, with each tank containing 250 L clean water. The single colony of *Aeromonas veronii* used in the challenge test was originally isolated from *M. salmoides* and reserved in the Guangzhou Key Laboratory of Aquatic Animal Diseases and Waterfowl in Guangzhou, Guangdong, China. A concentration of 1.8×10^7 CFU/mL, which resulted in approximately 50% fish mortality according to pre-experiment data, was injected intraperitoneally at a dose of 0.2 mL per fish. Fish mortality was observed and recorded daily for seven continuous days. During this period, each tank was continuously aerated and approximately 20% of the total water in each tank was renewed daily to ensure a qualified water environment for the experimental fish.

2.10. Statistical analysis

The data obtained were analyzed using the statistical software SPSS 23.0. The test data were expressed as mean ± standard error (SE). The results were analyzed using a one-way ANOVA followed by Duncan's multiple comparisons to determine the significance of differences between the data. A significant level of difference was set at $P < 0.05$.

With the dietary BSFO substitution ratio (%) as an independent variable (x), and the values of growth, flesh quality, and health status related parameters as dependent variables (y), one-variable linear regression analysis was performed to evaluate the effects of BSFO inclusion in diets on the growth and health status of fish. Initially, scatter plots with regression lines for each parameter were first generated, and the best-fit regression model was then selected based on the adjusted R^2 value. P-values < 0.05 indicate a significant influence of the independent variable on the dependent variable.

3. Results

3.1. Growth performance and body indices

As seen in Table 3, after the 8-week growth trial, the fish in all the BSFO substitution groups achieved a SR of 86% to 90%, which was significantly higher than of the control group (80%) ($P < 0.05$). Similarly, all the BSFO substitution groups showed a significant

Table 2
The sequences of gene primers used for RT-qPCR.

Gene	Prime sequence (5′-3′)	Fragment size, bp	Annealing temperature, °C
IL-1β	F: ATCATCTACAACTCAGTAGCGTTCA R: TTGCTTTCACAGACGGGATAGTC	119	56.7
TNF-α	F: CTTCGTCTACAGCCAGGCATCG R: TTTGGCACACCGACCTCACC	161	59.4
IL-10	F: CGGCACAGAAATCCCAGAGC R: CAGCAGGCTCACAAAATAAACATCT	119	55.3
IL-15	F: GTATGCTGCTTCTGTGCCTGG R: AGCGTCAGATTTCTCAATGGTGT	82	56.2
TGF-β	F: GCTCAAAGAGAGCGAGGATG R: TCCTCTACCATTCGCAATCC	118	55.3
Bcl-2	F: CCATCCACGACGAACCTG R: GGCGTATCGCTGCTCAAACT	75	57.1
Bcl-xL	F: CATCCTCCTTGGCTCTGG R: GGGTCTGTTTGCCTTTGG	141	54.5
BAX	F: AAATGTGGGAGCCAGACATC R: AGGCTCCTGGTCTCCTTCTC	112	53.3
BAD	F: CACATTTCGGATGCCACTAT R: TTCTGCTCTTCTGCGATTGA	116	53.3
caspase3	F: GCTTCATTCGTCTGTGTTC R: CGAAAAAGTGATGTGAGGTA	98	52.8
caspase8	F: GAGACAGACAGCAGACAACCA R: TTCCATTTCAGCAAACACATC	195	57.6
caspase9	F: CTGGAATGCCTTCAGGAGACGGG R: GGGAGGGGCAAGACAACAGGGTG	125	63.3
Beta-actin	F: TGGCTACTCCTTCACCACCACAG R: GAAGTCCAGGGCGACATAGCACA	82	61.6

IL-1β = interleukin-1β; *TNF-α* = tumor necrosis factor-α; *IL-10* = interleukin-10; *IL-15* = interleukin-15; *TGF-β* = transforming growth factor beta; *Bcl-2* = B-cell lymphoma-2; *Bcl-xL* = BCL-xl associated death promoter; *BAX* = BCL-2 associated X; *BAD* = BCL-2 associated death promoter; *caspase3* = cysteinyl aspartate specific proteinase 3; *caspase8* = cysteinyl aspartate specific proteinase 8; *caspase 9* = cysteinyl aspartate specific proteinase 9..

Table 3
Growth performance and body indices of *Micropterus salmoides* fed with six experimental diets.

Indicators	Diets					
	Control	BSFO20	BSFO40	BSFO60	BSFO80	BSFO100
IBW, g	16.50 ± 0.00	16.50 ± 0.00	16.50 ± 0.00	16.50 ± 0.00	16.50 ± 0.00	16.50 ± 0.00
FBW, g	58.63 ± 0.93^{bc}	59.41 ± 1.06^{abc}	60.52 ± 1.40^{abc}	58.05 ± 0.95^{c}	62.24 ± 1.88^{ab}	62.76 ± 0.67^{a}
SR, %	80.00 ± 5.00^{b}	90.00 ± 1.80^{a}	86.30 ± 3.30^{ab}	86.30 ± 3.10^{ab}	87.50 ± 2.00^{ab}	86.90 ± 1.90^{ab}
FI, g	1485.20 ± 20.71^{b}	1660.68 ± 53.98^{a}	1669.48 ± 38.42^{a}	1672.51 ± 14.70^{a}	1678.32 ± 14.77^{a}	1673.41 ± 13.81^{a}
FCR, g/g	1.04 ± 0.04	1.05 ± 0.01	1.04 ± 0.04	1.10 ± 0.07	1.02 ± 0.01	1.00 ± 0.01
WGR, %	186.15 ± 17.04^{b}	224.12 ± 9.78^{ab}	217.15 ± 18.98^{ab}	203.51 ± 12.59^{ab}	229.43 ± 3.74^{a}	230.18 ± 3.72^{a}
VSI, %	7.87 ± 0.12	7.68 ± 0.09	8.00 ± 0.21	7.51 ± 0.32	8.25 ± 0.08	8.29 ± 0.43
HSI, %	1.29 ± 0.07^{ab}	1.08 ± 0.04^{b}	1.31 ± 0.05^{ab}	1.16 ± 0.06^{ab}	1.34 ± 0.07^{a}	1.37 ± 0.12^{a}
CF, g/cm^3	2.08 ± 0.02^{ab}	2.02 ± 0.02^{b}	2.09 ± 0.04^{ab}	2.12 ± 0.01^{ab}	2.23 ± 0.07^{a}	2.28 ± 0.11^{a}

BSFO = black soldier fly oil; IBW = initial body weight; FBW = final body weight; SR = survival rate; FI = feed intake during the feeding trial; FCR = feed conversion ratio; WGR = weight gain rate; VSI = viscera index; HSI = hepatosomatic index; CF = condition factor.
Values are mean ± SE (n = 4). Different lowercase letters in the same row indicate statistical differences ($P < 0.05$).
SR (%) = final number of fish/initial number of fish × 100.
FCR = feed intake/(final body weight − initial body weight).
WGR (%) = (final body weight - initial body weight)/initial weight × 100.
VSI (%) = visceral weight/body weight × 100.
HSI (%) = liver weight/body weight × 100.
CF (%) = weight/body length^3 × 100.

increase (9.3% to 23.7%) in WGR compared to that of the control ($P < 0.05$). HSI and CF also appeared to increase with higher levels of BSFO, with both being significantly higher in the BSFO80 and BSFO100 groups than in the BSFO20 group ($P < 0.05$). However, there was no significant difference in FCR among the six experimental groups ($P > 0.05$).

3.2. Approximate composition and fatty acid profile of fish

As shown in Table 4, no significant differences were observed among the six groups in terms of moisture content, crude protein content, crude fat content, and crude ash content of the whole fish ($P > 0.05$). However, the fatty acid profiles of the fish muscle were significantly impacted by the compositions of the diets ($P < 0.05$). In particular, the fatty acid profiles in the dorsal muscle were significantly different ($P < 0.05$). Generally, the fatty acid profiles in the dorsal muscle seemed to mirror the dietary fatty acid profiles. The proportions of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in the dorsal muscle reduced slightly (from 27.2% to 25.2%, and 39.6% to 33.5%, respectively) in accordance with the levels in the diets ($P < 0.05$). Conversely, the proportions of saturated fatty acids (SFA), including C12:0, C14:0, C16:0, and C18:0, significantly increased (from 28.8% to 37.6%) as the levels in the diets increased ($P < 0.05$). Notably, although the dietary proportions of n3-PUFA declined linearly (from 15.1% to 11.2%) with increased substitution of BSFO, the proportions of these fatty acids, particularly C20:5n-3 and C22:6n-3, did not show any significant differences in the dorsal muscle ($P > 0.05$).

3.3. pH value, water holding capacity, and textural properties of flesh

The quality indicators of the dorsal muscle, including pH value and water holding capacity (the freezing loss rate, cooking loss rate, and drip loss rate) were measured. As shown in Table 5, the pH value of the dorsal muscle was not significantly affected by diets ($P > 0.05$). However, the freezing loss rate, cooking loss rate, and drip loss rate, all of which are associated with water-holding capacity, progressively decreased as the level of dietary BSFO increased.

Seven indicators related to textural property were determined in the dorsal muscle (Table 6). Except for springiness and resilience, the remaining five indicators were significantly influenced by diets. In comparison to the control group, all BSFO substitution groups exhibited higher values for hardness, adhesiveness, cohesiveness, gumminess, and chewiness ($P < 0.05$). Interestingly, the BSFO20 group had the highest values for all these indicators, outperforming the other BSFO substitution groups ($P < 0.05$).

3.4. Hematological indicators

A total of 10 hematological indicators of fish serum were measured (Table 7). Five of these indicators, TC, TBA, ALP, TG, and LDL, did not exhibit significant differences among the groups ($P > 0.05$). However, there was a notable decline in the activities of ALT and AST with the increasing levels of BSFO substitution ($P < 0.05$). In contrast, the concentration of HDL and FFA demonstrated a significant increase in most groups with BSFO substitution ($P < 0.05$). Conversely, the concentration of GLU was significantly reduced in the groups of BSFO60, BSFO80, and BSFO100 ($P < 0.05$) (Table 7).

3.5. Gene expression in the liver

The expression levels of 12 genes in the liver related to inflammatory cytokines and apoptosis were measured (Fig. 1). It was found that, compared with the control group, the expression of *IL-1β*, *TNF-α*, *Bcl-xl*, *BAX*, and *caspase8* genes was significantly down-regulated in all the BSFO substitution groups ($P < 0.05$), the former two genes were related to inflammatory cytokines, and the later three were related to apoptosis. Whereas, the expression of *IL-10* (gene related to inflammatory) was significantly up-regulated in the BSFO80 and BSFO100 groups ($P < 0.05$). No significant differences were observed in the expression of the remaining six genes (*IL-15*, *TGF-β*, *Bcl-2*, *BAD*, *caspase3*, and *caspase9*) among groups ($P > 0.05$).

3.6. Resistance against bacteria

After being exposed to bacteria, the experimental fish showed a cumulative SR of 10.0%, 26.7%, 13.3%, 40.0%, 60.0%, and 66.7% in the control, BSFO20, BSFO40, BSFO60, BSFO80, and BSFO100 groups, respectively (Fig. 2). The results demonstrate that dietary BSFO significantly improved the resistance of fish to *A. veronii* infection ($P < 0.05$). Furthermore, the effect of BSFO on enhancing resistance exhibited a notable dose-dependent relationship.

Table 4
Proximate composition of the whole body and fatty acid profiles of the dorsal muscle for *Micropterus salmoides* fed with six experimental diets.

Indicators	Diets					
	Control	BSFO20	BSFO40	BSFO60	BSFO80	BSFO100
Proximate composition of the whole body, % wet basis						
Dry matter	28.3 ± 0.3	28.4 ± 0.3	29.0 ± 0.4	28.4 ± 0.6	28.7 ± 0.8	28.4 ± 0.6
Crude protein	17.4 ± 0.2	17.2 ± 0.1	17.3 ± 0.2	16.9 ± 0.3	17.1 ± 0.3	17.2 ± 0.1
Crude fat	6.0 ± 0.1	6.4 ± 0.1	6.9 ± 0.2	6.4 ± 0.1	6.5 ± 0.1	6.2 ± 0.2
Crude ash	4.4 ± 0.1	4.3 ± 0.0	4.3 ± 0.1	4.4 ± 0.0	4.3 ± 0.1	4.4 ± 0.1
Fatty acid profile of the dorsal muscle, % total fatty acids						
C12:0	0.00^{f}	1.01^{e}	1.95^{d}	2.62^{c}	3.63^{b}	4.24^{a}
C14:0	2.48^{d}	2.93^{c}	3.35^{b}	3.35^{b}	3.83^{a}	3.91^{a}
C16:0	19.87^{e}	19.41^{e}	20.47^{d}	21.41^{c}	21.92^{b}	22.91^{a}
C16:1n-9	2.70^{b}	2.93^{ab}	3.04^{ab}	2.92^{ab}	3.18^{a}	3.15^{a}
C18:0	6.42	5.42	5.49	6.21	5.88	6.55
C18:1n-9	24.53^{a}	24.62^{a}	24.30^{a}	23.18^{b}	23.06^{b}	22.37^{b}
C18:2n-6	24.60^{ab}	25.54^{a}	23.96^{b}	21.65^{c}	20.40^{d}	18.21^{e}
C18:3n-3	2.23^{ab}	2.33^{a}	2.07^{b}	1.65^{c}	1.53^{c}	1.14^{d}
C20:4n-6	0.60^{bc}	0.53^{c}	0.57^{bc}	0.79^{ab}	0.76^{abc}	0.91^{a}
C20:5n-3	1.96	1.88	1.74	1.86	1.81	1.83
C22:6n-3	10.24	9.44	9.47	10.50	10.33	11.35
∑SFA	28.77^{e}	28.78^{e}	31.26^{d}	33.59^{c}	35.26^{b}	37.60^{a}
∑MUFA	27.22^{a}	27.55^{a}	27.34^{a}	26.09^{ab}	26.24^{ab}	25.52^{b}
∑PUFA	39.62^{a}	39.73^{a}	37.80^{b}	36.45^{b}	34.83^{c}	33.45^{c}
∑n3-PUFA	14.43	13.65	13.27	14.01	13.67	14.33
∑n6-PUFA	25.19^{a}	26.08^{a}	24.53^{b}	22.44^{b}	21.16^{c}	19.12^{c}

BSFO = black soldier fly oil; SFA = saturated fatty acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
Values are mean ± SE ($n = 3$). Individual fatty acids whose content below 1.0% are not list in the Table. Different lowercase letters in the same row indicate statistical differences ($P < 0.05$).

Table 5
pH value and water holding capacity of the dorsal muscle of *Micropterus salmoides* fed with six experimental diets.

Indicators	Diets					
	Control	BSFO20	BSFO40	BSFO60	BSFO80	BSFO100
pH	6.03 ± 0.02	6.02 ± 0.01	6.04 ± 0.01	6.06 ± 0.01	6.05 ± 0.01	6.05 ± 0.02
Freezing loss rate, %	3.97 ± 0.16^{a}	3.30 ± 0.35^{ab}	3.22 ± 0.40^{ab}	3.15 ± 0.9^{ab}	3.15 ± 0.44^{ab}	2.42 ± 0.34^{b}
Cooking loss rate, %	19.59 ± 2.46	19.92 ± 0.94	18.41 ± 1.8	17.62 ± 3.00	17.80 ± 2.36	17.14 ± 4.35
Drip loss rate, %	3.00 ± 0.14	3.01 ± 0.65	3.01 ± 0.15	2.71 ± 0.06	2.71 ± 0.27	2.63 ± 0.80

BSFO = black soldier fly oil.
Values are mean ± SE ($n = 3$). Different lowercase letters in the same row indicate statistical differences ($P < 0.05$).

Table 6
Textural properties of the dorsal muscle of *Micropterus salmoides* fed with six experimental diets.

Indicators	Diets					
	Control	BSFO20	BSFO40	BSFO60	BSFO80	BSFO100
Hardness, g	428.90 ± 22.93^{b}	517.24 ± 45.96^{a}	442.43 ± 13.53^{ab}	453.93 ± 3.46^{ab}	472.47 ± 22.81^{ab}	459.83 ± 17.84^{ab}
Adhesiveness, g	−47.53 ± 8.31^{ab}	−36.83 ± 3.00^{a}	−52.97 ± 9.39^{ab}	−60.28 ± 9.39^{b}	−37.55 ± 3.08^{a}	−46.92 ± 3.79^{ab}
Springiness, mm	0.82 ± 0.09	0.92 ± 0.02	0.81 ± 0.08	0.87 ± 0.05	0.91 ± 0.01	0.94 ± 0.01
Cohesiveness	0.31 ± 0.02^{b}	0.42 ± 0.05^{a}	0.31 ± 0.03^{b}	0.26 ± 0.01^{b}	0.30 ± 0.02^{b}	0.30 ± 0.02^{b}
Gumminess, g	145.67 ± 16.78^{b}	214.81 ± 38.84^{a}	138.38 ± 11.29^{b}	100.09 ± 4.40^{b}	125.59 ± 4.56^{b}	136.72 ± 12.53^{b}
Chewiness, mJ	100.42 ± 7.84^{b}	187.78 ± 43.54^{a}	103.54 ± 17.69^{b}	84.54 ± 9.30^{b}	114.71 ± 5.25^{b}	123.07 ± 14.54^{b}
Resilience	0.05 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00

BSFO = black soldier fly oil.
Values are mean ± SE ($n = 4$). Different lowercase letters in the same row indicate statistical differences ($P < 0.05$).

3.7. Statistical evaluation of dietary BSFO substitution levels on various parameters in fish

One variable linear regression analysis was conducted to evaluate the influence of dietary BSFO substitution levels on growth, flesh quality, and health status-related parameters. It was revealed that FBW, WGR, the expression of *IL-10*, and the cumulative survival rate after being exposed to bacteria were positively influenced by dietary BSFO substitution levels, while the freezing loss rate, the expression of *TNF-α*, *BAX*, and *BCL-xl* were negatively influenced by dietary BSFO substitution levels (Fig. 3). These results indicated that, the growth, flesh quality, and health status of fish were widely and significantly influenced by dietary BSFO in a dose-independent manner.

4. Discussion

4.1. Growth performance and body indices affected by dietary BSFO

The feasibility of replacing fish oil or soybean oil with BSFO has been evidenced in several studies on different fish species (Fawole et al., 2021; Hender et al., 2021; Hu et al., 2020; Xu et al., 2021). In

Table 7
Hematological indicators in serum of *Micropterus salmoides* fed with six experimental diets.

Indicators	Diets					
	Control	BSFO20	BSFO40	BSFO60	BSFO80	BSFO100
TC, mmol/L	6.99 ± 0.42	7.81 ± 0.37	8.44 ± 0.51	8.33 ± 0.61	8.39 ± 0.50	7.41 ± 0.52
ALT, U/L	9.00 ± 1.00^{a}	8.70 ± 2.19^{a}	7.30 ± 1.21^{ab}	5.00 ± 0.71^{b}	4.80 ± 0.48^{b}	4.50 ± 0.65^{b}
AST, U/L	163.00 ± 14.84^{a}	161.00 ± 11.48^{a}	127.30 ± 7.87^{ab}	112.80 ± 12.13^{b}	127.30 ± 6.56^{ab}	122.60 ± 15.68^{b}
TBA, mmol/L	40.80 ± 10.26	38.40 ± 4.15	43.70 ± 8.60	45.30 ± 22.53	44.70 ± 6.59	47.50 ± 12.73
ALP, U/L	137.30 ± 10.64	141.00 ± 6.15	157.30 ± 6.76	142.00 ± 12.38	153.80 ± 10.56	143.30 ± 6.69
GLU, mmol/L	8.80 ± 0.98^{a}	9.63 ± 0.38^{a}	9.10 ± 0.82^{a}	6.20 ± 0.64^{b}	5.55 ± 0.35^{b}	5.70 ± 0.76^{b}
TG, mmol/L	6.02 ± 0.34	5.63 ± 0.22	7.24 ± 0.53	6.12 ± 0.64	7.27 ± 0.45	6.09 ± 0.25
HDL, mmol/L	3.00 ± 0.11^{b}	3.40 ± 0.13^{ab}	3.34 ± 0.12^{ab}	3.56 ± 0.20^{a}	3.26 ± 0.14^{ab}	3.18 ± 0.13^{ab}
LDL, mmol/L	1.37 ± 0.06	1.65 ± 0.10	1.44 ± 0.14	1.50 ± 0.19	1.37 ± 0.22	1.40 ± 0.13
FFA, mmol/L	0.69 ± 0.05^{b}	0.78 ± 0.05^{ab}	0.78 ± 0.06^{ab}	0.81 ± 0.04^{ab}	0.92 ± 0.07^{a}	0.85 ± 0.07^{ab}

BSFO = black soldier fly oil; TC = total cholesterol; ALT = alanine aminotransferase; AST = aspartate aminotransferase; TBA = total bile acids; ALP = alkaline phosphatase; GLU = glucose; TG = triglyceride; HDL = high-density lipoprotein; LDL = low-density lipoprotein; FFA = free fatty acids.
Values are mean ± SE (n = 4). Different lowercase letters in the same row indicate statistical differences (P < 0.05).

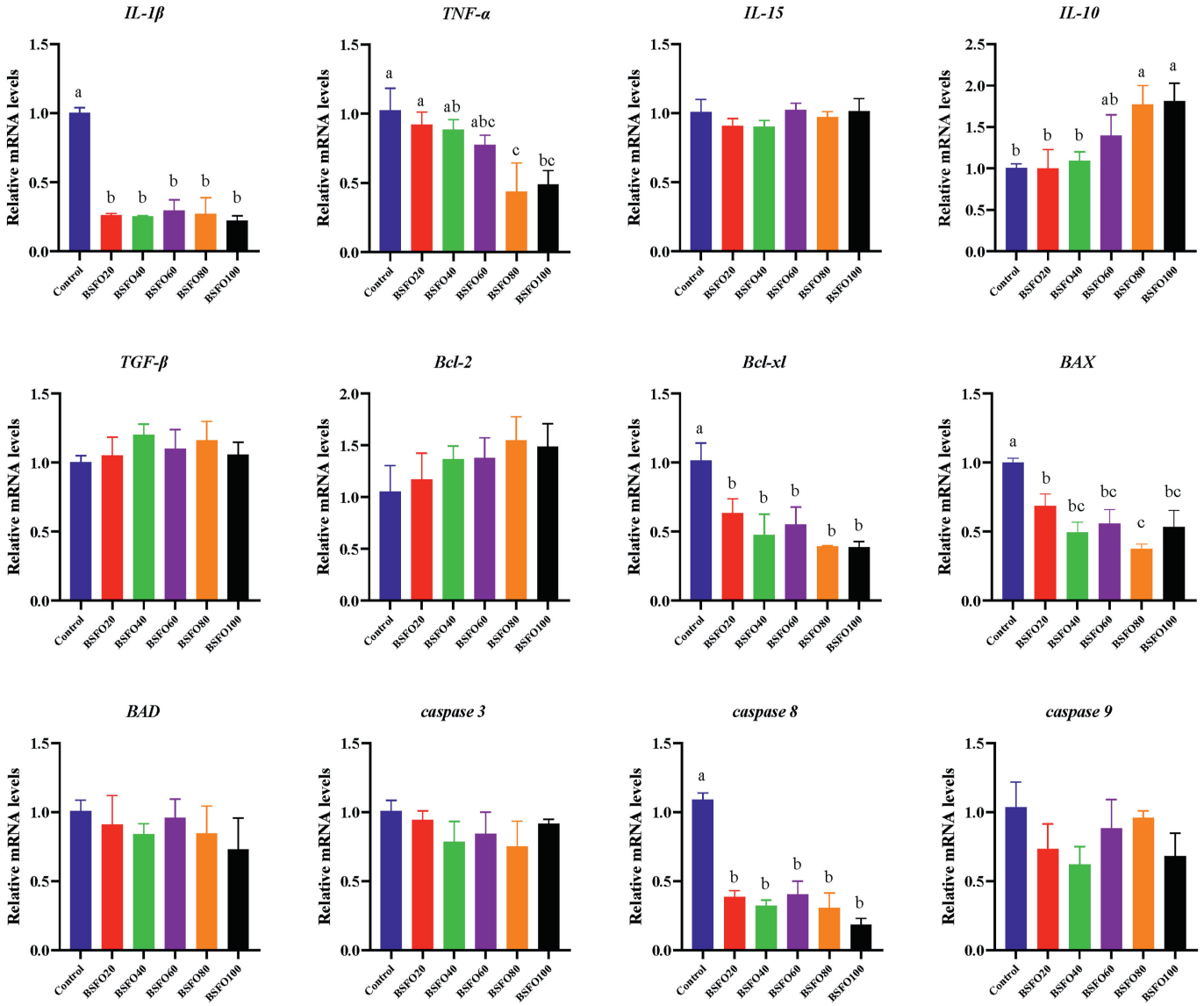

Fig. 1. Expression of 12 genes in the liver of *Micropterus salmoides* fed with six experimental diets. Interleukin-1β (*IL-1β*), tumor necrosis factor-α (*TNF-α*), interleukin-10 (*IL-10*), interleukin-15 (*IL-15*), and transforming growth factor beta (*TGF-β*) are genes related to inflammatory reactions, and B-cell lymphoma-2 (*Bcl-2*), BCL-xl associated death promoter (*Bcl-xl*), BCL-2 associated X (*BAX*), Bcl-2 associated death promoter (*BAD*), cysteinyl aspartate specific proteinase3 (*caspase3*), cysteinyl aspartate specific proteinase 8 (*caspase8*) and cysteinyl aspartate specific proteinase 9 (*caspase9*) are genes related to apoptosis. The gene expression data were compared with the control group and represented as the mean fold change ± standard error (SE) (n = 6). Bars with different lower cases means significant differences among groups (P < 0.05). BSFO = black soldier fly oil.

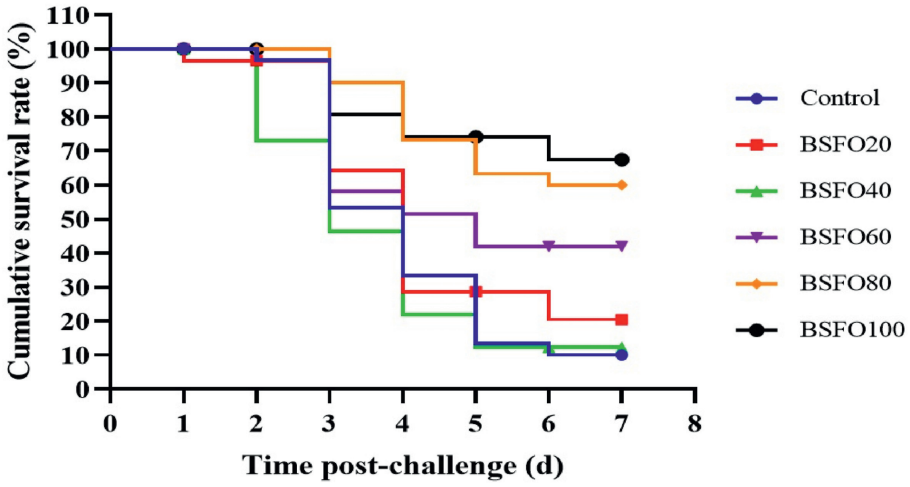

Fig. 2. The post-challenge survival rate of *Micropterus salmoides* infected with *Aeromonas veronii*. The survival rate of fish within seven days were recorded and analyzed, and the cumulative survival rate is expressed as the following: cumulative survival rate (%) = 100 × final number of fish survivor/initial number of inoculated fish. BSFO = black soldier fly oil.

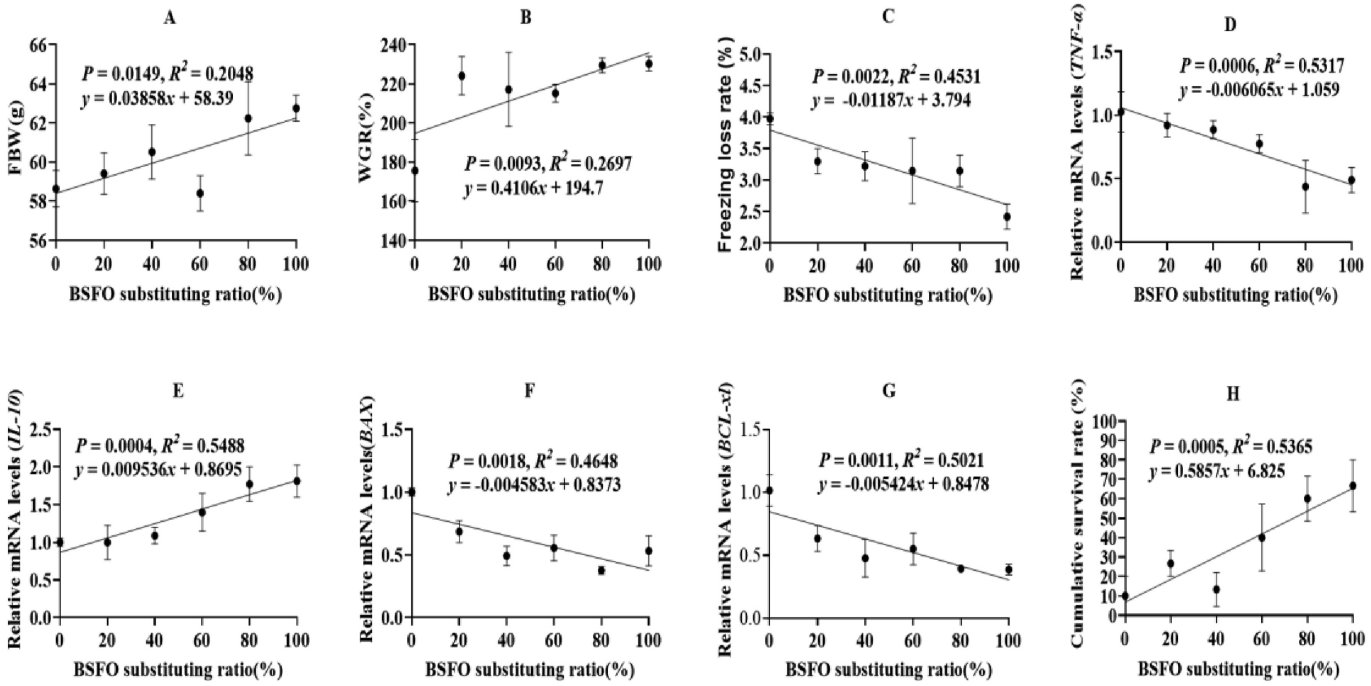

Fig. 3. Graphical representation of some parameters in fish in relation to dietary black soldier fly oil (BSFO) substituting ratio. (A) Final body weight (FBW) of fish in relation to dietary BSFO substituting ratio. (B) Weight gain rate (WGR) of fish in relation to dietary BSFO substituting ratio. (C) Freezing lose rate of fish muscle in relation to dietary BSFO substituting ratio. (D, E, F, G) The expression levels of tumor necrosis factor-α (*TNF-α*), interleukin-10 (*IL-10*), BCL-2 associated X (*BAX*), BCL-xl associated death promoter (*Bcl-xl*) in fish liver in relation to dietary BSFO substituting ratio. (H) The cumulative survival rate after being exposed to bacteria in relation to dietary BSFO substituting ratio. The linear regression equations are presented in each panel, accompanied with the fitting lines. *P*-values < 0.05 mean a significant influence by dietary BSFO substituting ratio.

the current study, the substitution of BSFO led to higher values of FBW, WGR, and SGR in *M. salmoides*, with a remarkable improvement of 23.1% and 23.7% in WGR in groups of BSFO80 and BSFO100 compared to the control group. The findings are consistent with the research on juvenile mirror carp, where the inclusion of 2.5% BSFO in the diets resulted in positive effects on fish growth (Xu et al., 2021). Similarly, Fawole et al. (2021) demonstrated that BSLO can be used as a complete replacement for fish oil and soybean oil in the diet of rainbow trout without adverse effects on growth performance, whole-body composition, or nutrient retention. Furthermore, Hender et al. (2021) found that when 30% fish meal was substituted with BSF protein and 30% fish oil was replaced with BSFO in the diets of *Lates calcarifer*, there was no significant impact on growth and feed utilization indices. The positive effects of dietary BSFO on growth enhancement may be attributed to its unique fatty acid profile. Specifically, BSFO is abundant in short- and medium-chain saturated fatty acids that are well-known for their rapid digestibility, absorption, and conversion into energy, ultimately promoting animal growth (Belghit et al., 2018; Schonfeld and Wojtczak, 2016).

Although the proximate composition of fish was not influenced by dietary BSFO, a general trend towards increased body indices

(VSI, HIS, and CF) was observed when BSFO was present in diets containing high levels of BSFO (BSFO80 and BSFO100). This increase may be attributed to the deposition of lipids in the tissues induced by the limited utilization by fish in effectively utilizing SFA, which accounted for 38.7% and 43.2% of the total fatty acids in the diets of BSFO80 and BSFO100, respectively. Similar findings have been reported in European sea bass, *T. macdonaldi*, *hybrid sturgeon*, and GIFT *Oreochromis niloticus*, where the incorporation of large amounts of fat sources rich in SFA, for example, lard oil, silkworm pupal oil, palm oil, beef fat, or black soldier fly oil, resulted in increased body indices (BAI et al., 2017; Li et al., 2021; Maldonado-Othón et al., 2022). Additionally, Xu (2014) found that the growth and liver health of Japanese seabass were impaired when 10% palmitic acid or stearic acid were used as the sole dietary lipid sources. Previous studies have primarily emphasized the practical impact of utilizing various fat sources in the diets of *M. salmoides*. However, there is a scarcity of data concerning the specific fatty acid requirements of this species, which necessitates further investigation to ensure the precise formulation of potential fat sources.

4.2. Flesh quality affected by dietary BSFO

The n-3 long-chain PUFA (LC-PUFA) content in the farmed fish is a major concern for consumers regarding flesh quality. Fatty acid profiles in fish tissue can be greatly influenced by the feed they receive. An encouraging observation of this study revealed that the main n-3 LC-PUFA, particularly C20:5n-3 and C22:6n-3, in the dorsal muscle of *M. salmoides* were consistently similar among different groups. It is generally accepted that freshwater fish have significant LC-PUFA biosynthetic capacity and that linoleic and linolenic acids can be converted to EPA and DHA by the desaturation and elongation reactions catalyzed by fatty acyl desaturases and very long-chain fatty acid proteins (Xie et al., 2021). Compared to PUFA, SFA and MUFA are preferentially oxidized for energy production, thus avoiding the energy metabolism of the n-3 LC-PUFA, a process known as the "omega-3 sparing effect", which also results in greater retention of the n-3 LC-PUFA in the muscle (Rombenso et al., 2021; Turchini et al., 2011). This study provides additional evidence that n-3 LC-PUFA could be synthesized within freshwater fish in vivo (Agaba et al., 2005), combined with the effects of the preferential oxidation of SFA, which therefore selectively retained n-3 LC-PUFA in the flesh (Trullas et al., 2017). In contrast, when dietary BSFO was introduced, there was a linear increase in the content of medium-chain SFA (MC-SFA) in the fish muscle, predominantly C12:0, C14:0, and C16:0. These findings imply that, even when fish oil and soybean oil are completely replaced by BSFO in *M. salmoides* diets, the flesh could provide sufficient n-3 LC-PUFA for consumers. Furthermore, the inclusion of MC-SFA in the fish flesh as a result of dietary BSFO offers the advantage of increasing the presence of "healthy fats" for consumers (Namkung et al., 2011; Ng and Koh, 2016; Wang et al., 2021).

Cooking loss rate, drip loss rate, and freezing loss rate are representative indicators for assessing the liquid holding capacity of meat, which make important contributions to the overall quality of freshen meat in terms of juiciness and tenderness (Brewer, 2014). They showed a linear decreased tendency with the increased substitution levels of BSFO, indicating the elevated liquid holding capacity resulted from dietary BSFO. It is well known that the characteristics of proteins (net charge, myofiber histology, etc.) in flesh are key factors that influence the water-holding capacity of flesh. However, fewer studies have focused on the impact of fatty acid profiles. According to the observations on poultry and livestock, the primary cause of a decrease in water-holding capacity due to feed fat sources is the peroxidation of fat, which damages the cell membrane and consequently impacts the cell's ability to retain water (Dilger et al., 2018; Lou, 2011; Lv et al., 2019; Jiang et al., 2021). Accordingly, the decreased water holding capacity with increasing levels of MUFA and PUFA in the current study seemed consistent with the above observations but needs further proof for confirmation.

The texture characteristics of flesh are commonly evaluated using the texture profile analysis method (Tang et al., 2023), which provides results based on indicators including hardness, adhesiveness, elasticity, cohesiveness, stickiness and chewiness. These indicators are influenced by various properties, such as muscle fiber diameter, muscle tissue density, collagen content in connective tissue, and intramuscular and intermuscular fat (Ye et al., 2022). Previous studies on various fish species have demonstrated that higher values of these indicators indicate superior flesh quality (Hashimoto et al., 2019; Yang et al., 2019; Zhao et al., 2023).

In the current study, it was found that the highest values of hardness, adhesiveness, stickiness, and chewiness were all achieved in the BSFO20 group, rather than in the groups with higher BSFO inclusion. This suggests that a small amount of dietary BSFO can lead to improved texture properties. This interesting finding could not be solely attributed to the increase of flesh SFA, which have higher melting points and can contribute to higher values of hardness, springiness, cohesiveness, and gumminess in the muscles of different fish. Instead, it is more likely to be the results of the combined effects of various fatty acids (particularly stearic acid and linoleic acid), water, and collagen protein concentration in the flesh (Fuentes et al., 2010; Marques et al., 2022; Stejskal et al., 2011; Xu et al., 2016). Therefore, further investigation is required to gather additional data and elucidate the specific effects of the biochemical ingredients present in the flesh on its texture characteristics.

4.3. The health status influenced by dietary BSFO

Hematological indicators could reflect the long-term or short-term status of fish health (Seibel et al., 2021). For instance, the serum AST and ALT activities are typically used as markers of liver health, with their elevation indicating abnormal liver function (Mohieldein et al., 2013). In the current study, a significant decrease in ALT and AST activities was noted as the levels of BSFO substitution increased. This observation is consistent with previous findings in juvenile mirror carp (Xu et al., 2021), Qihe crucian carp (Jia et al., 2022), and *Peltobagrus fulvidraco* (Hu et al., 2020), suggesting the liver function of the preferred fish is not negatively affected by the inclusion of dietary BSFO or even potentially improved.

The inclusion of BSFO was found to significantly reduce the concentration of serum glucose. This reduction may be attributed to the presence of MCFA in BSFO. Previous research has demonstrated that MCFA can decrease insulin resistance and improve glucose tolerance in both mammals and humans. This is achieved by stimulating insulin secretion from pancreatic β cells in response to glucose stimulation through the activation of GPR40, a type of G protein-coupled receptor highly expressed in pancreatic β cells (Defossa and Wagner, 2014; Hira et al., 2009). It is generally acknowledged that fish naturally have insufficient insulin secretion, leading to a poor tolerance to dietary carbohydrates (Stephan et al., 1996). Therefore, the inclusion of MCFA in BSFO may potentially contribute to the improvement of glucose intolerance and the utilization of dietary carbohydrates.

Hematological parameters related to lipid metabolism were minimally affected by diets, except for HDL and FFA. HDL, primarily synthesized by the liver, plays a crucial role in eliminating excess cholesterol from the bloodstream. A decrease in HDL is commonly associated with chronic liver disease. In this study, the inclusion of BSFO in the diets resulted in a slight increase in serum HDL

concentration. FFA in blood are lipid molecules those are not esterified with glycerol, cholesterol, or other lipids. Their increase in serum can be induced by various physiological or pathological factors, such as diabetes, hyperthyroidism, glycogen accumulation, liver damage, starvation, strenuous exercises, etc. In relation to other hematological indicators, the increase of FFA concentration in BSFO inclusion groups may be associated with the higher levels of MCFA present in BSFO. These MCFA can be rapidly hydrolyzed and released into the intestinal lumen, which is particularly important for animals with lipid metabolism disorders as it provides a supply of FFA that can be directly absorbed by the intestinal epithelial cells (Gadsby, 2002).

The expression of genes involved in inflammatory reactions provides valuable information for consideration of animal health status. Specifically, pro-inflammatory cytokines IL-1β and TNF-α were involved in host defense against microbial pathogens (Budhu and Wang, 2006; DePaolo et al., 2011; Wang and Secombes, 2013), while IL-10 acts as an anti-inflammatory cytokine, regulating immune responses and preventing excessive expression of pro-inflammatory cytokines such as *TNF-α*, *IL-1β*, and *IL-8* (Ip et al., 2017). In the present study, an increase in the dietary BSFO led to a decrease in the expression of *IL-1β* and *TNF-α*, along with an increase in the expression of *IL-10* in the liver of *M. salmoides*. These findings are consistent with those observed in *Cyprinus carpio* var. *Specularis* fed with diets containing BSFO (Xu et al., 2021). Another research that the consumption of BSFO induced an active state of inflammation development and inhibition through the increased expression of several anti-inflammatory and pro-inflammatory factors in the serum (Hu et al., 2020). Overall, these results highlight the potential benefits of dietary supplementation with BSFO in regulating the inflammation response of fish, which could be attributed to the increased concentration of C12:0 in the fish body that has anti-inflammatory effects by boosting cellular innate immunity (Spranghers et al., 2018; Stenberg et al., 2019), as observed in some fish species fed with lauric acid (Li et al., 2022b; Tang et al., 2023; Wang et al., 2022).

Genes associated with apoptosis play a vital role in regulating programmed cell death. In particular, caspases, a group of structurally similar proteases found in the cytoplasm, are closely linked to the process of apoptosis. Among these caspases, *caspase8* and *caspase9* act as initiators, while *caspase3* acts as the effector (Broker et al., 2005; Duprez et al., 2009; Van Opdenbosch and Lamkanfi, 2019). In this study, a significant decrease in the expression of the *caspase8* gene in all BSFO inclusion groups is observed. Similarly, the expression of *caspase9* and *caspase3* genes also showed a decrease, although not statistically significant. Furthermore, the expression of the pro-apoptotic gene *BAX* was significantly down-regulated in a dose-dependent manner in all the BSFO inclusion groups. These findings support our previous observations regarding the changes in inflammatory cytokines, as apoptosis and inflammation responses are deeply interconnected in pathological processes (James and Winkler, 1999).

An additional piece of evidence supporting the immune-enhancing properties of BSFO in this study is the significant increase in SR of fish following a bacterial attack. The SR are dependent on the substitution levels of dietary BSFO. Although there is limited research on the disease-resistance effects of dietary BSFO in fish, there have been reports suggesting its potential in this area. For instance, a study by Wu et al. (2021) found that BSFO exhibited inhibitory effects on the proliferation of respiratory syndrome virus in vitro. Besides the well-recognized active ingredient of MCFA rich in the BSFO that treat various diseases by regulating the way of tissue energy utilization, interfering with cell cycle, or activating immune cells (Roopashree et al., 2021), some specific insect-source ingredients in BSFO, such as icosanoic acids, oxylipids, and isoprene like compounds may also work (Richter et al., 2023), the underlying mechanism needs further research.

5. Conclusion

In conclusion, this study demonstrated that BSFO shows great promise as a dietary oil source for *M. salmoides*. By partially or completely replacing fish oil and soybean oil with BSFO, significant improvements were observed in growth performance, flesh quality (increased water holding capacity, improved texture properties), and overall health status (changes in hematological parameters and altered expression of inflammation and apoptosis-related genes, as well as enhanced resistance against bacteria), some of the parameters were dose-dependently changed with the dietary BSFO substituting ratio. To optimize the benefits of using BSFO, our study suggests a small amount of BSFO (around 20% of the dietary oil source) is economically sufficient for enhancing growth and flesh quality. However, in order to enhance immunity and increase resistance against pathogenic infections, a higher inclusion of BSFO in the diet is strongly recommended.

Author contributions

Hailin Yuan, Xiangce Li and **Qiuxuan Sun:** Methodology, Investigation, Data curation, Writing - original draft, Formal analysis. **Xiaohong Tan, Cuihong You and Yewei Dong:** Supervision, Formal analysis, Writing - review & editing. **Meng Zhou, Yanhua Huang** and **Junru Hu:** Supervision, Experiment design, Writing - review & editing, Conceptualization, Funding acquisition, Resources. All the authors read and agreed to publish the final version of the manuscript.

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.

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References

Agaba MK, Tocher DR, Zheng XZ, et al. Cloning and functional characterisation of polyunsaturated fatty acid elongases of marine and freshwater teleost fish. Comp Biochem Physiol B 2005;142(3):342–52.

AOAC. Official methods of analysis. 16th ed. Gaithersburg. MD: Association of Official Analytical Chemists; 2000.

Bai FJ, Luo L, Huang XZ, et al. Comparative study on effects of silkworm pupal oil and other six kinds of lipid on growth, body composition and lipid metabolism of GIFT *Oreochromis niloticus*. J Fish China 2017;41:258–70.

Belghit I, Liland NS, Waagbo R, et al. Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture 2018;491:72–81.

Brewer MS. Chemical and physical characteristics of meat. Water-holding capacity. 2nd ed. Encyclopedia of meat sciences; 2014. p. 274–82.

Broker LE, Kruyt FA, Giaccone G. Cell death independent of caspases: a review. Clin Cancer Res 2005;11(9):3155–62.

Budhu A, Wang XW. The role of cytokines in hepatocellular carcinoma. J Leukoc Biol 2006;80(6):1197–213.

Credence Research. Global black soldier fly market by product type (protein meal, Biofertilizers (Frass), whole dried larvae, larvae oil, and OthersLive, larvae, adult, cocoon, & pupa by application (animal feed, agriculture, pet food, and others) growth. Future Prospects 8Competitive Analysis 2023:2016–30.

Defossa E, Wagner M. Recent developments in the discovery of FFA1 receptor agonists as novel oral treatment for type 2 diabetes mellitus. Bioorg Med Chem Lett 2014;24(14):2991–3000.
DePaolo RW, Abadie V, Tang F, et al. Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. Nature 2011;471(7337):220–4.
do Couto MVS, da Costa Sousa N, Paixão PEG, et al. Is there antimicrobial property of coconut oil and lauric acid against fish pathogen? Aquaculture 2021;545:737234.
Dilger AC, Overholt MF, Boler DD, et al. Impact of feeding oxidized oils to induce oxidative stress on the quality of pork. J Anim Sci 2018;96:147.
Dumas A, Raggi T, Barkhouse J, et al. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (Oncorhynchus mykiss). Aquaculture 2018;492:24–34.
Duprez L, Wirawan E, Vanden Berghe T, et al. Major cell death pathways at a glance. Microbes. Infect 2009;11(13):1050–62.
Fawole FJ, Labh SN, Hossain MS, et al. Insect (black soldier fly larvae) oil as a potential substitute for fish or soy oil in the fish meal-based diet of juvenile rainbow trout (*Oncorhynchus mykiss*). Anim Nutr 2021;7(4):1360–70.
Fisheries Bureau of the Ministry of Agriculture and Rural Affairs. China Fishery statistical yearbook. Beijing: China Agricultural Press; 2022.
Fuentes A, Fernández-Segovia I, Serra JA, et al. Comparison of wild and cultured sea bass (*Dicentrarchus labrax*) quality. Food Chem 2010;119(4):1514–8.
Gadsby R. Epidemiology of diabetes. Adv Drug Deliv Rev 2002;54(9):1165–72.
Hashimoto K, Yamashita M. Seasonal variation in quality and chemicalcomposition of the muscles of the spotted mackerel *Scomber australasicus* and pacific mackerel *S. japonicus*. Fish Sci 2019;85:767–75.
Hender A, Siddik MAB, Howieson J, et al. Black soldier fly, Hermetia illucens as an alternative to fishmeal protein and fish oil: impact on growth, immune response, mucosal barrier status, and flesh quality of juvenile barramundi, *Lates calcarifer* (Bloch, 1790). Biology 2021;10(6).
Hira T, Mochid T, Miyashita K, et al. GLP-1 secretion is enhanced directly in the ileum but indirectly in the duodenum by a newly identified potent stimulator, zein hydrolysate, in rats. Am. J. Physiol. Am J Physiol Gastrointest Liver Physiol 2009;297(4):663–71.
Hu JR, Yi CJ, Wang GX, et al. Effects of dietary soybean oil replaced with black soldier fly larvae oil on growth performance, plasma biochemical indices and liver lipid droplets of juvenile yellow catfish. Acta Hydrobiol Sin 2020;44:717–27.
Ip WKE, Hoshi, Shouval DS, et al. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. Science 2017;356(6337):513–9.
James Ed, Winkler D. Apoptosis and inflammation. (pp256; DM228.). Basel: Birkhäuser Verlag; 1999. ISBN:3-7643-5795-9.
Jiang Y, Li DH, Tu JC, et al. Mechanisms of change in gel water-holding capacity of myofibrillar proteins affected by lipid oxidation: the role of protein unfolding and cross-linking. Food Chem 2021;34:12857.
Jia SZ, Lin MJ, L RH, et al. Effect of replacing soybean oil with black soldier fly (*Hermetia illucens*) larvae oil on the growth performance, serum biochemical index and intestinal digestive enzyme of Qihe crucian carp (*Carassius auratus*). J Henan Normal Univ (Philos Soc Sci Ed Bimon) 2022;50(2):129–35.
Kumar V, Fawole FJ, Romano N, et al. Insect (black soldier fly, *Hermetia illucens*) meal supplementation prevents the soybean meal-induced intestinal enteritis in rainbow trout and health benefits of using insect oil. Fish Shellfish Immunol 2021;109:116–24.
Li TT, Chu ZP, Li CJ, et al. Effect of different lipid sources in diet on growth performance, body composition, nutrient apparent digestibilities, liver lipid metabolism enzymes activities and serum biochemical parameters of juvenile hybrid sturgeon. Chinese J Ani Nutr 2021;33(6):3447–60.
Li XC, Dong YW, Sun QX, et al. Growth and fatty acid composition of black soldier fly *Hermetia illucens* (Diptera: Stratiomyidae) larvae are influenced by dietary fat sources and levels. Animals 2022a;12(4).
Li X, Ray GW, Kou S, et al. Glycerol monolaurate (GML), a medium-chain fatty acid derivative, ameliorate growth performance, immune function, disease resistance and intestinal microbiota in juvenile hybrid grouper (*Epinephelus fuscoguttatus* female symbol × *Epinephelus lanceolatus* male symbol). Fish Shellfish Immunol 2022b;128:634–43.
Li YY, Xie DZ, Wang SQ, et al. Theory and application of precise nutrition of fatty acids in fish. Beijing: Science Press; 2023.
Liang C, Zhao XY, Jiao L, et al. Effects of different lipid sources on growth performance, fatty acids composition in tissue and expression of genes related to lipid metabolism in largemouth bass (*Micropterus salmoides*). Aquaculture Rep 2022;23.
Lou P. The effects of different oils on performance and meat quality of broilers. Master's thesis. China: Henan University of Technology. Zhengzhou; 2011.
Lv Y, Chen L, Wu H, et al. (-)-Epigallocatechin-3-gallate-mediated formation of myofibrillar protein emulsion gels under malondialdehyde-induced oxidative stress. Food Chem 2019;285:139–46.
Maldonado-Othón CA, De La Re-Vega E, Perez-Velazquez M, et al. Replacement of fish oil by camelina and black soldier fly larvae oils in diets for juvenile T*otoaba macdonaldi* and their effect on growth, fatty acid profile, and gene expression of pancreatic lipases. Aquaculture 2022;552.
Marques A, Matos E, Aires T, et al. Understanding the interaction between terrestrial animal fat sources and dietary emulsifier supplementation on muscle fatty acid profile and textural properties of European sea bass. Aquaculture 2022;560.
Mohamed H, Marusich E, Afanasev Y, et al. Fatty acids-enriched fractions of *Hermetia illucens* (black soldier fly) larvae fat can combat MDR pathogenic fish bacteria Aeromonas spp. Int J Mol Sci 2021;22(16).
Mohieldein A, Hyder MA, Hasan M. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. Eur J Exp Biol 2013;3(2):280–4.
Muller A, Wolf D, Gutzeit HO. The black soldier fly, Hermetia illucens - a promising source for sustainable production of proteins, lipids and bioactive substances. Z Naturforsch, C: J Biosci 2017;72(9–10):351–63.
Namkung H, Yu H, Gong J, et al. Antimicrobial activity of butyrate glycerides toward *Salmonella typhimurium* and *Clostridium perfringens*. Poultry Sci 2011;90(10):2217–22.
Ng WK, Koh CB. The utilization and mode of action of organic acids in the feeds of cultured aquatic animals. Rev Aquacult 2016;9(4):342–68.
Nitbani FO, Tjitda PJP, Nitti F, et al. Antimicrobial properties of lauric acid and monolaurin in virgin coconut oil: a review. ChemBioEng Rev 2022;9(5):442–61.
Rabani V, Cheatsazan H, Davani S. Proteomics and lipidomics of black soldier fly (Diptera: Stratiomyidae) and blow fly (Diptera: calliphoridae) larvae. J Insect Sci 2019;19(3).
Ramos-Bueno RP, González-Fernández MJ, Sánchez-Muros-Lozano MJ, et al. Fatty acid profiles and cholesterol content of seven insect species assessed by several extraction systems. Eur Food Res Tech 2016;242:1471–7.
Richter H, Gover O, Schwartz B. Anti-inflammatory activity of black soldier fly oil associated with modulation of TLR Signaling: a metabolomic approach. Int J Mol Sci 2023;24:10634.
Rombenso AN, Turchini GM, Trushenski JT. The omega-3 sparing effect of saturated fatty acids: a reason to reconsider common knowledge of fish oil replacement. Aquaculture 2021;14:213–7.
Roopashree PG, Shetty SS, Kumari NS. Effect of medium chain fatty acid in human health and disease. J Funct Foods 2021;87:104724.
Schonfeld P, Wojtczak L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. J Lipid Res 2016;57(6):943–54.
Seibel H, Bassmann B, Rebl A. Blood will tell: what hematological analyses can reveal about fish welfare. Front Vet Sci 2021;8:616955.
Spranghers T, Michiels J, Vrancx J, et al. Gut antimicrobial effects and nutritional value of black soldier fly (*Hermetia illucens* L.) prepupae for weaned piglets. Anim Feed Sci Technol 2018;235:33–42.
Stejskal V, Vejsada P, Cepak M, et al. Sensory and textural attributes and fatty acid profiles of fillets of extensively and intensively farmed Eurasian perch (*Perca fluviatilis* L.). Food Chem 2011;129(3):1054–9.
Stenberg OK, Holen E, Piemontese L, et al. Effect of dietary replacement of fish meal with insect meal on in vitro bacterial and viral induced gene response in Atlantic salmon (*Salmo salar*) head kidney leukocytes. Fish Shellfish Immunol 2019;91:223–32.
Stephan G, Dreanno C, Guiollaume J, et al. Incidence of different amount s of proteins, lipids and carbohydrates in diets on the muscle lipid composition in the turbot (Scophthalmus maximus). Ichtyophysiol Acta 1996;19:11–30.
Subhadra B, Lochmann R, Rawles S, et al. Effect of fish-meal replacement with poultry by-product meal on the growth, tissue composition and hematological parameters of largemouth bass (*Micropterus salmoides*) fed diets containing different lipids. Aquaculture 2006;260:221–31.
Tang Y, Zhang Z, Weng M, et al. Glycerol monolaurate improved intestinal barrier, antioxidant capacity, inflammatory response and microbiota dysbiosis in large yellow croaker (*Larimichthys crocea*) fed with high soybean oil diets. Fish Shellfish Immunol 2023;141:109031.
Trullas C, Tres A, Saldo J, et al. Quality characteristics of fillets of rainbow trout fed acid or re-esterified rapeseed oils as dietary fat sources. Aquaculture 2017;480:22–31.
Turchini GM, Francis DS, Senadheera SPSD, et al. Fish oil replacement with different vegetable oils in Murray cod: evidence of an "omega-3 sparing effect" by other dietary fatty acids. Aquaculture 2011;315(3–4):250–9.
Ushakova NA, Brodski ES, Kovalenk AA, et al. Characteristics of lipid fractions of larvae of the black soldier fly *Hermetia illucens*. Dokl Biochem Biophys 2016;468(1):209–12.
Van Opdenbosch N, Lamkanfi M. Caspases in cell death, Inflammation, and disease. Immunity 2019;50(6):1352–64.
Wang C, Zhan C, Yu H, et al. Glycerol monolaurate and triglycerol monolaurate alleviated high-fat diet induced lipid accumulation and damage of liver in zebrafish (*Danio rerio*). Aquaculture 2022;561.
Wang T, Secombes CJ. The cytokine networks of adaptive immunity in fish. Fish Shellfish Immunol 2013;35(6):1703–18.
Wang YC, Abdlluh, Zhong H, et al. Dietary glycerol monolaurate improved the growth, activity of digestive enzymes and gut microbiota in zebrafish (*Danio rerio*). Aqu. Rep 2021;20:100670.
Wang YS, Shelomi M. Review of black soldier fly (*Hermetia illucens*) as animal feed and human food. Foods 2017;6(10).
Wu ZH, Liu Y, Hu WF, et al. The fat of Hermetia illucens L. larvae inhibited PRRSV proliferation in vitro. Anim Husb Vet Med 2021;53(8):79–85.
Xie DZ, Chen CY, Dong YW, et al. Regulation of long-chain polyunsaturated fatty acid biosynthesis in teleost fish. Prog Lipid Res 2021;82(1).
Xu H, Dong X, Ai Q, et al. Regulation of tissue LC-PUFA contents, Delta6 fatty acyl desaturase (FADS2) gene expression and the methylation of the putative FADS2 gene promoter by different dietary fatty acid profiles in Japanese seabass (*Lateolabrax japonicus*). PLoS One 2014;9(1):e87726.
Xu H, Dong X, Zuo R, et al. Response of juvenile Japanese seabass (*Lateolabrax japonicus*) to different dietary fatty acid profiles: growth performance, tissue lipid accumulation, liver histology and flesh texture. Aquaculture 2016;461:40–7.

Xu XX, Ji H, Belghit I, et al. Effects of black soldier fly oil rich in n-3 HUFA on growth performance, metabolism and health response of juvenile mirror carp (*Cyprinus carpio* var. specularis). Aquaculture 2021;533.
Yadav AK, Rossi W, Habte-Tsion HM, et al. Impacts of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) level and ratio on the growth, fatty acids composition and hepatic-antioxidant status of largemouth bass (*Micropterus salmoides*). Aquaculture 2020;529.
Yang F, Jia S, Li J, et al. The relationship between degradation of myofibrillar structural proteins and texture of superchilled grass carp (*Ctenopharyngodon idella*) fillet. Food Chem 2019;301:125–8.
Ye YT. Technology integration and innovation to improve the edible quality of freshwater cultured fish. Feed Industry 2022;43:1–11.
Zhao HH, Chong J, Li DP, et al. Integrated multiple-omics reveals the regulatory mechanism underlying the effects of artificial feed and grass feeding on growth and muscle quality of grass carp (*Ctenopharyngodon idellus*). Aquaculture 2023;562:738808.
Zhou D, Cui YN, Zhou DR, et al. A comparative analysis of the quality of meat from largemouth bass (*Micropterus salmoides*) reared under different culture modes. Jiangsu Agric Sci 2021;49(6):146–9.