fishes

MDPI

Article

Black Soldier Fly Larvae Meal as a Sustainable Fishmeal Substitute for Juvenile Hybrid Grouper: Impacts on Growth, Immunity, and Gut Health

Yan Chen [1,2,3,4], Wenfeng Li [1,2,3,4], Minyi Zhong [5], Jun Ma [1,2,3,4], Bing Chen [6], Junming Cao [6], Jiun-Yan Loh [7] and Hai Huang [1,2,3,4,*]

1 College of Fisheries and Life Science, Hainan Tropical Ocean University, Sanya 572000, China
2 Yazhou Bay Innovation Institute, Hainan Tropical Ocean University, Sanya 572024, China
3 Hainan Key Laboratory for Conservation and Utilization of Tropical Marine Fishery Resources, Sanya 572022, China
4 Key Laboratory of Utilization and Conservation for Tropical Marine Bioresources (Hainan Tropical Ocean University), Ministry of Education, Sanya 572022, China
5 YuYi Habitat consultation Co., Ltd., Guangzhou 510642, China
6 Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
7 Tropical Futures Institute, James Cook University Singapore, 149 Sims Drive, Singapore 387380, Singapore; james.loh@jcu.edu.au
* Correspondence: huanghai74@126.com or 1814010232@stu.hrbust.edu.cn; Tel.: +86-18689808119

Abstract

Background: Aquaculture increasingly seeks sustainable alternatives to fishmeal, a key protein source in fish diets. Black Soldier Fly Larvae (BSFL) meal is a promising substitute, but its effects on fish growth, immunity, and gut health need further investigation. This study aimed to evaluate the impact of varying BSFL inclusion levels on juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂), a widely farmed species in tropical aquaculture. **Methods:** Juvenile hybrid grouper were fed diets with four levels of BSFL substitution (0%, 10%, 30%, and 50%) over 56 days. Key metrics such as growth performance, immune function, antioxidant capacity, and gut transcriptome were analyzed. **Results:** Replacing fish meal with BSFL meal had no significant effect on the survival rate of hybrid grouper ($p > 0.05$) but significantly affected growth performance, immune function, and antioxidant capacity ($p < 0.05$). BSFL10 and BSFL30 groups showed good growth and elevated immune enzyme activity, with significantly higher HIS levels ($p < 0.05$); the Wf of the BSFL10 group was comparable to the control. However, excessive replacement (BSFL50) led to reduced growth (Wf significantly lower, $p < 0.05$) and increased oxidative stress, as indicated by higher CAT activity ($p < 0.05$). Transcriptomic analysis revealed upregulation of immune- and metabolism-related genes with increasing BSFL levels, with immune pathways notably activated in the BSFL50 group. **Conclusions:** BSFL meal is a promising alternative to fishmeal in juvenile hybrid grouper diets, with moderate inclusion (10–30%) being most beneficial. Excessive BSFL substitution (50%) may impair fish health, highlighting the need for careful formulation in aquaculture diets.

Keywords: black soldier fly larvae; fishmeal replacement; hybrid grouper; immune function; antioxidant capacity

Key Contribution: This study demonstrates that Black Soldier Fly Larvae (BSFL) meals can be an effective, sustainable alternative to fishmeal in juvenile hybrid grouper diets. The research highlights that moderate inclusion of BSFL (10–30%) improves growth, immune function, and antioxidant capacity, with a 30% substitution yielding the best overall results.

Academic Editor: Marina Paolucci

Received: 24 May 2025
Revised: 26 June 2025
Accepted: 27 June 2025
Published: 11 July 2025

Citation: Chen, Y.; Li, W.; Zhong, M.; Ma, J.; Chen, B.; Cao, J.; Loh, J.-Y.; Huang, H. Black Soldier Fly Larvae Meal as a Sustainable Fishmeal Substitute for Juvenile Hybrid Grouper: Impacts on Growth, Immunity, and Gut Health. *Fishes* **2025**, *10*, 344. https://doi.org/10.3390/fishes10070344

1. Introduction

In recent years, the rapid development of aquaculture has led to a continuous increase in global farmed fish production, driving a growing demand for high-quality protein. Fishmeal, known for its high nutritional value and excellent digestibility, remains a key protein source in aquafeeds [1]. However, its production relies heavily on marine resources [2], making it expensive and limited in supply, which restricts the sustainable growth of the aquaculture industry. Environmental concerns have also emerged, as harvesting large volumes of small fish disrupts marine ecosystems [3,4]. To address these challenges, the search for cost-effective and environmentally friendly alternatives to fishmeal has become urgent. Various substitutes, such as plant and insect proteins, have been explored. Plant proteins are limited by imbalanced amino acid profiles and anti-nutritional factors [1]. In contrast, insect proteins offer high nutritional value, low environmental impact, and fast growth, making them a promising focus in fishmeal replacement research [5].

The black soldier fly (BSF) has gained global attention due to its high content of protein, fatty acids, minerals, and antimicrobial peptides (AMPs) [6]. BSF larvae meal (BSFL) is considered a promising and sustainable alternative to fishmeal owing to its short life cycle, low production cost, adaptability, and ability to convert organic waste into valuable biomass [7]. Nutritionally, BSFL is comparable to fishmeal and contains abundant unsaturated fatty acids and AMPs, offering potential antimicrobial and immunomodulatory benefits [8,9]. As a novel protein source, BSFL has been widely explored in aquafeeds. It provides essential nutrients for fish growth while exhibiting immune-enhancing and antimicrobial properties [6,10,11]. Studies on species such as seabass and carp demonstrate that low to moderate levels of BSFL replacement significantly promote growth and immune function [11,12]. AMPs, composed of 7–100 amino acids, are key components of the innate immune system and act by disrupting microbial membranes or interfering with metabolism [13,14]. Unsaturated fatty acids, including monounsaturated (e.g., oleic acid) and polyunsaturated types (e.g., linoleic acid, α-linolenic acid, EPA, DHA), play crucial roles in lipid regulation, anti-inflammation, and neural development [15,16].

BSFL has shown positive effects in replacing fishmeal in species like gilthead seabream and largemouth bass, enhancing growth performance and immune responses, including increased alkaline phosphatase, acid phosphatase, and lysozyme activities, as well as upregulation of cytokines (interleukins, interferons). BSFL also supports gut health by promoting beneficial microbiota, improving microbial balance, and enhancing intestinal morphology and barrier integrity through AMP secretion [11,17,18]. However, the physiological effects of BSFL vary with substitution levels. High inclusion rates may lead to adaptive challenges. Thus, further research is needed to elucidate its underlying mechanisms and optimize its application as a fishmeal alternative.

Key indicators such as growth performance, biochemical parameters, immune status, and antioxidant capacity are central to evaluating the effectiveness of alternative aquafeeds. These metrics reflect overall fish health and help assess the sustainability of feed formulations [19,20]. Growth metrics like weight gain, specific growth rate, and feed conversion ratio directly reflect nutritional quality and digestibility, while serum biochemical and antioxidant indicators reveal effects on metabolism, immune function, and oxidative balance [21,22]. With advances in molecular biology, transcriptomics has become a widely used tool in feed evaluation [23]. It enables the identification of gene expression changes in the gut and elucidates molecular mechanisms underlying the effects of alternative feeds on immune, antioxidant, and metabolic pathways [24].

BSFL, a promising fishmeal substitute, exhibits a “double-edged” effect depending on inclusion levels. Moderate replacement improves growth, immunity, and antioxidant capacity [25], while high inclusion levels may induce oxidative and immune stress [26].

This may be linked to BSFL's lipid profile, including indigestible components and high saturated fat content, which can burden the gut and immune system [27,28]. Excessive replacement may disrupt gut microbiota, impairing intestinal and immune health. These findings underscore the gut's critical role in nutrient absorption and immune defense and highlight the need to optimize inclusion levels for balanced immune modulation. Despite its potential, BSFL research remains early, with most studies focusing on low replacement levels. Systematic investigation into the physiological risks associated with higher inclusion rates is still lacking. Further studies are essential to understand the dual effects of BSFL at varying levels and to clarify the mechanisms behind its impact on fish health.

This study systematically investigates the effects of BSFL as a fishmeal substitute on the growth performance, immune function, antioxidant capacity, and gut gene expression of juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂) at different inclusion levels. A total of 480 uniform-sized juveniles were randomly assigned to four dietary groups: a control group (CK) fed a diet containing 40% fishmeal and no BSFL, and three treatment groups with 10% (BSFL10), 30% (BSFL30), and 50% (BSFL50) of the fishmeal replaced by BSFL, respectively. Growth, biochemical, and immune parameters were assessed alongside transcriptomic analysis to elucidate physiological responses. This research provides critical insights into the dual effects of BSFL on grouper health and offers a scientific basis for its rational use in aquafeed formulation.

2. Materials and Methods

2.1. Experimental Materials

The BSFL used in this experiment was provided by Guangzhou Fiet Biotechnology Co., Ltd. BSFL is a high-protein, high-fat feed additive rich in amino acids, fatty acids, and mineral elements. It contains 35% crude protein, 32% crude fat, 14.8% ash, and 5.6% chitin. The amino acid profile includes lysine (1.76%), arginine (2.31%), histidine (0.97%), aspartic acid (3.75%), glutamic acid (5.65%), glycine (3.18%), alanine (3.29%), valine (2.41%), leucine (3.21%), isoleucine (1.64%), phenylalanine (1.65%), proline (2.37%), tryptophan (0.78%), tyrosine (2.06%), serine (1.66%), methionine (0.56%), and threonine (1.49%). The control group (CK) was fed a diet containing 40% fishmeal and no BSFL, which also served as the basal formulation for the other treatments. Based on this diet, fishmeal was partially replaced with BSFL at levels of 10% (BSFL10), 30% (BSFL30), and 50% (BSFL50) by weight, resulting in five isonitrogenous and isolipidic diets with crude protein content ranging from 36.45% to 37.75%, crude fat content from 14.10% to 14.90%, and total energy from 17.17 to 17.56 kJ g^{-1}. The feed formulation followed established protocols [29]. All feed ingredients were ground and passed through a 60-mesh (250 μm) standard sieve to ensure uniform particle size. The sieved materials were mixed thoroughly with the formulation and processed into 2.0 mm pellets using a twin-screw extruder. During extrusion, the temperature was maintained at 90–100 °C to preserve the nutritional integrity of the feed. The finished pellets were dried at 55 °C, vacuum-sealed, and stored at −20 °C to prevent oxidation and spoilage. This study received approval from the Animal Ethics Committee of ["Institutional Animal Care and Use Committee of Hainan Tropical Ocean University" and the "Hainan Key Laboratory for the Conservation and Utilization of Tropical Marine Fishery Resources"] with ethics approval number [20191111A1]. All experimental procedures adhered to ethical guidelines to ensure animal welfare. Anesthesia and sampling were conducted in accordance with protocols to minimize animal discomfort and pain.

2.2. Feeding Management and Experimental Design

The experiment was conducted at a commercial aquaculture facility in Lingshui, China. Juvenile hybrid groupers (*Epinephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂)

were sourced from Delin Chengxin Aquaculture Co., Ltd. (Lingshui, China), with uniform size and an average body weight of 56.49 ± 0.34 g (8–12 weeks of age). Before the trial, fish were acclimated for one week under identical rearing conditions and fed the same control diet used in the CK, containing 40% fishmeal and no BSFL. After acclimation, 480 healthy juveniles were randomly assigned to four dietary groups, each with four replicates (30 fish per replicate), and distributed into 16 tanks (500 L each). Fish were fed the corresponding experimental diets for 56 days using apparent satiation feeding at 08:00 and 18:00. Uneaten feed was removed 30 min post-feeding to prevent overfeeding. Feed intake and water quality parameters were monitored daily. Water temperature was maintained at 27–30 °C, dissolved oxygen > 6.0 mg/L, ammonia < 0.10 mg/L, nitrite < 0.01 mg/L, and pH 7.6–7.9.

2.3. Sample Collection

After the feeding trial, groupers were fasted for 24 h and then anesthetized with eugenol (1:10,000) for sampling. Ten groupers were randomly selected from each group to measure growth performance indicators, including condition factor (CF), hepatosomatic index (HSI), and intraperitoneal fat ratio (IPR). Blood samples were collected for serum antioxidant and immune indicator analysis. Gut samples were immediately frozen in liquid nitrogen and stored at −80 °C for subsequent RNA extraction and transcriptome sequencing.

2.4. Indicator Measurement and Data Processing

Growth performance was assessed using survival rate (SR), CF, HSI, and IPR. The calculation formulas are as follows:

- Survival Rate (SR, %) = (N1/N0) × 100%;
- Condition Factor (CF, g/cm^3) = Wf/L^3;
- Viscerosomatic Index (VSI, %) = (W2/Wf) × 100%;
- Hepatosomatic Index (HSI, %) = (W3/Wf) × 100%.

Three fish from each tank were randomly selected for dissection, and their viscera and liver were weighed to calculate CF, HSI, and IPR. In these formulas, N1 and N0 represent the total number of fish at the end and start of the experiment, respectively; Wi and Wf represent the average initial and final body weight (g), respectively; L is fish length (cm); and W2 and W3 are the average weights of the viscera and liver (g), respectively.

2.5. Serum Immunity and Antioxidant Indicator Measurement

Serum total protein (TP), globulin (GLB), and albumin (ALB) levels were measured using an automated biochemical analyzer (Hitachi 7180, Chiyoda, Japan). Antioxidant parameters, including superoxide dismutase (SOD) activity, lysozyme (LZY) activity, malondialdehyde (MDA) content, glutathione peroxidase (GSH-Px) activity, and catalase (CAT) activity, were determined using commercial assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), following the manufacturer's protocols. These kits are specifically designed to quantify enzyme activity or oxidative products. The catalog numbers were as follows: SOD (A001-1-2), LZY (A050-1-1), MDA (A003-1-2), GSH-Px (A005-1-2), and CAT (A007-1-1).

2.6. Gut Transcriptome Sequencing and Data Analysis

Intestinal samples were subjected to transcriptome sequencing using the Illumina NovaSeq 6000 platform (San Diego, CA, USA) with a read length of 150 bp. Raw sequencing data were quality-checked using FastQC (v0.11.9) and trimmed with Trimmomatic (v0.39) for adapter removal and quality filtering.

Differentially expressed genes (DEGs) were identified using the DESeq2 package (v1.36.0) in R, with thresholds set at $|\log_2 FC| > 1$ and adjusted p-value < 0.05. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using the ClusterProfiler package (v4.4.4).

2.7. Statistical Analysis

Experimental data were analyzed using SPSS 20.0 software (IBM, New York, NY, USA). Normality was assessed with the Shapiro–Wilk test, and homogeneity of variances was tested with Levene's test to confirm the suitability for variance analysis. For data meeting normal distribution and homogeneity of variance assumptions, one-way analysis of variance (ANOVA) was used to compare group differences, with Duncan's post hoc multiple comparisons method. For variables that did not meet the assumptions of normality or homogeneity of variance, the Kruskal–Wallis test was used to detect overall group differences. When significant, pairwise comparisons were conducted using Dunn's post hoc test with Bonferroni correction. Results are presented as mean ± standard error (mean ± SE), with statistical significance set at $p < 0.05$. Significant differences among groups are indicated by letters in tables and figures.

3. Results

3.1. Effects of Replacing Fishmeal with Black Soldier Fly Larvae Meal on Survival Rate and Final Weight in Grouper

To evaluate the impact of BSFL as a fishmeal substitute on grouper survival and growth, a 56-day feeding trial was conducted. Survival rates remained near 100% across all groups, indicating no adverse effects from BSFL substitution (Table 1). The CK had the highest Wf (108.70 g), followed by BSFL10 (107.19 g) and BSFL30 (95.36 g), indicating good growth. However, BSFL50 showed a marked decline (76.55 g), suggesting that high substitution levels may impair growth. CF showed little variation (1.55–1.61). In contrast, the HSI was significantly higher in all BSFL groups compared to the control, indicating increased liver energy storage; however, no significant differences were observed among the BSFL treatments (BSFL10, BSFL30, and BSFL50). The IPR was lower in BSFL10 and BSFL30, suggesting reduced fat accumulation with moderate substitution.

Table 1. Growth and biometry of hybrid grouper fed diets containing various concentrations of FM replacement with BSFL.

Items	Diets			
	CK	**BSFL 10**	**BSFL 30**	**BSFL 50**
		Growth indices		
W_i (g)	56.50 ± 0.00	56.47 ± 0.04	56.23 ± 0.05	56.58 ± 0.06
W_f (g)	108.70 ± 0.24 a	107.19 ± 0.25 b	95.36 ± 0.04 c	76.55± 0.17 d
Survival (%)	100.0 ± 0.00	100.0 ± 0.00	98.20 ± 1.55	99.10 ± 1.55
		Biometric indices		
CF (g cm^{-3})	1.61 ± 0.11	1.58 ± 0.12	1.57 ± 0.14	1.55 ± 0.08
HSI (%)	3.38± 0.19 b	5.51 ± 0.35 a	5.99 ± 0.41 a	5.77 ± 0.42 a
IPR (%)	5.68 ± 0.33 a	3.55 ± 0.46 b	3.76 ± 0.42 b	4.42 ± 0.40 b

Note: Values (mean ± SD, $n = 3$) within the same row with different letters are significantly different ($p < 0.05$). Absence of letters indicates no significant difference between treatments. CF: condition factor; HSI: hepatosomatic index; IPR: index of ponderosity of fat.

In summary, moderate BSFL replacement supports growth and reduces fat deposition, but excessive levels may hinder growth and increase liver lipid accumulation. Although

no pathological changes were observed, long-term high-fat diets may pose risks such as hepatic steatosis or oxidative stress, warranting further monitoring in future studies.

3.2. Positive Effects of Serum Biochemical Indicators, Immune Response, and Antioxidant Capacity in Grouper by 30% BSFL Replacement

To evaluate the effects of BSFL meal as a fishmeal substitute on protein metabolism, immune function, and antioxidant capacity in grouper, serum biochemical, immune, and antioxidant parameters were assessed across different substitution levels (Table 2). In serum biochemistry, the BSFL30 group showed the highest total protein (TP, 53.31 g/L) and globulin (GLB, 26.40 g/L) levels ($p < 0.05$), indicating enhanced protein metabolism and immune function. ALB was also significantly higher in BSFL30 (26.91 g/L, $p < 0.05$), while the ALB/GLB remained unchanged among groups. Regarding lipid profiles, BSFL50 exhibited the highest total cholesterol (TC, 8.32 mmol/L) and low-density lipoprotein (LDL, 5.27 mmol/L) levels ($p < 0.05$), suggesting increased lipid accumulation at high substitution. BSFL30 showed elevated high-density lipoprotein (HDL, 2.62 mmol/L, $p < 0.05$), indicating a beneficial effect on lipid metabolism. Immune analysis revealed that LZY activity was highest in BSFL30 (35.80 U/mL, $p < 0.05$), followed by BSFL10, while BSFL50 was comparable to the control, suggesting immune enhancement at moderate substitution.

Table 2. Serum biochemistry of hybrid grouper fed different diets.

Items	CK	BSFL 10	BSFL 30	BSFL 50
Serum Chemistry				
TP (g/L)	34.79 ± 1.26 b	40.82 ± 2.90 ab	53.31 ± 0.50 a	36.39 ± 1.30 b
GLB (g/L)	21.38 ± 0.07 c	24.33 ± 0.34 b	26.40 ± 0.68 a	19.46 ± 0.56 d
ALB (g/L)	13.41 ± 1.21 c	16.49 ± 3.22 b	26.91± 6.40 a	16.94 ± 1.29 b
ALB/GLB	0.63 ± 0.10	0.68 ± 0.15	1.02 ± 0.25	0.87 ± 0.07
TC (mmol/L)	5.81 ± 1.29	6.65 ± 1.3	5.47 ± 1.21	8.32 ± 1.91
HDL (mmol/L)	2.42 ± 0.07	2.34 ± 0.12	2.62 ± 0.16	2.30 ± 0.07
LDL (mmol/L)	2.89 ± 0.20 d	3.63 ± 0.69 b	3.00 ± 0.63 bc	5.27 ± 0.22 a
Anti-oxidation				
SOD (U/mL)	174.27 ± 2.36 a	146.79 ± 2.76 b	208.19 ± 12.25 a	142.69± 3.40 b
LZY (U/mL)	34.00 ± 1.81 b	35.05 ± 1.46 a	35.80± 1.64 a	34.00± 2.11 b
MDA (nmol/mL)	92.32 ± 2.63 d	109.20 ± 4.83 b	128.13 ± 2.41 a	107.68 ± 1.83 c
GSH-Px (U/mL)	1089.38 ± 171.11 b	1100.31 ± 75.16 bc	1246.88 ± 108.20 a	1185.63 ± 27.79 c
CAT (U/mL)	24.82 ± 8.46 c	18.03 ± 8.50 d	29.22 ± 3.82 b	34.56 ± 3.93 a

Note: Values (mean ± SD; $n = 3$) within the same row with different letters were significantly different ($p < 0.05$). The absence of letters indicates no significant difference between treatments. ALB, albumin; ALT, alanine transaminase; AST, aspartate aminotransferase; TC, total cholesterol; GLB, globulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LZY, serum lysozyme; SOD, superoxide dismutase; TP, total protein; CAT, catalase. MDA, malondialdehyde; GSH-Px, glutathione peroxidase.

In antioxidant assays, BSFL30 had the highest SOD activity (208.19 U/mL, $p < 0.05$), indicating enhanced antioxidant defense. However, MDA levels were also highest in BSFL30 (128.13 nmol/mL, $p < 0.05$), suggesting increased oxidative stress. GSH-Px activity in BSFL30 was significantly elevated (1246.88 ± 108.20 U/mL, $p < 0.05$), potentially reflecting a compensatory antioxidant response to higher metabolic demands, warranting further investigation into long-term effects. BSFL50 showed significantly increased catalase (CAT) activity (34.56 U/mL, $p < 0.05$), but also elevated oxidative stress markers ($p < 0.05$), indicating potential adverse effects at high substitution levels.

3.3. Impact of BSFL Substitution on Gut Gene Expression and Differential Analysis in Juvenile Hybrid Grouper

To evaluate the effects of BSFL meal substitution on intestinal gene expression in hybrid grouper juveniles, transcriptomic analysis was conducted comparing the control group (CK) with the BSFL10, BSFL30, and BSFL50 groups. Volcano plots (Figure 1A) showed that the number of DEGs increased significantly with higher BSFL inclusion levels, with the greatest number observed in the BSFL50 group. Box plots (Figure 1B) indicated significantly elevated gene expression levels in BSFL50 compared to other groups. Enrichment analysis identified significant involvement in metabolic pathways ($p < 0.05$), although functional experiments are needed for further validation. The proportion of upregulated genes was 52% in BSFL10, 46% in BSFL30, and 66% in BSFL50 (Figure 1C). BSFL50 also showed significant upregulation of immune-related genes such as irf3, irak4, and ikbke, as well as signaling genes cd40, plcg2, and npk11 ($p < 0.05$). These results demonstrate that high-level BSFL substitution significantly alters intestinal gene expression, particularly in pathways related to immune and metabolic processes.

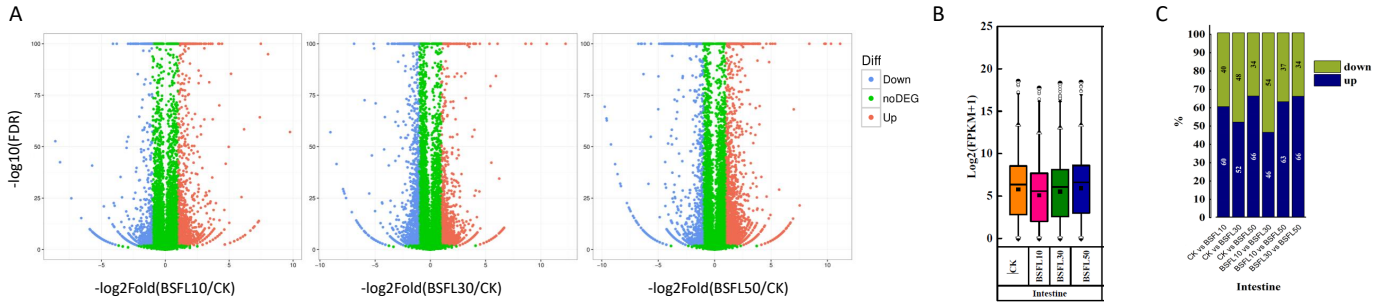

Figure 1. Analysis of DEGs in the gut tissue of juvenile hybrid grouper following BSFL substitution. Note: (**A**) Volcano plots show the gene expression differences between the CK and the BSFL substitution groups (BSFL10, BSFL30, and BSFL50). Red dots indicate upregulated genes (Up), blue dots represent downregulated genes (Down), and green dots denote non-significantly differentially expressed genes (noDEG). DEGs were selected based on a p-value < 0.05 and $|\log_2\text{FoldChange}| > 1$. (**B**) Box plots display the normalized expression levels of DEGs across the feed groups (CK, BSFL10, BSFL30, and BSFL50). Data are based on three biological replicates per group and are analyzed using Log_2(FPKM+1) values. The box plots illustrate the median, interquartile range, and outlier distribution of gene expression levels. (**C**) Distribution of up- and downregulated DEGs across feed groups, comparing the CK with each substitution group (CK vs. BSFL10, CK vs. BSFL30, CK vs. BSFL50). Green represents the proportion of downregulated genes, while purple indicates upregulated genes. Statistical significance was determined by Fisher's exact test, with a significance level set at $p < 0.05$.

3.4. GO Functional Enrichment Analysis of DEGs in the Gut of Hybrid Grouper Following BSFL Substitution

To investigate the effects of BSFL meal replacement on intestinal gene expression in hybrid grouper, Gene Ontology (GO) enrichment analysis was performed to identify significantly affected biological processes (BP), cellular components (CC), and molecular functions (MF) under different substitution levels. As shown in Figure 2, each comparison (CK vs. BSFL10, CK vs. BSFL30, and CK vs. BSFL50) revealed distinct sets of significantly enriched GO terms. In BSFL10 (Figure 2A), enriched BP terms included RNA metabolism, DNA repair, and redox processes. BSFL30 (Figure 2B) enriched ATP synthesis, ribosomal structure, and lipid metabolism. BSFL50 (Figure 2C) significantly enriched immune response, inflammation, and oxidative stress-related terms.

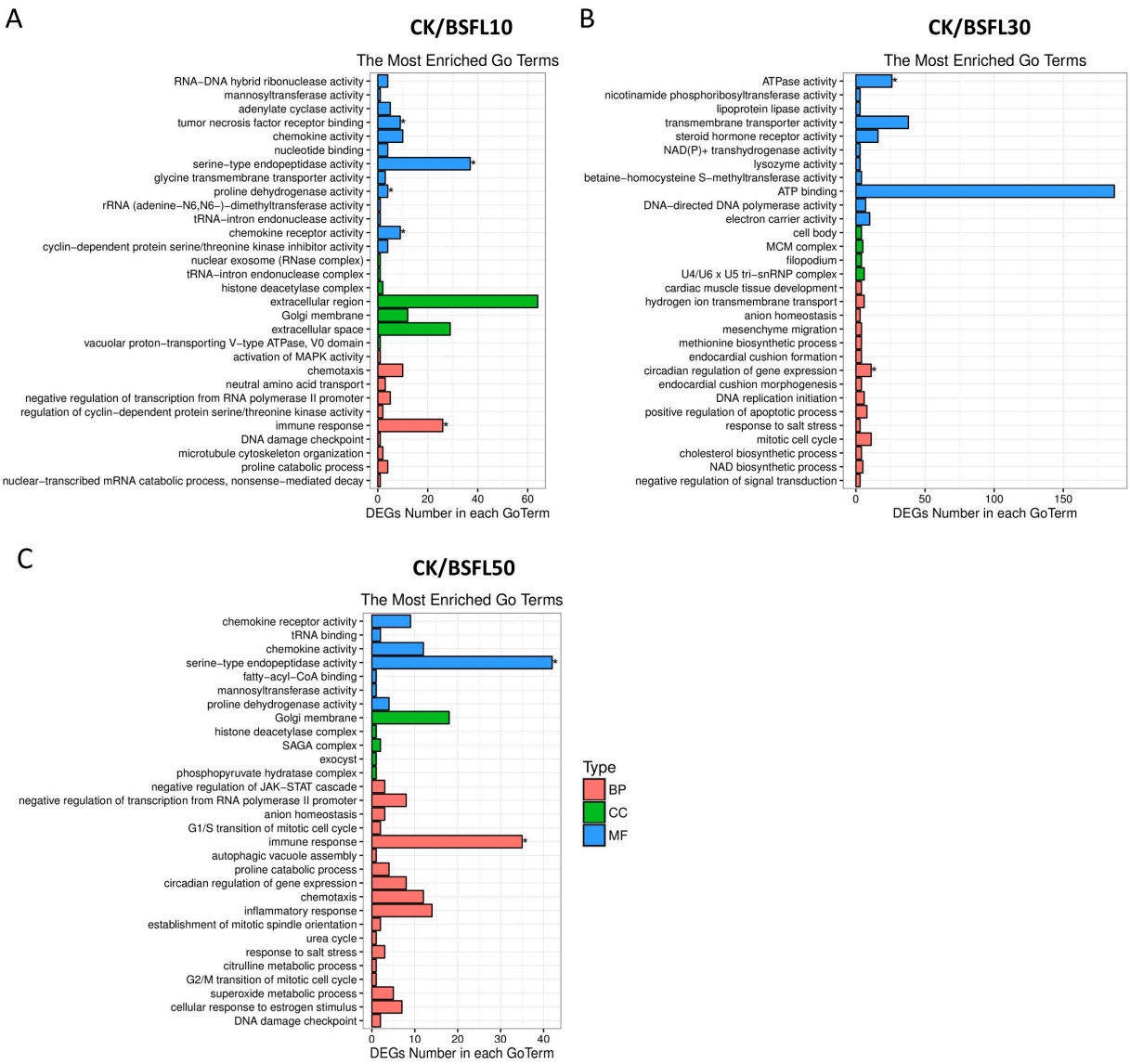

Figure 2. GO functional enrichment analysis of DEGs in the gut of hybrid grouper across different BSFL substitution levels. Note: (**A**) GO functional enrichment results for DEGs between the CK and the low substitution group (BSFL10). (**B**) GO functional enrichment results for DEGs between the CK and the medium substitution group (BSFL30). (**C**) GO functional enrichment results for DEGs between the CK and the high substitution group (BSFL50). The analysis includes categories for BP, CC, and MF. * Significance was determined based on a p-value < 0.05.

3.5. KEGG Pathway Enrichment Analysis of DEGs in the Gut of Hybrid Grouper Following BSFL Substitution

KEGG pathway enrichment analysis was conducted to identify significantly affected pathways at different substitution levels to further clarify the biological impact of BSFL substitution on intestinal gene expression in hybrid grouper. In the BSFL10 group (Figure 3A), significantly enriched pathways included glycolysis/gluconeogenesis and cell cycle, indicating effects on fundamental metabolic and proliferative processes. In the BSFL30 group (Figure 3B), enrichment was observed in pathways related to ATP synthesis, protein metabolism, and ribosome structure, reflecting energy and protein processing changes. In the BSFL50 group (Figure 3C), significantly enriched pathways were associated with immune response, inflammation, and apoptosis, suggesting activation of immune and stress-related signaling. These findings demonstrate that BSFL substitution alters key intestinal pathways in a level-dependent manner, with lower levels affecting metabolism

and higher levels influencing immune and stress responses. Further investigation is needed to optimize substitution ratios for health and performance outcomes.

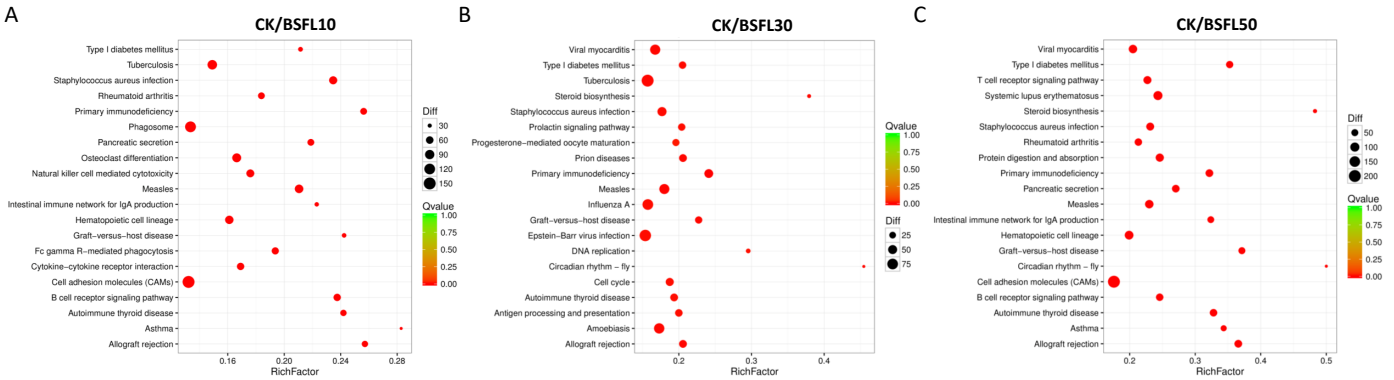

Figure 3. KEGG pathway enrichment analysis of DEGs in the gut of hybrid grouper across different BSFL substitution levels. Note: (**A**) KEGG pathway enrichment results for DEGs between the CK and the low substitution group (BSFL10). (**B**) KEGG pathway enrichment results for DEGs between the CK and the medium substitution group (BSFL30). (**C**) KEGG pathway enrichment results for DEGs between the CK and the high substitution group (BSFL50). The *x*-axis represents the Rich Factor, indicating the proportion of DEGs enriched in each pathway; the *y*-axis shows the names of significantly enriched pathways. Bubble color represents the Q-value (adjusted *p*-value), with colors ranging from green to red indicating increasing significance levels; bubble size reflects the number of enriched genes. Significance was determined based on a Q-value < 0.05.

3.6. Tissue-Specific Regulation of Immune-Related Gene Expression in the Intestine of Hybrid Grouper Following BSFL Substitution

To assess the impact of BSFL meal substitution on the intestinal immune system of hybrid grouper, the expression levels of immune-related genes were analyzed across different diet groups (CK, BSFL10, BSFL30, BSFL50) (Figure 4). In the BSFL50 group, key immune genes such as tlr (Toll-like receptor), nfb (NF-kappa B), and interleukins (il-1, il-8) were significantly upregulated ($p < 0.05$). These genes play critical roles in innate immunity, pathogen recognition, inflammatory response, and immune cell recruitment. The TNF signaling gene (TNF) was also significantly upregulated, indicating enhanced intestinal immune activation under this substitution level. In the BSFL10 and BSFL30 groups, expression of tlr and nfb also increased but to a lesser extent, suggesting that lower substitution levels can activate immune-related pathways without inducing a strong inflammatory response. In summary, BSFL substitution diets significantly influenced intestinal immune gene expression in hybrid grouper, with the strongest activation observed in the BSFL50 group, indicating enhanced stimulation of innate immune and anti-inflammatory pathways at higher substitution levels.

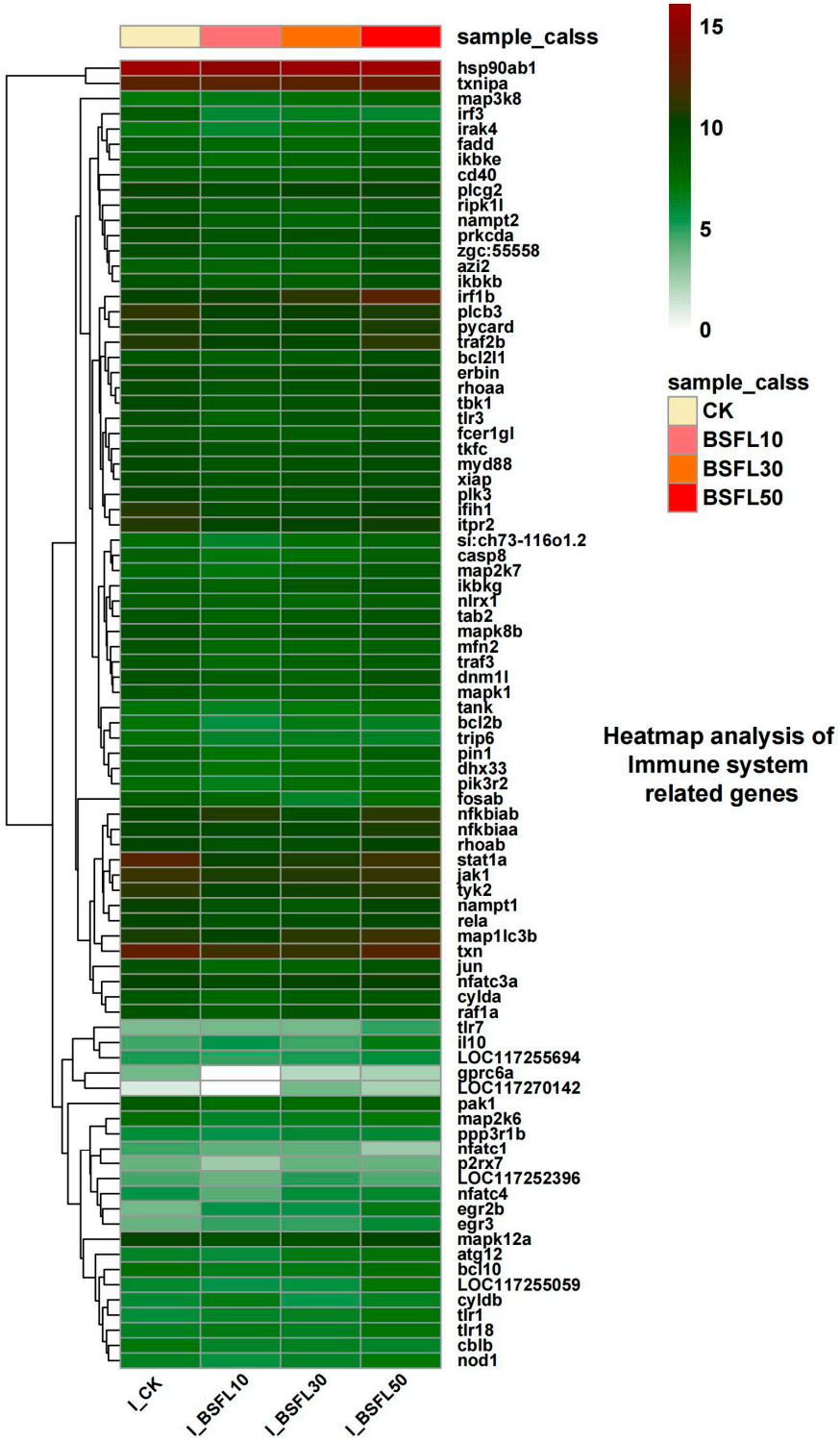

Figure 4. Heatmap analysis of immune-related gene expression in different tissues of hybrid grouper following BSFL substitution. Note: The heatmap shows the expression levels of immune-related genes in intestinal tissues across different feed groups (CK, BSFL10, BSFL30, BSFL50). The color gradient from green to red indicates the range of gene expression levels (green for low expression, red for high expression). The *x*-axis represents the feed groups and tissue sample types, while the *y*-axis lists immune-related genes. Gene expression levels are based on normalized data calculated using $\log_2$(FPKM+1) values, with hierarchical clustering applied for both genes and samples.

4. Discussion

In this study, BSFL was incorporated into the diet of juvenile hybrid grouper to assess its effects at different substitution levels, addressing a research gap in this specific species. Multiple substitution levels were designed to systematically evaluate the physiological impacts of BSFL on grouper, providing new data to support its broader application in aquaculture.

The results of this study indicate that the growth performance of hybrid grouper in the BSFL10 and BSFL30 groups was superior to that of the CK, while the BSFL50 group exhibited a significant decline in growth performance. This finding aligns with other studies showing that low to moderate substitution levels of BSFL can promote fish growth [30]. Previous research has reported that an appropriate level of BSFL substitution not only provides sufficient nutrients for fish but also enhances feed conversion rates, accelerating growth [31]. However, the observed reduction in growth performance in the BSFL50 group, with a 32.15 g decrease in final body weight and a 1.26% reduction in IPR compared to the control group, suggests that a high substitution level may increase metabolic burden and potentially impair feed digestion and absorption efficiency. This result is consistent with findings in other fish studies, where high substitution levels negatively impacted growth performance, reinforcing the need to carefully regulate BSFL usage levels in aquaculture [32].

Regarding immune function, the BSFL10 and BSFL30 groups exhibited higher immune enzyme activity, indicating that moderate BSFL substitution can effectively enhance grouper immune function. This finding is consistent with previous studies that observed positive effects of moderate BSFL substitution on the immune system [12,13]. Research suggests that BSFL is rich in unsaturated fatty acids and antimicrobial peptides, which boost fish immune function. However, immune stress was observed in the BSFL50 group, likely due to an excessive immune response triggered by the high substitution level, resulting in additional metabolic strain. Transcriptome analysis showed upregulation of several immune-related genes in the BSFL50 group, suggesting that a high substitution level may activate excessive immune pathways, negatively affecting the immune system. This observation aligns with findings from other studies where high substitution levels adversely affected the immune system, underscoring the importance of controlling BSFL substitution ratios appropriately [6].

Regarding antioxidant capacity, the BSFL10 and BSFL30 groups exhibited higher serum antioxidant enzyme activity, suggesting that moderate BSFL substitution can enhance the antioxidant capacity of the fish. This may be attributed to the antioxidant properties of fatty acids and trace elements in BSFL, a phenomenon also supported by other fish studies [33]. However, a decrease in antioxidant capacity was observed in the BSFL50 group, indicating that high substitution levels may induce oxidative stress, leading to the accumulation of reactive oxygen species (ROS) and disrupting the balance of the antioxidant system. Previous studies have shown that high saturated fatty acid intake levels can exacerbate oxidative stress and increase the risk of tissue damage [34]. The results of this study align with these findings, providing new evidence on the impact of BSFL substitution ratios on the antioxidant system.

Through transcriptome analysis, this study found that different levels of BSFL substitution significantly impacted gut gene expression in hybrid grouper, particularly in the BSFL50 group, where multiple immune- and metabolism-related pathways were notably activated. The gut plays a critical role in fish immunity and metabolism, and changes in feed composition directly influence gut health and gene expression. Our results indicate that a high substitution level may lead to the overexpression of immune genes in the gut, triggering immune stress responses. Additionally, we identified the activation of several

KEGG pathways related to immunity and metabolism, suggesting that BSFL has a dual role in modulating gut immune pathways. This finding is consistent with previous observations of changes in gut gene expression, providing new insights into the impact of BSFL substitution on gut health.

The results of this study indicate that BSFL meal, as a fishmeal substitute at varying inclusion levels, significantly affects the growth performance, immune function, and antioxidant capacity of juvenile grouper. Particularly at moderate replacement levels (10–30%), with 30% being optimal, BSFL significantly enhances growth performance and immune enzyme activity and optimizes the antioxidant system, further improving fish health. However, a high replacement level (50%) may induce oxidative stress and excessive immune responses, potentially negatively impacting fish health. Transcriptomic analysis of the intestine revealed that higher BSFL replacement activated immune- and metabolism-related genes, suggesting that excessive substitution may burden intestinal health.

This study reveals the "double-edged effect" of BSFL as a fishmeal replacement: moderate replacement (10–30%) positively affects growth, immunity, and antioxidant capacity, with 30% being optimal. However, excessive replacement levels (50%) can lead to oxidative stress and immune burden. Similar findings have been reported in studies on other fish species [35], suggesting that over-replacement should be avoided when applying BSFL in aquaculture. Based on our results, we recommend maintaining BSFL replacement at 10–30% in aquaculture to ensure optimal growth and health outcomes. For effective utilization of BSFL, future applications should consider the specific needs and growth stages of different fish species in formulation design to avoid potential negative effects associated with high replacement levels. This study has several limitations. The 56-day experimental period was relatively short, and the fish did not achieve full weight doubling, potentially limiting detection of long-term physiological effects. Growth performance declined even at moderate BSFL inclusion levels (10% and 30%), indicating the need for extended feeding trials. The limited number of inclusion levels (0%, 10%, 30%, 50%) restricted the use of dose–response regression analysis, and comparisons were confined to ANOVA-based group differences. Future studies should adopt finer, continuous inclusion gradients to support regression modeling and dose optimization. The focus on growth, antioxidant capacity, and selected immune markers excluded other physiological systems such as the gut microbiota. Broader assessments, including microbiome profiling and longer-term monitoring, are recommended. Nonetheless, the findings provide valuable evidence for the development of sustainable aquaculture feeds.

5. Conclusions

This study demonstrates that BSFL meal, as a fishmeal substitute, significantly affects hybrid grouper juveniles' growth performance, immune function, and antioxidant capacity (Figure 5). A substitution level of 10–30% notably improved growth, immune enzyme activity, and antioxidant function ($p < 0.05$), with the 30% group showing the most favorable results. In contrast, the 50% substitution group showed reduced growth performance, with a 32.15 g decrease in final weight and a 1.26% reduction in IPR compared to the control, along with signs of oxidative stress and heightened immune activation, suggesting increased metabolic burden and compromised gut health.

The transcriptomic analysis further confirmed that high substitution levels significantly upregulated immune and metabolism-related genes, indicating potential physiological stress. A substitution ratio of 10–30% is recommended to balance growth promotion and immune regulation. These findings provide scientific support for the rational use of BSFL meal in aquafeeds and offer a basis for its application in sustainable aquaculture.

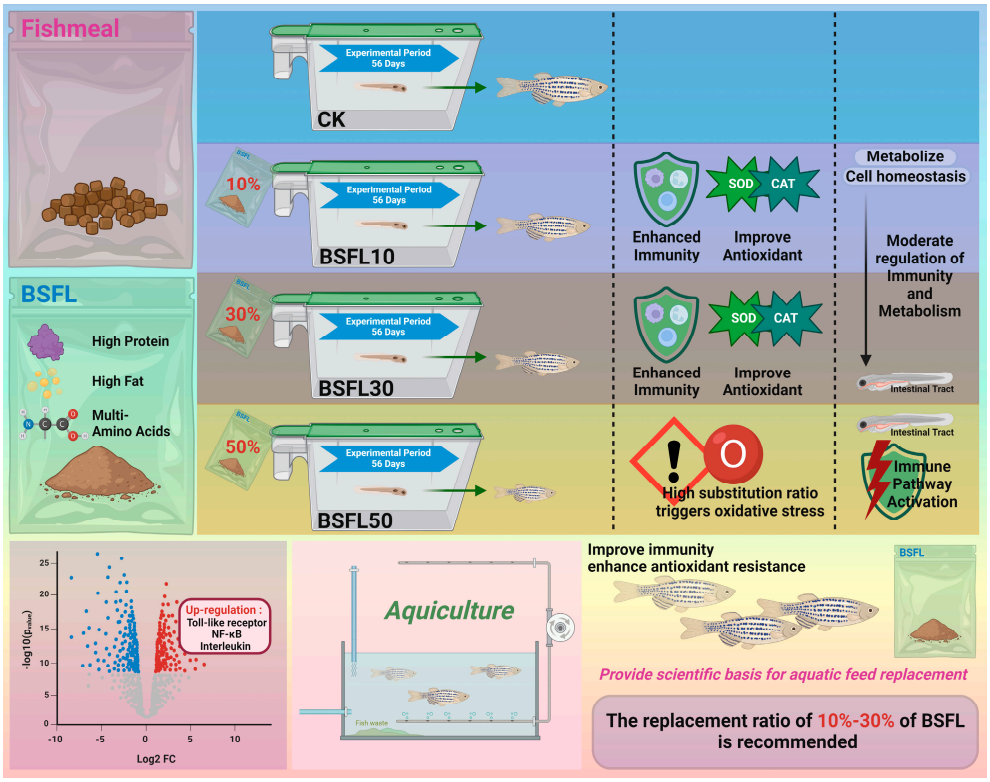

Figure 5. Comprehensive Effects of BSFL as Fishmeal Substitute on Grouper Growth, Immunity, and Gut Gene Expression.

Author Contributions: Y.C., W.L. and H.H. conceived and designed the study. Y.C., W.L., M.Z. and J.M. conducted the feeding trials and sample collection. B.C. and J.C. performed the biochemical and immunological analyses. J.-Y.L. contributed to the transcriptomic analysis and data interpretation. Y.C. and W.L. drafted the manuscript. H.H. supervised the project and revised the manuscript critically. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Hainan Province Science and Technology Special Fund (SQ2024XDNY0083), the Major Science and Technology Program of Yazhou Bay Innovation Institute of Hainan Tropical Ocean University (2022CXYZD001), the National Natural Science Foundation of China (32160861) and the Major Science and Technology Plan of Hainan Province (ZDKJ2021017, ZDYF2022XDNY331).

Institutional Review Board Statement: This study received approval from the Animal Ethics Committee of [Institutional Animal Care and Use Committee of Hainan Tropical Ocean University" and the "Hainan Key Laboratory for the Conservation and Utilization of Tropical Marine Fishery Resources] with ethics approval number [20191111A1].

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated or analyzed for this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest: Author Minyi Zhong was employed by the company YuYi Habitat consultation. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

ALB	albumin
ANOVA	analysis of variance
BP	biological processes
BSF	black soldier fly
BSFL	black soldier fly larvae meal
CAT	catalase
CC	cellular components
CF	condition factor
CK	control group
DEGs	differentially expressed genes
GLB	globulin
GO	Gene Ontology
GSH-Px	glutathione peroxidase
HIS	hepatosomatic index
IPR	intraperitoneal fat ratio
KEGG	Kyoto Encyclopedia of Genes and Genomes
LZY	lysozyme
MDA	malondialdehyde
Mean ± SE	mean ± standard error
MF	molecular functions
nfb	NF-kappa B
noDEG	non-significantly differentially expressed genes
PAMPs	Pathogen-Associated Molecular Patterns
ROS	reactive oxygen species
SOD	superoxide dismutase
SR	survival rate
tlr	Toll-like receptor
TNF	Tumor Necrosis Factor
TP	total protein
VSI	viscerosomatic index
Wi	initial weight
Wf	final weight

References

1. Ido, A.; Ali, M.-F.-Z.; Takahashi, T.; Miura, C.; Miura, T. Growth of Yellowtail (*Seriola quinqueradiata*) Fed on a Diet Including Partially or Completely Defatted Black Soldier Fly (*Hermetia illucens*) Larvae Meal. *Insects* **2021**, *12*, 722. [CrossRef] [PubMed]
2. Goglio, P.; Van Den Burg, S.; Kousoulaki, K.; Skirtun, M.; Espmark, Å.M.; Kettunen, A.H.; Abbink, W. The Environmental Impact of Partial Substitution of Fish-Based Feed with Algae- and Insect-Based Feed in Salmon Farming. *Sustainability* **2022**, *14*, 12650. [CrossRef]
3. Ferrer Llagostera, P.; Kallas, Z.; Reig, L.; Amores de Gea, D. The Use of Insect Meal as a Sustainable Feeding Alternative in Aquaculture: Current Situation, Spanish Consumers' Perceptions and Willingness to Pay. *J. Clean. Prod.* **2019**, *229*, 10–21. [CrossRef]
4. Malcorps, W.; Kok, B.; van't Land, M.; Fritz, M.; van Doren, D.; Servin, K.; van der Heijden, P.; Palmer, R.; Auchterlonie, N.; Rietkerk, M.; et al. The Sustainability Conundrum of Fishmeal Substitution by Plant Ingredients in Shrimp Feeds. *Sustainability* **2019**, *11*, 1212. [CrossRef]
5. Belghit, I.; Liland, N.S.; Gjesdal, P.; Biancarosa, I.; Menchetti, E.; Li, Y.; Waagbø, R.; Krogdahl, Å.; Lock, E.-J. Black Soldier Fly Larvae Meal Can Replace Fish Meal in Diets of Sea-Water Phase Atlantic Salmon (*Salmo salar*). *Aquaculture* **2019**, *503*, 609–619. [CrossRef]
6. Tippayadara, N.; Dawood, M.A.O.; Krutmuang, P.; Hoseinifar, S.H.; Doan, H.V.; Paolucci, M. Replacement of Fish Meal by Black Soldier Fly (*Hermetia illucens*) Larvae Meal: Effects on Growth, Haematology, and Skin Mucus Immunity of Nile Tilapia, Oreochromis Niloticus. *Animals* **2021**, *11*, 193. [CrossRef] [PubMed]
7. Jahan, R.; Tipu, M.M.H.; Haque, M.M.; Salam, M.A. Black Soldier Fly (*Hermetia illucens*) Larvae Meal as a Fish Meal Replacement in Diets for 1 Nursing Common Carp (*Cyprinus carpio*) Fry. *AgriRxiv* **2021**, *2021*, 20210012413.

8. Weththasinghe, P.; Rocha, S.D.C.; Øyås, O.; Lagos, L.; Hansen, J.Ø.; Mydland, L.T.; Øverland, M. Modulation of Atlantic Salmon (*Salmo salar*) Gut Microbiota Composition and Predicted Metabolic Capacity by Feeding Diets with Processed Black Soldier Fly (*Hermetia illucens*) Larvae Meals and Fractions. *Anim Microbiome* **2022**, *4*, 9. [CrossRef]
9. Xia, J.; Ge, C.; Yao, H. Antimicrobial Peptides from Black Soldier Fly (*Hermetia illucens*) as Potential Antimicrobial Factors Representing an Alternative to Antibiotics in Livestock Farming. *Animals* **2021**, *11*, 1937. [CrossRef]
10. Lu, S.; Taethaisong, N.; Meethip, W.; Surakhunthod, J.; Sinpru, B.; Sroichak, T.; Archa, P.; Thongpea, S.; Paengkoum, S.; Purba, R.A.P.; et al. Nutritional Composition of Black Soldier Fly Larvae (*Hermetia illucens* L.) and Its Potential Uses as Alternative Protein Sources in Animal Diets: A Review. *Insects* **2022**, *13*, 831. [CrossRef]
11. Hender, A.; Siddik, M.; Howieson, J.; Fotedar, R. 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*, 505. [CrossRef]
12. Lanes, C.F.C.; Pedron, F.A.; Bergamin, G.T.; Bitencourt, A.L.; Dorneles, B.E.R.; Villanova, J.C.V.; Dias, K.C.; Riolo, K.; Oliva, S.; Savastano, D.; et al. Black Soldier Fly (*Hermetia illucens*) Larvae and Prepupae Defatted Meals in Diets for Zebrafish (*Danio rerio*). *Animals* **2021**, *11*, 720. [CrossRef] [PubMed]
13. Zhang, H.; Lv, J.; Ma, Z.; Ma, J.; Chen, J. Advances in Antimicrobial Peptides: Mechanisms, Design Innovations, and Biomedical Potential. *Molecules* **2025**, *30*, 1529. [CrossRef] [PubMed]
14. Rahman, M.R.T.; Guay, L.-D.; Fliss, I.; Biron, E. Structure–Activity Study of the Antimicrobial Lipopeptide Humimycin A and Screening Against Multidrug-Resistant *Staphylococcus aureus*. *Antibiotics* **2025**, *14*, 385. [CrossRef]
15. Casillas-Vargas, G.; Ocasio-Malavé, C.; Medina, S.; Morales-Guzmán, C.; Del Valle, R.G.; Carballeira, N.M.; Sanabria-Ríos, D.J. Antibacterial Fatty Acids: An Update of Possible Mechanisms of Action and Implications in the Development of the next-Generation of Antibacterial Agents. *Prog. Lipid Res.* **2021**, *82*, 101093. [CrossRef] [PubMed]
16. Obukhova, E.S.; Murzina, S.A. Mechanisms of the Antimicrobial Action of Fatty Acids: A Review. *Appl. Biochem. Microbiol.* **2024**, *60*, 1035–1043. [CrossRef]
17. Xiao, Y.; Zhu, L.; Liang, R.; Su, J.; Yang, J.; Cao, X.; Lu, Y.; Yu, Y.; Hu, J. A Meta-analysis of the Effects of Black Soldier Fly Meal on Fish Immune Response and Antioxidant Capacity. *Comp. Immunol. Rep.* **2024**, *7*, 200162. [CrossRef]
18. Ruiz, A.; Andree, K.B.; Furones, D.; Holhorea, P.G.; Calduch-Giner, J.À.; Viñas, M.; Pérez-Sánchez, J.; Gisbert, E. Modulation of Gut Microbiota and Intestinal Immune Response in Gilthead Seabream (*Sparus aurata*) by Dietary Bile Salt Supplementation. *Front. Microbiol.* **2023**, *14*, 1123716. [CrossRef]
19. Fadel, A.; Khafage, A.; Abdelsalam, M.; Abdel-Rahim, M.M. Comparative Evaluation of Three Herbal Extracts on Growth Performance, Immune Response, and Resistance against Vibrio Parahaemolyticus in Litopenaeus Vannamei. *BMC Vet. Res.* **2025**, *21*, 166. [CrossRef]
20. Taklu, M.; Islami, H.R.; Shekarabi, S.P.H.; Mousavi, S.A.; Jourdehi, A.Y. Supplemental Effect of Dietary Nucleotides on Hematological Profile, Hepatic Biomarkers, Antioxidant Capacity, and Digestive Functions in Sterlet Sturgeon, Acipenser Ruthenus. *Sci. Rep.* **2025**, *15*, 11408. [CrossRef]
21. Hervé, M.K.; Calice, M.D.; Dzepe, D.; Djouaka, R.F.; Chia, S.Y.; Efole, T.; Ndindeng, S.A. Black Soldier Fly (*Hermetia illucens*) Larvae Improve Growth Performance and Flesh Quality of African Catfish (*Clarias gariepinus*). *Discov. Anim.* **2025**, *2*, 9. [CrossRef]
22. Muurmann, A.T.; Eriksen, N.T.; Rasmussen, J.A.; Limborg, M.T.; Tomberlin, J.K.; Gilbert, M.T.P.; Bahrndorff, S. Growth and Metabolic Performance of House Fly and Black Soldier Fly Larvae Differ across Densities and Waste-Based Growth Substrates. *Waste Manag.* **2025**, *193*, 529–538. [CrossRef] [PubMed]
23. Zarantoniello, M.; Bruni, L.; Randazzo, B.; Vargas, A.; Gioacchini, G.; Truzzi, C.; Annibaldi, A.; Riolo, P.; Parisi, G.; Cardinaletti, G.; et al. Partial Dietary Inclusion of Hermetia Illucens (*Black soldier fly*) Full-Fat Prepupae in Zebrafish Feed: Biometric, Histological, Biochemical, and Molecular Implications. *Zebrafish* **2018**, *15*, 519–532. [CrossRef] [PubMed]
24. Weththasinghe, P.; Lagos, L.; Cortés, M.; Hansen, J.Ø.; Øverland, M. Dietary Inclusion of Black Soldier Fly (*Hermetia illucens*) Larvae Meal and Paste Improved Gut Health but Had Minor Effects on Skin Mucus Proteome and Immune Response in Atlantic Salmon (*Salmo salar*). *Front. Immunol.* **2021**, *12*, 599530. [CrossRef] [PubMed]
25. Radhakrishnan, G.; Liland, N.S.; Koch, M.W.; Lock, E.-J.; Philip, A.J.P.; Belghit, I. Evaluation of Black Soldier Fly Larvae Meal as a Functional Feed Ingredient in Atlantic Salmon (*Salmo salar*) under Farm-like Conditions. *Front. Aquac.* **2023**, *2*, 1239402. [CrossRef]
26. Chen, Y.; Zhuang, Z.; Liu, J.; Wang, Z.; Guo, Y.; Chen, A.; Chen, B.; Zhao, W.; Niu, J. Effects of Hermetia Illucens Larvae Meal on the Pacific White Shrimp (*Litopenaeus vannamei*) Revealed by Innate Immunity and 16S RRNA Gene Sequencing Analysis. *Comp. Biochem. Physiol. Part D Genom. Proteom.* **2023**, *46*, 101080.
27. Fatima, S.; Afzal, A.; Rashid, H.; Iqbal, S.; Zafar, R.; Khalid, K.; Rauf, A.; Majeed, M.; Malik, A.; Carter, C.G. Dietary Replacement of Soybean Meal with Black Soldier Fly Larvae Meal in Juvenile Labeo Rohita and Catla Catla: Effects on Growth, Nutritional Quality, Oxidative Stress Biomarkers and Disease Resistance. *PLoS ONE* **2023**, *18*, e0294452. [CrossRef]

28. Kamalii, A.; Antony, C.; Ahilan, B.; Uma, A.; Prabu, E. Dietary Protein Replacement of Fish Meal with Black Soldier Fly Larvae Meal: Effects on Growth, Whole-Body Composition, Digestive Enzyme Activity, Muscle-Growth-Related Gene Expression and Haemato-Biochemical Responses of Juvenile Goldfish, Carassius Auratus. *Turk. J. Fish. Aquat. Sci.* **2022**, *23*, TRJFAS21837.
29. Chen, Y.; Ma, J.; Yong, Y.-S.; Chen, Y.; Chen, B.; Cao, J.; Peng, K.; Wang, G.; Huang, H.; Loh, J.-Y. Impacts of Black Soldier Fly (*Hermetia illucens*) Larval Meal on Intestinal Histopathology and Microbiome Responses in Hybrid Grouper (*Epinephelus fuscoguttatus* ♀× E. Lanceolatus ♂): A Comprehensive Analysis. *Animals* **2024**, *14*, 3596. [CrossRef]
30. Nankervis, L.; Cobcroft, J.M.; Nguyen, N.V.; Rimmer, M.A. Advances in Practical Feed Formulation and Adoption for Hybrid Grouper (*Epinephelus fuscoguttatus* ♀× *E. Lanceolatus* ♂) Aquaculture. *Rev. Aquac.* **2021**, *14*, 288–307. [CrossRef]
31. Limbu, S.M.; Shoko, A.P.; Ulotu, E.E.; Luvanga, S.A.; Munyi, F.M.; John, J.O.; Opiyo, M.A. Black Soldier Fly (*Hermetia illucens* L.) Larvae Meal Improves Growth Performance, Feed Efficiency and Economic Returns of Nile Tilapia (*Oreochromis niloticus* L.) Fry. *Aquac. Fish Fish.* **2022**, *2*, 167–178. [CrossRef]
32. Rawles, S.D.; Riche, M.; Gaylord, T.G.; Webb, J.; Freeman, D.W.; Davis, M. Evaluation of Poultry By-Product Meal in Commercial Diets for Hybrid Striped Bass (*Morone chrysops* ♀× *M. Saxatilis* ♂) in Recirculated Tank Production. *Aquaculture* **2006**, *259*, 377–389. [CrossRef]
33. Jiang, B.; Sun, Y.; Li, W.; Liu, C.; Wen, C.; Li, A.; Huang, Y.; Su, Y. Effects of Dietary Black Soldier Fly (*Hermetia illucens linnaeus*) on the Disease Resistance of Juvenile Grouper (*Epinephelus coioides*). *Fish Amp. Shellfish. Immunol.* **2022**, *123*, 136–141. [CrossRef] [PubMed]
34. Simões-Alves, A.C.; Costa-Silva, J.H.; Bassot, A.; Leandro, C.G.; Pirola, L.; Fernandes, M.P.; Morio, B. Diet Enriched in Saturated Fatty Acids Induces Liver Oxidative Stress and Elicits Inflammatory Pathways Prior to Metabolic Disruption in Perinatal Protein Undernutrition. *Nutr. Res.* **2023**, *118*, 104–115. [CrossRef]
35. Xiao, X.; Jin, P.; Zheng, L.; Cai, M.; Yu, Z.; Yu, J.; Zhang, J. Effects of Black Soldier Fly (*Hermetia illucens*) Larvae Meal Protein as a Fishmeal Replacement on the Growth and Immune Index of Yellow Catfish (*Pelteobagrus fulvidraco*). *Aquac. Res.* **2018**, *49*, 1569–1577. [CrossRef]