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Effects of Dietary Black Soldier Fly (*Hermetia illucens*) Oil Supplementation on Flesh Quality of Largemouth Bass (*Micropterus salmoides*)

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Effects of Dietary Black Soldier Fly (*Hermetia illucens*) Oil Supplementation on Flesh Quality of Largemouth Bass (*Micropterus salmoides*)

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Abstract

Black soldier fly (*Hermetia illucens*) larvae are a promising source of insect oil, characterized by rapid fatty acid accumulation and a high lauric acid content. This study investigated the effects of dietary black soldier fly oil (BSFO) on muscle quality in largemouth bass (*Micropterus salmoides*). Experimental diets were formulated to be isonitrogenous, isolipidic, and isophosphoric, with 1.0% and 2.0% BSFO partially replacing soybean oil. A control group received 2.3% soybean oil without BSFO or glycerol monolaurate (GML), while positive controls were supplemented with 0.35% and 0.7% GML. Fish (initial weight: 25.08 ± 0.12 g) were cultured in pond cages for 56 days.Muscle quality and nutritional traits were evaluated, including proximate composition, fatty acid profiles, texture properties, fiber diameter, hydroxyproline content, antioxidant capacity, and expression of genes related to muscle development, atrophy, apoptosis, and mTOR signaling. Compared with the control, the 2.0% BSFO group showed a significant increase in muscle hydroxyproline content ($p < 0.05$), while GML supplementation led to a significant decrease ($p < 0.05$). In the 1.0% BSFO group, muscle SFA and MUFA contents were unchanged ($p > 0.05$), but n-3/n-6 PUFA ratio and HUFA levels were significantly elevated ($p < 0.05$). The dietary supplementation of BSFO enhanced the levels of high-quality fatty acids in the muscle tissue. Antioxidant capacity was also significantly enhanced in the 1.0% BSFO group ($p < 0.05$), but reduced in the GML groups ($p < 0.05$). Texture analysis showed that BSFO significantly improved muscle hardness, elasticity, chewiness, and gumminess ($p < 0.05$). Gene expression analysis revealed no significant effects of BSFO on genes related to myogenesis (*myod, myog*) and muscle atrophy (*mstn, murf1*), or apoptosis-related genes (*caspase8, caspase9, caspase3*) ($p > 0.05$); mTOR signaling pathway-related genes (*s6k1, akt1*) were significantly upregulated in the 2.0% BSFO group ($p < 0.05$). In contrast, 0.7% GML significantly upregulated genes related to myogenesis (*myod, myf5, myog*), muscle atrophy (*mstn, fbxo32, murf1*), and apoptosis (*caspase8, caspase9, caspase3*) ($p < 0.05$). In summary, dietary supplementation with 1.0% BSFO effectively enhances muscle quality in largemouth bass without negatively impacting muscle development.

Keywords: black soldier fly; insect lipid; largemouth bass; flesh quality; texture property

Key Contribution: Our study aimed to assess the impact of substituting soybean oil with black soldier fly (*Hermetia illucens*) oil on various aspects of largemouth bass (*Micropterus salmoides*), including overall body composition, muscle characteristics, fatty acid profile, textural properties, muscle fiber diameter, muscle antioxidant capacity, and the expression levels of genes associated with muscle development and apoptosis. Notably, dietary supplementation with BSFO effectively enhances muscle quality in largemouth bass.

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1. Introduction

Lipids are indispensable in fish nutrition, serving as a vital source of energy and essential nutrients that are fundamental to the growth and health of aquatic species. Traditionally, soybean oil has been a preferred lipid source in commercial fish feed due to its high quality and nutritional value [1]. However, the extensive use of plant-based fats, such as soybean oil, has raised concerns over its competition with human land resources and the associated environmental implications, including deforestation and the excessive application of fertilizers and pesticides. For achieving the sustainable development of aquaculture, it has become important to find new sources of high-quality fats with low-cost and less impact on environment.

Insects have garnered extensive attention from researchers as a potential feed source for some aquatic animals in recent years [2]. Insects have their advantages such as high fertility, fast growth rate, renewable resources and the ability to utilize organic waste. The black soldier fly (*Hermitia illucens*) is one of the resource insects designated by the Food and Agriculture Organization (FAO) of the United Nations due to its nutritional comprehensiveness, well tolerance and ease of factory farming [3]. Black soldier fly larvae are rich in medium-chain fatty acids, with lauric acid constituting for 21.4-49.3% of total fatty acids [4]. Initially considered a by-product, black soldier fly oil (BSFO) has now attracted attention as a suitable lipid source due to the high lipid content and unique fatty acid profile [5].

Currently, black soldier fly oil has been reported as a fat source for aquatic animal feed. Fawole et al. [6] found that feeding BSFO did not adversely affect the normal performance of juvenile rainbow trout (*Oncorhynchus mykiss*) and fatty acid deposition in muscle. At the same time, adding BSFO to feed could promot growth, effectively prevent the development of enteritis, and improve the immunity of rainbow trout [5]. Sudha et al. [7] found that BSFO significantly enhanced the expression levels of myod and myog in the white muscle of juvenile striped catfish (*Pangasianodon hypophthalmus*), thereby promoting muscle development. Furthermore, adding BSFO to feed affects the fatty acid composition of fish because of the high content of lauric acid in BSFO [8–10]. So far, BSFO has been less studied in largemouth bass (*Micropterus salmoides*). Xia et al. [11] found that replacing fish oil with BSFO in largemouth bass feed had no negative effects on growth and decreased liver glycogen content. Conversely, adding BSFO to feed was beneficial to the immunity promotion of largemouth bass.

Largemouth bass (*M. salmoides*), known for its excellent growth performance, strong resilience, and low disease incidence, has become a key economic freshwater fish species [12]. FAO statistics reveal that global annual production of largemouth bass increased from 621 kilotonnes to 804 kilotonnes between 2020 and 2022 [13]. With the expansion of largemouth bass farming, there is a growing imperative to develop new feed lipid sources. Moreover, as living standards improve, consumer demand for high-quality aquatic products is increasing, making the flesh quality of fish a critical consideration when seeking lipid substitutes.

In this study, we investigated the effects of partially replacing soybean oil with BSFO in the feed on the body composition and texture of largemouth bass. Our findings aim to provide data supporting the substitution of soybean oil with BSFO in feed and contribute to the development of new, low-carbon feed options.

2. Materials and Methods

2.1. Experimental Feed and Groups

Five isonitrogenous and isolipidic feeds with 53% crude protein and 8.6% crude lipid were formulated by using fishmeal and soybean meal as the main protein sources, soybean oil , BSFO and glyceryl monolaurate as the main lipid sources. BSFO free diet was as Control. Experimental diets were formulated to be isonitrogenous, isolipidic, and isophosphoric, with 1.0% and 2.0% BSFO partially replacing soybean oil. The control diet contained 2.3% soybean oil with neither BSFO nor glycerol monolaurate (GML), whereas the positive control diets were supplemented with 0.35% and

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0.7% GML. Feed ingredients were thoroughly pulverized to pass through a 60-mesh sieve, full mixing, mixed oil and the right amount of water added to make pellets. The pellets feed were dried at 40 ℃ and stored at -20 ℃ until use. The experimental diet formulation and proximate composition were shown in Table 1.

Table 1. Largemouth bass experimental diets composition (g/kg) and nutrient composition.

Items	Control	BSFO1	BSFO2	GML0.35	GML0.7
Tapioca	100	100	100	100	100
Full-Fat Rice Bran	116.5	116.5	116.5	116.5	116.5
Cotton meal	80	80	80	80	80
Corn gluten meal	35	35	35	35	35
Fish meal	500	500	500	500	500
Hemoglobin powder	30	30	30	30	30
Chicken meal	90	90	90	90	90
Black soldier fly oil	0	10	20	0	0
Soybean oil	23	13	3	19.5	16
glyceryl monolaurate	0	0	0	3.5	7
Ca $(H_2PO_4)_2$	15	15	15	15	15
Premix[1]	10	10	10	10	10
Ruiantai[2]	0.5	0.5	0.5	0.5	0.5
Proximate analysis (%, DM)					
Moisture	10.67	6.56	5.51	11.23	10.09
Crude protein	53.30	53.40	53.12	53.37	53.22
Crude protein	8.66	8.64	8.77	8.66	8.70
Ash	13.51	13.33	13.43	13.39	13.40

[1]Premix contained the following per kilogram of feed:vitamin A 8mg,vitamin B1 18mg,vitamin B2 8mg,vitamin B6 12mg,vitamin B12 0.02mg,vitamin C 300mg,vitamin D3 3mg,vitamin K3 5mg, folic acid 5 mg, pantothenic acid 25 mg, niacin 25 mg, inositol 100 mg,Mg 96mg,Fe 64mg,Zn 19mg,Mn 13mg,Cu 2.5mg,I 0.021mg,Se 0.07mg,Co 0.016mg,K 0.05mg. [2]Ruiantai is natutal plant-based additives.

Black soldier fly larvae were rinsed three times with tap water and ultrapure water respectively, and then dried using vacuum freeze-drying. The dried BSFL were subjected to mechanical pressing to extract oil. The extracted oil was centrifuged at 13,000 rpm for 10 minutes at 40 °C, and the clear supernatant was collected as the BSFO used in this study.

2.2. Experimental Fish and the Feeding Trial

The experimental fish were feed in net cages (1.8 m × 1.5 m × 1.8 m) in a pond covering an area of 10,000 m2. The experimental fish were feed with the diet of the control group for two weeks for acclimation. 600 healthy largemouth bass were randomly divided into 15 net cages (1.8m × 1.5m × 1.8m) with 40 fish per cage, with an initial average body weight of 25.08 ± 0.12 g. During the eight-week culture trial, fish were fed twice daily (06:30 and 17:00) with 3% of body weight. During farming, the water temperature is maintained at 24-35 ℃, dissolved oxygen > 6 mg/L, pH 7.6±0.5, NH4+-N < 0.01 mg/L.

2.3. Sample Collection

After 8-week feeding trial, the fish were fasted for 24 h and anesthetized with an appropriate amount of MS-222. Three fish were taken from each net cage and stored at -20 °C for body component analysis. Nine fish in each net cage were randomly selected for dissection, and muscles above the left lateral line were collected, frozen in liquid nitrogen and stored at -80 °C for biochemical analysis and gene expression analysis. Another 3 fish were dissected, the muscle above the left lateral line were stored in a 4% paraformaldehyde solution at 4 ℃ for histologic section preparation. Three fish were

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taken from each net cage to remove scales and skin, and the muscle below the dorsal fin and above the left lateral line was cut into 1 cm × 1 cm × 1 cm square muscle blocks for texture analysis.

2.4. Proximate Analysis of Body and Muscle Composition

The moisture, crude protein, crude fat and crude ash was calculated according to National Standards, GB5009.3-2016, GB6432-2018, GB/T14772-2008 and GB5009.4-2016 respectively. The fatty acid composition was determined with an fatty acid profile analyzer (6980N, Agilent Technologies, USA)., and glycogen and hydroxyproline content of the muscle were determined using the A043-1-1 and A030-2-1 commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Muscle Antioxidant Capacity

Total superoxide dismutase (T-SOD) and total antioxidant capacity (T-AOC) ain muscle were measured by colorimetric method using the A001-1-2 and A015-1-2 commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.6. Texture Properties of Muscle

Six textural parameters including hardness, elasticity, chewiness, cohesiveness, gumminess and resilience were measured using a texture analyzer (Rapid TA+, Shanghai Tengba Instrument Technology, China). The analyzer was operated with the following parameters: P/36R probe diameter of 3.6 mm, test speeds of 5 mm/s, downward pressure distance of 30% of the sample thickness at 4 sec intervalsand trigger force setting of 5 g.

2.7. Histology of Muscle

Muscle samples were dehydrated in ethanol, infiltrated in xylene, embedded in paraffin, cut serially (5 μm thick) and then stained with hematoxylin and eosin. Section were observed using a light microscope (DM500, Leica, German) and photographed. The diameters of the muscle fibers were expressed as the longest axis length using the imaging software (ImageJ, USA).

2.8. RT-qPCR Analysis

Total RNA was extracted from muscle using Trizol reagent. RNA concentration was determined using a micro UV spectrophotometer (NanoDrop 2000, Thrrmo, USA). The total RNA was reverse-transcribed into cDNA with the PrimerScriptTM RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China) and then stored at -20℃. The relative expression levels of gapdh used as an internal reference, and the relative expression levels of the target genes were calculated using the $2^{-\Delta\Delta CT}$method [14]. Primers designed by Primer Premier 5.0 and the sequences were shown in Table 2.

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

Gene	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
myf5	GAGGAGGACGAGCATGTCAG	GAGGTGCAACGTCTCAAAGC
myod	GCGACTAAGCAAGGTGAACG	CTGCAGGGACTCGATGTAGC
myog	GAGTTGGGGTGACAGGAACA	TCTGGTTTGGGTTCATCAGG
mstn	TGATTGCTTTGGGTCCAGTC	CCTTCATTCGCAGTTTGCTC
fbxo32	GACATGGCTGCCAAGAAGAG	TGAAGGCCTCTCCCAGTGTA
MuRF1	TGGACGACACGTGTAAGCTG	GTGGACACCTTCTGCTGCTC
caspase7	CCATGGTGAAGAGGGAATGA	CGGACCCGAATCTGTCTGTA
caspase8	GAGGGGACAAAGAGGTGGAG	GAGCCTGTGGAAGTGTTTCG
caspase9	TCGGGCCTTTCCATTATTTC	AACAACCTGAGTGGGTGCTG
akt1	AGGACGCTACTACGCCATGA	CCGCTCCTCTGAGAACACAC
s6k1	GGCTCATCCCTTCTTTCGAC	GGCTTGATTCGCACTCTCAC
4ebp1	GACTGCCAGAAGACCACTGC	CAGCAGGAACTTTCGGTCAT

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gapdh	AAGGGTGGTGCCAAGAGAGT	AGTCTTCTGAGTGGCGGTGA

2.9. RT-qPCR Analysis

SPSS 26 was used to statistical analysis. One-way ANOVA was carried out with Duncan's multiple range test at a significance level of 0.05. All the result are presented as the mean ± s tandard errors (SEM). Bar graphs were produced using Origin 2021 software.

3. Results

3.1. The Effect of Black Soldier Fly Oil Supplementation on the Body and Muscle Composition

The composition of fish body and muscle fed with BSFO replacing different levels of soybean oil was shown in Table 3. Fish fed with different levels of BSFO had no significant impact on crude lipid, crude protein and ash of whole body and muscle of M. salmoides ($p > 0.05$).

Table 3. Effects of black soldier fly oil supplementation on body and muscle composition (dry weight, %).

Items	Control	BSFO1	BSFO2
Whole fish body			
Crude lipid (%)	20.71±0.98	23.06±1.67	24.59±1.84
Crude protein (%)	61.12±1.12	59.96±2.52	58.68±2.24
Ash (%)	15.29±0.72	13.95±0.46	13.95±0.12
Muscle			
Crude lipid (%)	16.51±0.83	17.04±1.06	19.80±1.75
Crude protein (%)	80.09±0.91	79.49±2.72	79.09±1.94
Ash (Ash,%)	5.87±0.27	5.90±0.12	6.27±0.12

Control, BSFO1 and BSFO2 represent diets with BSFO inclusion levels of 0%, 1%, and 2%, respectively. All data are expressed as mean ± SEM.

3.2. The Effect of Black Soldier Fly Oil and Glyceryl Monolaurate Supplementation on the Glycogen and Hydroxyproline Contents of Muscle

The effect of black soldier fly oil and glyceryl monolaurate replacing soybean oil on the glycogen and hydroxyproline content of M. salmoides muscle was shown in Table 4. There were no significant effect on glycogen contents in the groups of BSFO and GML compared with the control group ($p > 0.05$). Hydroxyproline content was significantly increased in the group BSFO2, whereas it was decreased in both GML0.35 and GML0.7 groups ($p < 0.05$).

Table 4. Effect of black soldier fly oil and glyceryl monolaurate supplementation on the glycogen and hydroxyproline content of muscle.

Items	Control	BSFO1	BSFO2	GML0.35	GML0.7
Glycogen (mg/g)	1.06±0.08	1.11±0.08	1.1±0.16	1.09±0.02	1.06±0.04
Hydroxyproline (μg/ml)	0.56±0.01^{b}	0.6±0.02^{b}	0.76±0.05^{a}	0.41±0.02^{c}	0.41±0.02^{c}

Control, BSFO1 and BSFO2 represent diets with BSFO inclusion levels of 0%, 1%, and 2%, respectively. GML0.35 and GML0.7 represent diets with GML inclusion levels of 0.35% and 0.7% respectively. All data are expressed as mean ± SEM. Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.3. The Effect of Black Soldier Fly Oil Supplementation on the Fatty Acid Composition of Body and Muscle

Table 5 showed the effects on the fatty acid composition of body and muscle of *M. salmoides* by black soldier fly oil supplementation. C12:0 (lauric acid), C14:0 and ∑SFA in the body were significantly more content in the BSFO supplementation group compared with the control group ($p < 0.05$), and C20:5n-3 (EPA), C22:6n-3 (DHA), ∑n-3 PUFA and ∑HUFA were significantly lower

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content in the BSFO groups compared with the control group ($p < 0.05$). The n-3/n-6 PUFA ratio was not significantly different in all groups ($p > 0.05$).

In the muscle, EPA, HUFA and∑n-3 PUFA were significantly higher content in the BSFO groups compared with the control group ($p < 0.05$), and the contents of DHA and ∑n-6 PUFA were significantly less than the control group ($p < 0.05$). The n-3/n-6 PUFA ratio was significantly higher in all BSFO groups than the control group ($p < 0.05$).

Table 5. Effect of black soldier fly oil supplementation on the fatty acid composition of whole fish and muscle (% total fatty acid).

Items		**Body**			**Muscle**	
Fatty acid	Control	BSFO1	BSFO2	Control	BSFO1	BSFO2
C12:0	0.18±0.06^{c}	4.47±0.1^{b}	6.23±0.46^{a}	0.14±0^{c}	2.93±0.22^{b}	4.84±0.33^{a}
C13:0	0.09±0^{a}	0.09±0^{a}	0.06±0^{b}	0.04±0^{a}	0.03±0^{b}	0.02±0^{b}
C14:0	5.43±0.11^{b}	6.59±0.14^{a}	6.36±0.19^{a}	3.24±0.01^{c}	4.07±0.08^{b}	4.42±0.18^{a}
C15:0	0.59±0.02^{a}	0.53±0.03^{b}	0.42±0.03^{c}	0.34±0^{a}	0.3±0.01^{b}	0.29±0^{b}
C16:0	35.51±0.75^{a}	34±0.5^{ab}	32.64±0.86^{b}	25.71±0.04^{a}	23.72±0.63^{b}	23.62±0.05^{b}
C17:0	0.59±0.02	0.53±0.05	0.49±0.05	0.35±0^{a}	0.29±0.01^{c}	0.32±0^{b}
C18:0	7.03±0.02^{a}	6.38±0.27^{b}	6.04±0.28^{b}	5.36±0.02^{a}	4.12±0.04^{b}	4.23±0.18^{b}
C21:0	0.14±0.03^{a}	0.1±0.02^{ab}	0.07±0.01^{b}	0.48±0.0^{5}	0.41±0.04	0.43±0.01
C23:0	0.43±0.03^{a}	0.3±0.04^{b}	0.24±0.04^{b}	0.4±0.06^{a}	0.1±0.01^{b}	0.11±0.01^{b}
∑SFA	50.01±0.73^{b}	53.05±0.22^{a}	52.61±0.77^{a}	36.43±0.03^{b}	36.37±0.43^{b}	38.64±0.34^{a}
C16:1n-7	6.9±0.12	6.96±0.31	6.67±0.26	5.37±0^{c}	5.85±0.24^{a}	5.45±0.17^{ab}
C18:1n-9	33.97±0.17^{ab}	32.63±1^{b}	34.51±0.72^{a}	29.03±0.1^{a}	26.44±0.92^{b}	27.09±0.2^{b}
C20:1n-9	0.39±0.02^{a}	0.31±0.01^{b}	0.25±0.01^{c}	0.28±0.04^{a}	0.19±0.01^{b}	0.18±0^{b}
C24:1n-9	0.18±0.03	0.2±0.1	0.17±0.03	5.45±0.03^{b}	7.67±0.25^{a}	7.88±0.29^{a}
∑MUFA	41.5±0.1	40.19±1.03	41.7±0.46	40.3±0.14	40.34±0.79	40.8±0.31
C18:2n-6	6.18±0.72^{a}	4.95±0.82^{ab}	4.08±0.39^{b}	18.38±0.02^{a}	16.91±0.72^{b}	14.93±0.02^{c}
C18:3n-6	0.15±0.02	0.11±0.04	0.08±0.02	1.33±0.03^{a}	1.45±0.1^{a}	1.16±0.01^{b}
C20:2n-6	0.06±0.01^{a}	0.04±0^{ab}	0.03±0^{b}	0.04±0.01^{a}	0.04±0^{ab}	0.03±0^{b}
C20:3n-6	0.04±0	0.03±0.01	0.02±0	0.2±0.01^{b}	0.35±0.02^{a}	0.44±0.07^{a}
C20:4n-6	0.21±0.01^{a}	0.14±0.02^{b}	0.09±0.02^{c}	0.14±0.02	0.14±0.01	0.14±0.01
∑n-6 PUFA	6.64±0.75	5.28±0.88	4.3±0.4	20.1±0.01^{a}	18.89±0.83^{b}	16.7±0.04^{c}
C18:3n-3	1.23±0.06^{a}	1.13±0.03^{ab}	1.06±0.09^{b}	0.97±0.04^{a}	0.78±0.04^{b}	0.91±0.03^{a}
C20:3n-3	0.2±0.05^{a}	0.13±0.02^{b}	0.1±0.01^{b}	1.07±0.01^{b}	1.42±0.18^{a}	1.11±0.08^{b}
C20:5n-3 (EPA)	0.16±0.03^{a}	0.07±0.03^{b}	0.1±0.02^{ab}	1.02±0.12^{c}	2.08±0.09^{a}	1.72±0.03^{b}
C22:6n-3 (DHA)	0.26±0.06^{a}	0.14±0^{b}	0.12±0.02^{b}	0.11±0.01	0.12±0.02	0.12±0.01
∑n-3 PUFA	1.85±0.09^{a}	1.48±0.01^{b}	1.38±0.12^{b}	3.17±0.09^{c}	4.4±0.28^{a}	3.86±0.02^{b}
∑PUFA	8.48±0.81^{a}	6.76±0.88^{ab}	5.69±0.34^{b}	23.27±0.11^{a}	23.29±1.11^{a}	20.56±0.04^{b}
∑HUFA	0.82±0.1^{a}	0.51±0.02^{b}	0.43±0.01^{b}	2.54±0.05^{c}	4.11±0.27^{a}	3.54±0.1^{b}
n-3/n-6 PUFA	0.28±0.02	0.29±0.05	0.33±0.05	0.16±0^{b}	0.23±0^{a}	0.23±0^{a}

All data are expressed as mean ± SEM. Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.4. The Effect of Black Soldier Fly Oil and Glyceryl Monolaurate Supplementation on the Antioxidant Capacity of Muscle

There was no significant difference in T-SOD activity among the groups ($p > 0.05$) shown in Table 6. The T-AOC in the muscle was not affected by BSFO supplementation ($p > 0.05$), and was significantly lower in the GML0.35 and GML0.7 groups compared with the control group ($p < 0.05$). MDA content in the muscle was not affected by BSFO1 supplementation ($p > 0.05$), and increased significantly in BSFO2, GML0.35 and GML0.7 groups ($p < 0.05$).

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Table 6. Effects of black soldier fly oil and glyceryl monolaurate supplementation on the antioxidant capacity of muscle.

Items	Control	BSFO1	BSFO2	GML0.35	GML0.7
T-SOD (U/mgprot)	33.97±0.89	33.67±3.05	31.43±1.94	33.31±1.85	31.34±1.12
T-AOC (U/mgprot)	2.63±0.12^{a}	2.7±0.27^{a}	2.95±0.24^{a}	1.76±0.18^{b}	1.94±0.11^{b}

All data are expressed as mean ± SEM. Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.5. The Effect of Black Soldier Fly Oil Supplementation on the Texture of Muscle

As shown in Table 7, the hardness, elasticity, chewiness and gumminess of muscle significantly increased compared with the control ($p < 0.05$), and cohesiveness and resilience were not affected by BSFO supplementation ($p > 0.05$).

Table 7. Texture of largemouth bass muscle by partially substituting soybean oil with black soldier fly oil.

Items	Control	BSFO1	BSFO2
Hardness,g	218.88±24.03^{b}	448.18±46.62^{a}	351.87±33.88^{a}
Elasticity,mm	0.58±0.01^{b}	0.64±0.01^{a}	0.62±0.01^{a}
Chewiness,mJ	79.55±11.86^{b}	178.73±18.88^{a}	142.16±17.82^{a}
Gummeness,g	135.83±18.11^{b}	276.26±27.27^{a}	226.89±24.68^{a}
Cohesiveness	0.62±0.02	0.67±0.01	0.64±0.03
Resilience	0.47±0.02	0.5±0.01	0.48±0.02

All data are expressed as mean ± SEM. Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.6. The Effect of Black Soldier Fly Oil Supplementation on the Histology of Muscle

As shown in Figure 1, compared with the control, the mean diameter of myofibers and the distribution of them in the muscle were not affected by BSFO supplementation. ($p > 0.05$). The histological morphological structure of M. salmoides myofiber was similar in both the soybean oil feed group and the BSFO groups.

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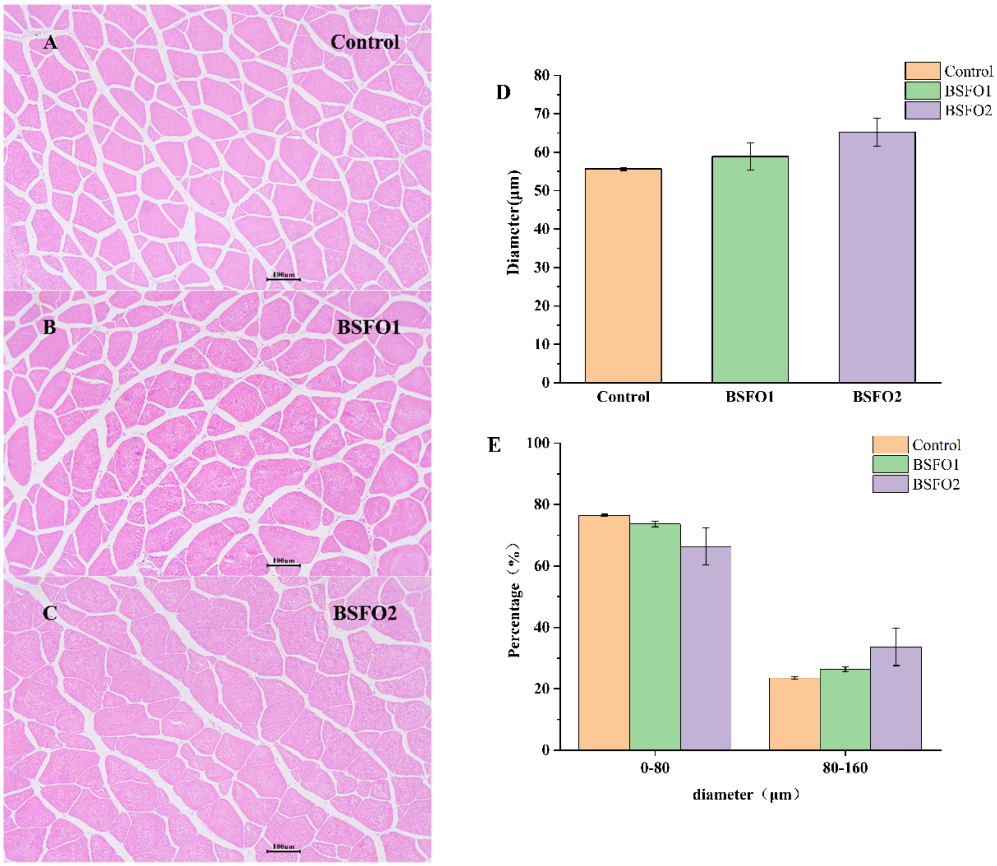

Figure 1. The effect of black soldier fly oil replacing soybean oil on the histology of muscle. (A), (B), (C) Transversal section of the white muscle of fish fed different experimental diets. Hematoxylin-eosin staining. Photomicrographs (magnification 100×) and scale bar (100μm); (D) Average diameter of myofibers; (E) the distribution of myofiber diameters.

3.7. The Effect on the Gene Relative Expression Levels in the Muscle by Fat Source Supplementation

The relative expression levels of myogenesis genes, TOR path-related genes and muscle apoptosis-related genes of *M. salmoides* were shown in Figure 2. Compared with the control group, the relative expression levels of *myf5* related to *myog* was significantly lower in the BSFO1 group ($p < 0.05$), and the relative expression levels of *fbxo32* related to muscle atrophy was significantly higher in the BSFO2 group ($p < 0.05$). Adding BSFO to diet reduced the relative expression levels of *4ebp1* significantly ($p < 0.05$). The relative expression levels of *akt1* and *s6k1* were significantly higher in the BSFO2 group ($p < 0.05$). The results showed that the addition of BSFO in the diet had no significant effect on the relative expression levels of apoptosis-related genes ($p > 0.05$).

Compare with the control group, the relative expression levels of all genes related to myogenesis, TOR path and muscle apoptosis were significantly higher in the GML0.7 group ($p < 0.05$), and the relative expression levels of *fbxo32*, *s6k1*, *caspase8* and *caspase9* were significantly lower in the GML0.35 group ($p < 0.05$).

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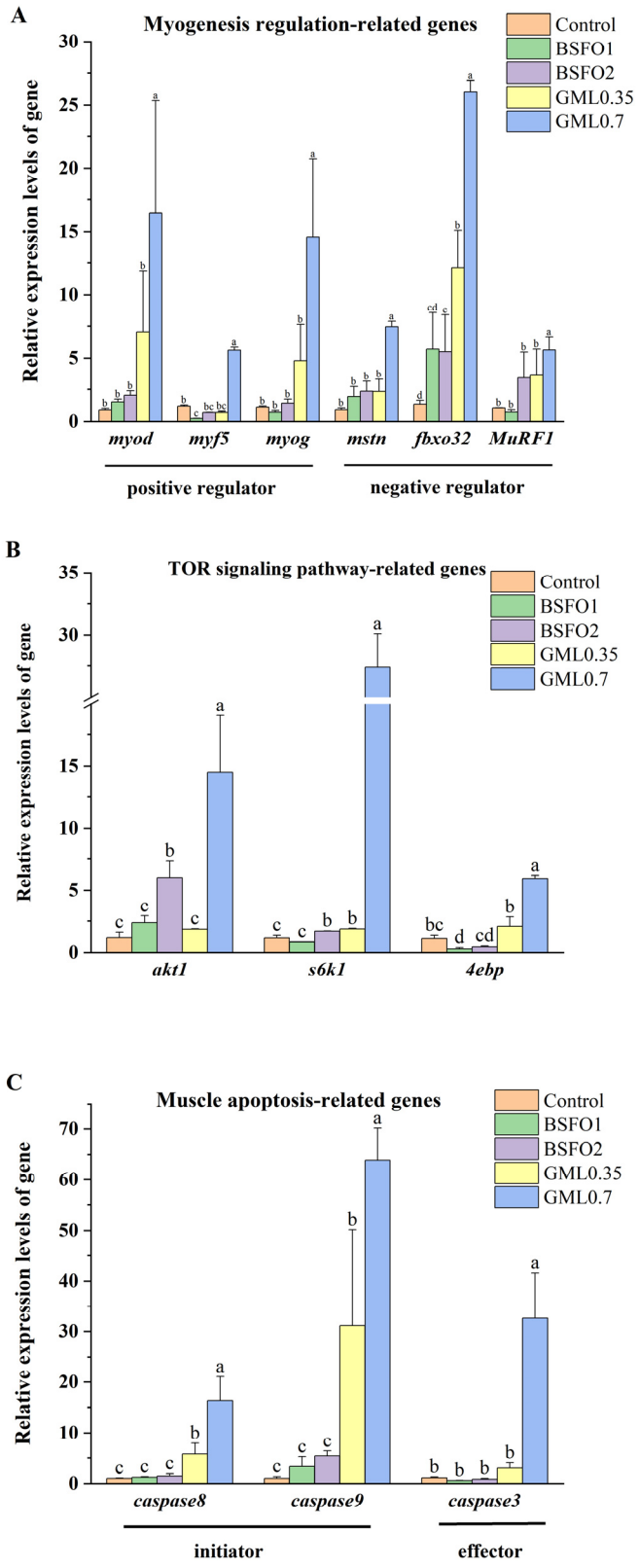

Figure 2. Effects of black soldier oil replacing soybean oil on the regulation of myogenesis, TOR path-related genes and apoptosis-related genes of largemouth bass. (A) myogenesis regulation-related genes (B) TOR path-related genes (C) apoptosis-related genes.

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4. Discussion

4.1. Effect on Flesh Quality of Largemouth Bass by BSFO Replacing Soybean Oil

The flesh textural characteristics, pivotal in quality assessment, are typically evaluated using texture profile analysis method [15]. This method quantifies key textural parameters such as hardness, elasticity, chewiness, cohesiveness, gumminess, and resilience, mimicking the human chewing process. These parameters are influenced by several factors, including intramuscular and intermuscular fat content, collagen content in connective tissues, and myofiber diameter and density [16]. Notably, hardness is a critical parameter for consumers, often reflecting the meat perceived value [17].Previous studies on fish have shown that the addition of black soldier fly oil alters the texture of flesh in terms of changes in hardness, elasticity, chewiness and gumminess [18]. In this study, we found that hardness, elasticity, chewiness and gumminess reached the higher values in the BSFO1 group, while they decreased in the BSFO2 group which had a higher degree of substitution compared with the BSFO1 group, but were still higher than the control group. This result is similar to previous discovery in *M. salmoides* where the addition of BSFO to the diet significantly increased flesh hardness [18]. Among the texture characteristics of flesh, especially hardness, are closely related to muscle lipid content [19]. In the present study, muscle lipid content of *M.salmoides* did not change significantly when the fish fed with BSFO instead of soybean oil. Previous studies in *O. mykiss*, *Cyprinus carpio var. Jian* and *M.salmoides* have also found that the usage of BSFO in diets could not vary lipid content of muscle [6,8,11].

In addition, the characteristics of fish myofibers influence the quality of the flesh, particularly the hardness, which is directly related to myofiber diameter and density [20]. Previous studies have shown that hardness and chewiness of flesh decreased as myofiber diameter increases and myofiber density decreases [21,22]. However, in this study, the increase in muscle hardness was not attributed to alterations in myofiber diameter, as substituting soybean oil with BSFO did not yield significant changes in either the diameter or density of largemouth bass myofiber. It was contrary to the reported by Xia et al., who found that when BSFO replaced more than 50% of fish oil, there was an increase in the diameter of *M.salmoides* myofiber; conversely, no significant change occurred when the replacement ratio remained below 50% [11]. It is possible that the difference in results is due to the difference between the fish oil and soybean oil used in the control group. Studies have shown that caspase mediated apoptosis was negatively correlated with myofiber diameter [23]. In this study, fish fed BSFO had no significant impact on the relative expression levels of apoptosis initiator *caspase8* and *caspase9* and apoptosis effector *caspase3*. However, the relative expression levels of *caspase8*, *caspase9* and *caspase3* was significantly upregulated in the GML0.35 and GML0.7 group. This suggested that lauric acid can induce apoptosis. Interestingly, despite the high lauric acid content in BSFO, its inclusion in fish diets did not exhibit these adverse effects, highlighting the potential of BSFO as a valuable feed component, even with its high concentration of lauric acid.

Furthermore, collagen content also affects the hardness of fish muscles. As a key component of the extracellular matrix, collagen is crucial for sustaining muscle growth and texture in fish [24]. Hydroxyproline, constituting 13.4% of the total amino and imine content in collagen and present in trace amounts in elastin, is absent in other proteins, making it a reliable indicator of collagen levels in muscle tissues [25]. In this study, we found that hydroxyproline content increased in the muscle of *M.salmoides* fed BSFO instead of soybean oil , which indicated that the increase in collagen amount is a reason of the increase in the flesh hardness.

In order to explore the mechanisms behind the observed changes in the muscle texture of *M.salmoides*, we examined the expression levels of genes associated with muscle growth. Østbye et al. [26] found a positive correlation between muscle hardness and myogenesis in Salmo salar. The process of fish muscle growth is regulated by a variety of factors, including myogenic regulatory factors (MRFs) and atrophy factors. MRFs are a family of basic helix-loop-helix transcription factors including *myod*, *myf5*, *mrf4* and *myog*, and can convert a large number of different cell type into muscle [27]. When the inhibition of myogenesis and protein degradation by atrophy factors exceed protein

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synthesis, muscle protein loss occurs, leading to a corresponding decrease in muscle hardness [28]. The genes *mstn*, *fbxo32* and *MuRF1* are three reliable atrophy sign to indicate muscle protein losing [29–31]. After an 8-week feeding trial with dietary BSFO, we assessed its impact on the relative expression levels of *myod*, *myf5*, *myog*, *mstn*, *fbxo32*, and *MuRF1* in the muscle. Previous studies have shown that some of the trophic factors affected the expression levels of one or more genes in MRFs, such as methionine levels of the dietary for rainbow trout juveniles [32], lysine and histidine levels of the dietary for *Oreochromis niloticus* [33], plant protein levels of the dietary for Solea senegalensis [34]. Sudha et al. [7] found that replacing fish oil with BSFO increasing the expression levels of genes related to muscle myogenesis in *Pangasianodon hypophthalmus* while reducing myostatin expression levels. However, another study on replacing fish oil in *M.salmoides* diets with BSFO found that there were no significant differences in mRNA expression levels of *myod1*, *murf1*, *myos*, *myog*, *myf5* and *paxbp-1* [11]. In this study, we replaced soybean oil in largemouth bass diets with BSFO, and found that the relative expression levels of genes related to muscle myogenesis and atrophy were not affected in the group BSFO2 compared with the control group. In the BSFO1 group, the expression level of *myf5* decreased significantly, while the expression level of *fbxo32* increased significantly, which revealing the primary cause why muscle hardness in BSFO1 group was higher than that in the control group and the BSFO2 group.

In this study, the relative expression levels of genes related to muscle myogenesis and atrophy were up-regulated in the GML0.7 group compare with the control. Despite the substantial lauric acid content in BSFO, the relative expression levels of these genes in the BSFO group were significantly lower than those in the GML group. This suggests that certain components within BSFO may mitigate the adverse effects typically associated with lauric acid, highlighting the potential for BSFO to modulate muscle gene expression in a beneficial manner.

The target of rapamycin (TOR) pathway integrates signals from extracellular and intracellular agents, which can regulate protein synthesis and promote cell survival, proliferation and growth [35]. In the TOR pathway, kinase akt activates TOR target proteins, phosphorylates kinase S6K1 and eukaryotic translation initiation factor 4E-BP1 regulate protein synthesis to promote muscle growth [36]. In the present study, the relative expression levels of *atk1* and *s6k1* was significantly up-regulated, suggesting that BSFO promotes cell growth and may lead to increased hardness in muscle.

4.2. Effect of Diet BSFO on Fatty Acid Profile of Largemouth Bass

The fatty acid profile in fish is directly affected by the fatty acid composition of the feed [37]. The fatty acid composition of BSFO is predominantly SFA, the majority of which is lauric acid [38]. The results showed that as the level of BSFO supplementation in the feed increased, the content of lauric acid increased in whole body and muscle of largemouth bass. Similar results have been reported in gilthead seabream [9]. In addition, feeding BSFO instead of soybean oil can resulte in an increase of C22:6n-3 (DHA) in muscle in Jian carp [8]. However, the fatty acid profile in rainbow trout was not consistent with them, where the usage of BSFO replacing soybean oil had no significant change in the DHA content of the muscle [6]. In the present study, replacing soybean oil with BSFO did not cause DHA content change in largemouth bass muscle, but significantly elevated C20:5n-3 (EPA) content in muscle, which may be attributed to the fact that lauric acid can be preferentially oxidized, allowing EPA to be better retained in muscle. The result that whole body contains more lauric acid than muscle seems to support this idea. In addition, the n-3/n-6 ratio is an important parameter for assessing the nutrition of flesh. Studies have shown that a high n-3/n-6 ratio is favorable for human health because n-3 PUFA can reduces inflammatory responses, whereas n-6 PUFA causes inflammation contrarily [39]. In the present study, n-3/n-6 levels were also significantly higher in the BSFO1 and BSFO2 groups than in the control group. This is in agreement with the results of previous studies [6,10]. Thus, the addition of BSFO to the diet increased EPA levels and n-3/n-6 ratio in *M.salmoides* muscle, thereby improved fatty acid quality accordingly.

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4.3. Effect of Diet BSFO on Antioxidant Capacity of Largemouth Bass

T-AOC is the total level of various antioxidant macromolecules, antioxidant small molecules and enzymes within a system [40,41]. T-SOD is an antioxidant enzyme that protects cells from peroxidative damage [42]. In the present study, replacing soybean oil with BSFO had no significant effect on both T-AOC and T-SOD activities in muscle. The results showed that inclusion of BSFO up to 2% in the diet had no significant effect on the antioxidant capacity of largemouth bass. Similar results have been reported, such as in the juvenile yellow catfish, BSFO as a substitute for soybean oil has no significant effect on the antioxidant capacity [43]. However, another study found that the addition of BSFO to largemouth bass diets resulted in an increase in T-SOD activities in the liver, suggesting that BSFO improves the antioxidant capacity of largemouth bass [11]. Because different studies have produced conflicting results, to investigate how BSFO affects the antioxidant capacity of largemouth bass, we fed largemouth bass using GML partially substitution of soybean oil. The results showed that the total antioxidant capacity of largemouth bass was significantly reduced. However, this may not be a universal phenomenon. For example, the study in hybrid grouper indicated GML increased the activity of SOD [44]. In *Trachinotus ovatus*, 0.15% GML significantly increased total antioxidant capacity and superoxide dismutase activities [45]. Similarly, the dietary addition of GML significantly increased the SOD activity in the juvenile grouper [46]. The different results may be due to the different effects of GML on different species of fish. In the present study, total antioxidant capacity was significantly lower in the GML groups than in the control, whereas the BSFO groups remained comparable to the control, suggesting that certain components in BSFO might mitigate the GML induced impairment of antioxidant capacity in largemouth bass.

5. Conclusions

This paper objective estimated the effects of dietary BSFO supplementation on the whole fish body composition, muscle composition, and fatty acid content, texture characteristics, diameter of muscle fibers, collagen content, antioxidant capacity of muscle, and mRNA expression levels of related genes involved in muscle generation, atrophy and apoptosis of largemouth bass. Our results showed that the addition of BSFO to the diet increased muscle hardness, enhanced flesh quality, also increased EPA levels and n-3/n-6 ratio in largemouth bass muscle and improved fatty acid quality. BSFO had no effect on the antioxidant capacity. In summary, it is feasible to use BSFO in the feed for largemouth bass.

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Institutional Review Board Statement: This study was conducted according to the SOPs of the Provincial Aquatic Animal Nutrition Key Laboratory of Soochow University and approved by the Animal Welfare Ethics Committee of Soochow University (Approval No.SUDA20250320A12).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

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