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Comparative effect of dietary full-fat or defatted Hermetia illucens larvae on growth and fatty acid profiles of juvenile Oncorhynchus mykiss

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Abstract

Although black soldier fly (BSF) larvae meal (BSFL) has been a promising alternative ingredient, limited information is available on full-fat BSF larvae meal (FBSF), which may offer cost-savings in aquafeed. This study evaluated the effects of FBSF compared with defatted BSF larvae meal (DBSF) as fishmeal substitutes on the growth performance and biochemical responses of juvenile rainbow trout. A control diet contained fishmeal at 360 g/kg diet, and four experimental diets replaced fishmeal with FBSF or DBSF at 36 or 108 g/kg diet (designated as FBSF10, FBSF30, DBSF10, and DBSF30, respectively). Following an 8-week feeding trial, only FBSF30 resulted in reduced growth and an increased feed conversion ratio compared to the control. Proximate compositions of carcass and viscera were unaffected. Tissue fatty acid profiles mirrored dietary composition; polyunsaturated fatty acids (including C20:5n-3 and C22:6n-3) decreased with higher BSFL inclusion, whereas saturated fatty acids increased, with C12:0 levels significantly elevated in the FBSF30 group. Principal component analysis associated FBSF30 strongly with saturated fatty acid vectors (C12:0 and C14:0), demonstrating that high FBSF inclusion markedly shifts tissue lipid composition toward saturation. Serum cortisol, glutathione peroxidase, superoxide dismutase, lysozyme, and immunoglobulin M levels were unaffected across treatments. Overall, up to 30% DBSF and 10% FBSF can replace fishmeal in juvenile rainbow trout diets without compromising growth or physiological health. While FBSF offers economic advantages, inclusion levels must be optimized to prevent growth impairment and lipid alterations.

1. Introduction

While aquaculture continues to grow rapidly, its sustainable expansion is constrained by environmental challenges and a depleting supply of fishmeal, making exploring stable and high-quality alternative ingredients critical for the industry's future (Fisher et al. 2020; Limbu et al. 2022; Roberts et al. 2024). The availability and quality of alternative protein sources in compound feeds have become key determinants of growth performance and health in farmed fish (Zheng et al. 2012). Locally producible feed ingredients have been studied to secure a sustainable supply in African countries (Adéyèmi et al. 2020; Chisoro et al. 2023). Similarly, the Norwegian government has established a goal to ensure that all feeds come from sustainable sources and to increase the inclusion rate of domestically produced raw materials from 8% to 25% by 2034 (Myrseth 2024). In 2025, the total production of compound feed in South Korea amounted to 21,791,617 tons (Ministry of Agriculture, Food and Rural Affairs 2025). As one of the largest importers of feed ingredients worldwide, South Korea relies heavily on foreign sources to support its livestock and aquaculture industry. Thus, exploring alternative feed resources is essential for a sustainable future in aquaculture (Fantatto et al. 2024; Roberts et al. 2024).

Various fishmeal alternatives have been proposed, including fisheries and aquaculture by-products, insects, and microbial biomass (Hua et al. 2019). Among these, insects and fisheries by-products have been identified as particularly promising due to their economic feasibility, stable supply, and regulatory compatibility (Hua et al. 2019). Insect meal, in particular, offers advantages over conventional plant and animal protein sources by requiring less land, energy, and labor, while also enabling efficient recycling of

organic waste streams (Liu et al. 2017; Nam et al. 2022; Wilfart et al. 2023). Moreover, large-scale insect production can support a circular economy by converting food and feed waste into high-value protein and lipid resources through bioconversion processes (DiGiacomo and Leury 2019).

The black soldier fly (BSF, *Hermetia illucens*) has emerged as a leading candidate for fishmeal replacement in aquafeeds. Its potential as an alternative protein source has been demonstrated in more than 17 species, which have shown relatively high levels of substitution without compromising fish growth or health (Gougbedji et al. 2022). The nutritional composition of BSF larvae meal (BSFL) typically includes 30–40% protein, 20–40% lipid, approximately 16% ash, 8% crude fiber, and 6–10% chitin (Lu et al. 2022), although these values vary depending on larval rearing substrates such as livestock manure and food waste (Liu et al. 2017). Notably, BSFL exhibits substantial nutritional plasticity, with its composition responding dynamically to dietary inputs (Barragán-Fonseca et al. 2021; Fitriana et al. 2022). This adaptability allows for the targeted enhancement of its nutritional value through substrate manipulation (Ewusie et al. 2019). In addition, BSFL are rich in chitin and lauric acid (C12:0), which have been associated with improved gut microbiota diversity, antioxidant capacity, and immune responses in fish (Do Couto et al. 2021; Makkar et al. 2014; Triunfo et al. 2022; Zhan et al. 2024). Previous studies have reported variable outcomes in fishmeal substitution, influenced by factors including inclusion level, nutrient digestibility, and availability of bioactive compounds (English et al. 2021; Menoyo et al. 2003; Radhakrishnan et al. 2023).

Despite its nutritional potential, economic feasibility remains a critical consideration in feed formulation (Bystricky et al. 2023). Fishmeal is relatively cost-effective because it is produced through simple processing methods, including the freezing and grinding of forage fish without extensive refinement (Jannathulla et al. 2019; Miles and Chapman 2006). In contrast, the high lipid content and fatty acid profile of BSFL may negatively affect nutrient digestibility and increase susceptibility to lipid oxidation (Belghit et al. 2019; Dumas et al. 2018; Menoyo et al. 2003; Tocher et al. 2000). To address these issues, defatting processes are often applied to BSFL, resulting in improved protein concentration and digestibility (Gasco et al. 2021; Nogales-Mérida et al. 2019). However, defatting processes in other insects, such as mealworms, have been shown to increase furosine content, a product of the Maillard reaction (Jensen et al. 2019), and the processing method itself can affect oxidative quality and stability (Hurtado-Ribeira et al. 2023). In addition, defatting processes add significant operational expenses, potentially limiting the price competitiveness of BSFL against fishmeal. Indeed, the high production cost of BSFL remains a substantial hurdle for its widespread application as a fishmeal replacement. While small-scale production in low-labor environments may be feasible for producing BSFL at prices competitive with fishmeal (Barragán-Fonseca et al. 2023; Gougbedji et al. 2021), medium- to large-scale production requires specialized equipment and entails high energy costs. Although official data regarding the market price of BSFL in South Korea are unavailable, industry representatives indicate that defatted BSFL is traded at approximately 3,500–4,000 KRW/kg for use as feed ingredients. Consequently, the use of full-fat BSFL represents a more economically viable alternative for the aquaculture industry (Weththasinghe et al. 2021). While numerous studies have evaluated growth performance and optimal replacement levels using defatted BSFL (Dumas et al. 2018; Hwang et al. 2021;

Xu et al. 2020), the nutritional quality and structural characteristics of full-fat BSFL remain relatively underexplored (Zozo et al. 2022). Although several studies have reported the potential of full-fat BSFL in diets for Pacific white shrimp (Ko et al. 2025), Nile tilapia (Sangsawang et al. 2024), turbot (Zhao et al. 2023), tambaqui (Monteiro dos Santos et al. 2023), Siberian sturgeon (Caimi et al. 2020; Rawski et al. 2021), Atlantic salmon (Willora et al. 2025), and rainbow trout (Bruni et al. 2022; Riesenbach et al. 2026), information regarding their nutritional utilization, specifically the relationship between fatty acid profiles and growth performance of rainbow trout at early life stages, remains limited. Furthermore, comparative studies between full-fat and defatted BSFL are scarce.

In South Korea, rainbow trout has been cultivated for nearly six decades since 1965 and is considered a practical alternative to Atlantic salmon, supported by well-established aquaculture technologies (Paek and Park 2016; Yoon et al. 2023). The domestic production of BSFL, combined with their contribution to circular economy frameworks, highlights their potential as a sustainable feed ingredient for local rainbow trout aquaculture. Therefore, this study aimed to investigate the effects of substituting dietary fishmeal with BSFL in full-fat and defatted forms on the growth performance and biochemical responses of rainbow trout. By evaluating parameters such as growth performance, feed efficiency, and biochemical markers, this research sought to assess the feasibility of BSFL as a sustainable and functional alternative protein source.

2. Materials and methods

2.1. Ethics statement

All procedures involving animals were reviewed and approved by the Animal Experiment Ethics Committee (Institutional Animal Care and Use Committee) of Kongju National University, South Korea (approval no. KNU_2023-06; approved 26 July 2023), and were performed in accordance with the Animal Protection Act and the Laboratory Animal Act of the Republic of Korea and the institutional guidelines for the care and use of laboratory animals.

2.2. Ingredients and experimental diets

The BSFL used in this study were reared on food waste, oven-dried, and mechanically screw-pressed to extract oil. These processes were conducted by a local commercial producer (ENTOMO Co., Ltd., Cheongju, South Korea), and the final products, full-fat BSF larvae meal (FBSF) and defatted BSF larvae meal (DBSF) were provided. Fatty acid profiles of BSFL before and after oil extraction are presented in Table 1. Other ingredients were purchased from a domestic feed supplier. The proximate composition of the major feed ingredients was analyzed and is presented in Table 2. All the ingredients were finely ground into a powder using a commercial blender (VMK 200B, VAIMARKOREA Co., Ltd., Seoul, South Korea) prior to mixing and pelleting.

Table 1
Fatty acid profiles of black soldier fly larvae meal.

Fatty acids (% total fatty acids)	FBSF	DBSF
C6:0	0.03	0.08
C8:0	0.04	0.05
C10:0	2.15	1.90
C12:0	30.6	34.9
C14:0	4.53	4.69
C15:0	0.14	0.12
C16:0	15.0	14.1
C17:0	0.22	0.20
C18:0	2.98	3.03
C21:0	0.14	0.13
C22:0	0.09	0.12
C24:0	0.03	ND
∑ SFA	55.9	59.3
C14:1	0.09	0.10
C15:1	0.02	0.03
C16:1	2.92	2.42
C17:1	0.22	0.17
C18:1n-9	20.3	18.6
C20:1n-9	0.46	0.34
C22:1n-9	0.03	0.07
C24:1n-9	0.05	0.04
∑ MUFA	24.1	21.8
C18:2n-6	16.0	15.8
C18:3n-6	0.24	0.18
C18:3n-3	1.78	1.40

Abbreviation: FBSF, full-fat black soldier fly larvae meal; DBSF, defatted black soldier fly larvae meal; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Fatty acids (% total fatty acids)	FBSF	DBSF
C20:3n-3	0.01	0.03
C20:4n-6	0.32	0.28
C20:5n-3	1.48	0.99
C22:2n-6	0.03	0.06
C22:6n-3	0.22	0.17
∑ PUFA	20.1	18.9
∑ PUFA n-3	3.49	2.59
∑ PUFA n-6	16.6	16.3
∑ PUFA n-9	20.8	19.0
n-3/n-6	0.21	0.16

Abbreviation: FBSF, full-fat black soldier fly larvae meal; DBSF, defatted black soldier fly larvae meal; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ND, not detected.

Table 2
Analyzed proximate composition of diet ingredients.

Ingredients (% dry matter)	Moisture	Crude protein	Crude lipid	Ash	Crude fiber	ADF	ADL	Chitin
Fishmeal	7.31	76.3	12.5	14.7	0.28	-	-	-
DBSF	1.06	57.2	4.85	23.2	13.0	15.3	4.2	11.1
FBSF	4.72	42.8	31.7	15.9	8.28	8.72	2.38	6.34
Soybean meal	8.31	51.2	2.80	8.04	2.88	-	-	-
Wheat gluten meal	5.16	85.0	1.31	0.85	0.32	-	-	-
Wheat flour	9.84	17.9	2.64	1.56	1.24	-	-	-

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; ADF, acid detergent fiber fraction; ADL, acid detergent lignin.

The formulation and proximate composition of the experimental diets are presented in Table 3. The control diet met the nutrient requirements of rainbow trout (NRC 2011) and contained fishmeal at a concentration of 360 g/kg diet. Four experimental diets were prepared by replacing 10% (36 g/kg diet) or 30% (108 g/kg diet) of the fishmeal in the control diet with FBSF or DBSF, respectively. These were designated as FBSF10, FBSF30, DBSF10, and DBSF30. The dry ingredients were mixed according to the

diet formulations and processed into experimental diets using a mixer machine. Dietary nutrients and energy levels were adjusted by soybean meal, wheat gluten and wheat flour. Compared to fishmeal, the methionine and lysine contents of the other ingredients were lower, so they were added proportionally (Schiavone et al. 2017; Zheng et al. 2012). Combined mixtures were refined using a sieve and any remaining pieces were blended and combined into the mixture. After adding fish oil, the mixture was mixed for 10 min using a mixer machine, and 30% primary distilled water was added to make a dough. The mixed feed base was used to produce feed by a meat grinder (MN-22S, Hankook Fujee Industries Co., Ltd., Gyeonggi, South Korea). The finely fragmented feed was dried for 72 h using an electric fan in a laboratory where the room temperature was maintained at 18°C. The resulting diets were stored in plastic bags at 4°C until needed for feeding the fish, and the sampling feed was stored at -20°C.

Table 3
Ingredient composition and chemical properties of the experimental diets.

Ingredients (% dry matter)	Control	DBSF10	DBSF30	FBSF10	FBSF30
Fishmeal	36.0	32.4	25.2	32.4	25.2
BSF (defatted)	-	3.60	10.8	-	-
BSF (full-fat)	-	-	-	3.60	10.8
Soybean meal	21.0	17.5	15.5	17.5	15.0
Wheat gluten meal	7.00	10.0	13.5	10.0	15.0
Wheat flour	20.0	19.8	17.1	20.8	19.4
L-ascorbate-2-phosphate	0.50	0.50	0.50	0.50	0.50
DL-α-tocopheryl acetate	0.50	0.50	0.50	0.50	0.50
Vitamin premix[1]	0.80	0.80	0.80	0.80	0.80
Mineral premix[2]	0.80	0.80	0.80	0.80	0.80
Monocalcium phosphate	1.00	1.00	1.00	1.00	1.00
Lecithin	0.50	0.50	0.50	0.50	0.50
L-lysine sulfate	0.30	0.40	0.50	0.40	0.50
DL-methionine	0.30	0.40	0.50	0.40	0.50
Taurine	0.30	0.30	0.30	0.30	0.30
Fish oil	11.0	11.5	12.5	10.5	9.20
Analyzed chemical properties					
Moisture	16.4	15.7	15.2	15.4	14.7
Crude protein	47.5	47.9	48.8	47.3	47.7

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; ADF, acid detergent fiber fraction; ADL, acid detergent lignin

[1] Vitamin mixture composition (unit/kg): retinol acetate, 5,000,000 IU; cholecalciferol, 1,000,000 IU; tocopherol acetate 37,500 mg; menadion sodium bisulfite, 2,500 mg; ascorbic acid, 6,400 mg; thiamine nitrate, 5,000 mg; riboflavin 10,000 mg; pyridoxine hydrochloride, 5,000 mg; folic acid, 2,500 mg; biotin, 50 mg; cyanocobalamin, 25 mg; inositol, 75,000 mg; nicotinic acid, 37,500 mg; calcium pantothenate, 17,500 mg

[2] Mineral mixture composition (g/kg): ferrous fumarate, 12.5; manganese sulfate, 11.25; ferrous sulfate, 20; cupric sulfate, 1.25; cobaltous sulfate, 0.75; zinc sulfate, 13.75; calcium iodate, 0.75; magnesium sulfate, 80.2; aluminum hydroxide, 0.75

Ingredients (% dry matter)	Control	DBSF10	DBSF30	FBSF10	FBSF30
Crude lipid	17.9	17.7	17.1	17.9	19.3
Ash	9.33	9.85	9.67	8.98	8.94
Crude fiber	3.47	1.42	2.36	1.42	1.53
ADF	1.14	1.28	2.12	1.31	1.91
ADL	0.20	0.26	0.48	0.26	0.54
Chitin (ADF-ADL)	0.93	1.02	1.64	1.05	1.37
Peroxide value (meq/kg)	13.0	9.16	9.06	8.17	7.86

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; ADF, acid detergent fiber fraction; ADL, acid detergent lignin

[1] Vitamin mixture composition (unit/kg): retinol acetate, 5,000,000 IU; cholecalciferol, 1,000,000 IU; tocopherol acetate 37,500 mg; menadion sodium bisulfite, 2,500 mg; ascorbic acid, 6,400 mg; thiamine nitrate, 5,000 mg; riboflavin 10,000 mg; pyridoxine hydrochloride, 5,000 mg; folic acid, 2,500 mg; biotin, 50 mg; cyanocobalamin, 25 mg; inositol, 75,000 mg; nicotinic acid, 37,500 mg; calcium pantothenate, 17,500 mg

[2] Mineral mixture composition (g/kg): ferrous fumarate, 12.5; manganese sulfate, 11.25; ferrous sulfate, 20; cupric sulfate, 1.25; cobaltous sulfate, 0.75; zinc sulfate, 13.75; calcium iodate, 0.75; magnesium sulfate, 80.2; aluminum hydroxide, 0.75

2.3. Experimental fish, culture system and feeding regime

Rainbow trout (*Oncorhynchus mykiss*) were obtained from the Ewhajung fish farm (Sangju, South Korea) and transported to the Aquaculture Laboratory of Kongju National University, Yesan, South Korea. The ambient temperature in the laboratory was maintained at 17°C using an air conditioner. The fish were acclimatized in a 1.2-ton cylindrical tank before the start of the feeding trial. Water quality was monitored using a portable water temperature, dissolved oxygen (DO) meter (ProDSS, YSI inc., Yellow Springs, OH, USA) and a pH sensor (8100 Plus, Eti, Worthing, UK) and it was maintained at 16.7 ± 0.1°C, DO 9.0 ± 0.4 mg/L, and pH 7.94 ± 0.05. During the acclimatization period, fish were offered a commercial feed (Woosung feed, Daejeon, South Korea). After one week of acclimation, fish were randomly distributed using a completely randomized design into fifteen acrylonitrile-butadiene-styrene (ABS) tanks (rectangle, 30 L capacity) at a density of 20 fish/tank. The experimental tanks were set up in a semi-recirculating water flow system consisting of aeration, a physical filter, a bio filter, LED lights control, a UV sterilizer, and a thermo-control system. The experiment was conducted using fish with an average weight of 12.1 ± 0.2 g. Over the course of the feeding trial, water quality was maintained at 16.5 ± 0.1°C, DO 8.89 ± 0.39 mg/L, and pH 7.94 ± 0.05. The photoperiod was controlled using a white LED auto-controller (13:11 h, light/dark cycle). Fish behavior, feeding response, external signs of disease, and water quality were monitored and recorded several times a day. Humane endpoints followed the approved protocol: fish

showing abnormal swimming, cessation of feeding, or severe external lesions were to be removed and euthanized immediately; no fish reached these criteria during the trial.

Fish were starved for 72 h before the initiation of the first feeding with the experimental diets. Feeding regime was managed as feeding for 6 days followed by fasting for 1 day on a weekly basis. During the first half of the feeding trial (week 1 to 4), fish were hand-fed *ad libitum* with one of the experimental diets three times a day (8:00, 13:00, 18:00). Waste matter was removed three times a day from all tanks by siphoning and flushing. As a result, the biomass increased roughly 2.4-fold, approaching the maximum capacity of the culture tank (with the biomass reaching roughly 1.9% of the water volume). Consequently, ten fish were sampled to allow for the continued growth of the remaining fish within the tank limit. For the remainder of the trial (week 5 to 8), the feeding method was identical except that feeding frequency was reduced to twice daily (8:00, 18:00).

2.4. Sample collection

After 24 h of starvation, fish were anesthetized with 2-phenoxyethanol (0.3 mL/L; Sigma-Aldrich, St. Louis, MO, USA) and the fish in each tank were bulk-weighed and counted to determine mean weight. At the end of the feeding trial, five fish from each tank (n = 15 per dietary treatment) were randomly collected and deeply anesthetized (2-phenoxyethanol, 0.5 mL/L). Blood samples were taken from the caudal vein under anesthesia, after which the fish were euthanized by an overdose of 2-phenoxyethanol (> 3 g/L). The ten fish per tank removed at week 4 and all fish remaining at the end of the trial were euthanized in the same way, and carcasses were disposed of through a licensed waste contractor in accordance with institutional regulations. Blood samples were allowed to clot at room temperature for 30 min and centrifuged for 10 min at 5,500 rpm using a microcentrifuge to obtain serum. The serum samples collected from each tank were pooled, mixed using a vortex mixer, and stored at -20°C until ELISA analysis. Body weight and total length of fish were then individually measured. These individuals were subsequently dissected to obtain the weight of liver and viscera. Carcass and viscera were separated and chopped with a knife. Each portion was homogenized with liquid nitrogen using a high-speed blender (RT-N04, Rong Tsong Precision Technology Co., Taichung, Taiwan) at 25,000 rpm. The prepared samples were stored at -20°C until analysis of proximate composition and fatty acids.

2.5. Biochemical analysis of ingredients, feeds, viscera, and carcass

Proximate composition analysis (moisture, crude protein, crude lipid, crude fiber, and ash) of major ingredients, experimental diets, fish carcass, and viscera was conducted using the methodology of the Association of Official Analytical Chemists (AOAC 2005) at the Institute of Feed Nutrition Research, Pukyong National University, South Korea. Moisture content was determined by drying samples to a constant weight at 105°C. Ash content was determined by combustion at 550°C for 3 h. Crude protein was determined from the nitrogen content (N × 6.25) through acid digestion using the Kjeldahl method (2300 Auto Analyzer, Foss Tecator AB, Höganäs, Sweden). Crude lipid was analyzed by ether extraction using the Soxtec System 1046 (Foss Tecator AB). The chitin content of the BSFL was calculated by

subtracting the acid detergent lignin (ADL) from the acid detergent fiber (ADF) fraction (Triunfo et al. 2022).

2.6. Fatty acid analysis

The fatty acid composition of experimental diets, fish carcass, and viscera was analyzed using a gas chromatograph at the Institute of Agricultural Science, Chungnam National University, South Korea. Fatty acid composition was determined by extracting total lipids from approximately 10 g of the ground sample using a chloroform-methanol mixture (2:1, v/v; ca. 100 mL) for 24 h at room temperature, following the method of Folch et al. (1957). The extract was concentrated under reduced pressure and then methylated using a 14% BF_3-MeOH solution (Sigma-Aldrich, St. Louis, MO, USA). Fatty acid methyl esters (FAMEs) were analyzed using a gas chromatograph (HP 6890, Hewlett-Packard, Palo Alto, CA, USA) equipped with an HP-88 capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness; Agilent Technologies, Santa Clara, USA). The column oven temperature was initially held at 140°C for 5 min, increased to 240°C at a rate of 4°C/min, and finally maintained at 240°C for 20 min. The injector and flame ionization detector (FID) temperatures were maintained at 260°C and 270°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min, with a split ratio of 1:50. Individual fatty acid peaks were identified by comparing their retention times with those of standard fatty acid methyl esters. The fatty acid composition was calculated based on the relative peak area and expressed as a percentage of the total identified fatty acids.

2.7. ELISA analysis

ELISA analysis was performed to assess the physiological responses of fish fed the experimental diets. ELISA kits for cortisol (CSB-E08487f), superoxide dismutase (SOD; CSB-E15929Fh), glutathione peroxidase (GPx; CSB-E15930Fh), lysozyme (LZM; CSB-E17296FH), and immunoglobulin M (IgM; CSB-E12045Fh) were purchased from CUSABIO Technology LLC (Houston, USA) and prepared according to the manufacturer's protocols. All ELISA tests were conducted using pooled serum samples. The absorbance was measured using a BioTek Synergy H1 Plate Reader (BioTek Instruments, Inc., Winooski, VT, USA). The results of five ELISA assays were processed and verified using the CurveExpert software (Hyams Development, Chattanooga, TN, USA).

2.8. Calculations and statistical analysis

Calculations were performed using the following equations.

Weight gain (WG, %) = [final weight (g) - initial weight (g)] / initial weight (g) × 100.

Specific growth rate (SGR, %) = [ln (final weight, g) - ln (initial weight, g)] / days × 100.

Feed conversion ratio (FCR) = diet consumed (g) / weight gained (g).

Condition factor (CF, %) = body weight (g) / total length (cm^3) × 100.

Hepato-somatic index (HSI, %) = liver weight (g) / fish weight (g) × 100.

Viscera-somatic index (VSI, %) = viscera (without liver, g) / fish weight (g) × 100.

Statistical analysis was performed using SPSS for Windows, version 27 (IBM, Armonk, USA). Data were analyzed by one-way analysis of variance (ANOVA) to evaluate the effects of the dietary treatments on growth and biochemical parameters, and differences among means were compared using Tukey's HSD test. For all statistical analyses, the significance level was set at $P < 0.05$. Results were expressed as means with the pooled standard error of the mean (Pooled SEM), with each tank serving as the experimental unit (n = 3 tanks per treatment). Relationships between dietary and tissue (Carcass and viscera) levels of C12:0, C18:3n-3, C20:5n-3, and C22:6n-3 were examined by linear regression using the treatment means (n = 5 diets), and the significance of each regression was tested by F-test. Principal component analysis (PCA) of the carcass fatty acid composition was conducted to visualize multivariate patterns and to identify major sources of variation among dietary treatments. PCA was performed using Python (version 3.X) in a Jupyter Notebook environment.

3. Results

3.1. Growth performance and biological indices

All fish readily accepted and vigorously consumed the experimental diets throughout the feeding trial. During the first feeding period (weeks 1–4), body weight increased approximately 2.4 times from 12 g to 29 g. No differences were observed in survival rate (SR), WG, SGR, feed intake (FI), FCR, or CF (Table 4). However, VSI and HSI differed significantly among the groups. The VSI of fish in the FBSF30 group was significantly higher than that of the FBSF10 group ($P < 0.05$), but there was no difference when compared to the control group. The FBSF30 group exhibited a significantly higher HSI than the other dietary groups, except for DBSF30 ($P < 0.05$). Furthermore, the HSI tended to increase with increasing dietary BSFL fat content and fishmeal replacement rates.

Table 4
Growth performance and biological indices of fish fed experimental diets.

Groups	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
Feeding period from the start to week 4						
IW (g/fish)	11.9	12.0	12.1	12.1	12.3	0.89
FW (g/fish)	28.7	28.8	28.3	29.3	28.3	0.40
SR (%)	96.7	93.3	100	95	95	0.29
WG (%)	140	140	133	141	130	0.28
SGR (%)	3.13	3.13	3.02	3.14	2.97	0.26
FI (g/fish)	14.4	14.9	14.5	15.0	14.6	0.39
FCR	0.86	0.89	0.90	0.88	0.91	0.10
CF	1.02	1.02	1.08	1.02	1.05	0.06
VSI (%)	10.4^{ab}	9.80^{ab}	9.90^{ab}	9.70^{a}	10.6^{b}	0.05
HSI (%)	1.40^{ab}	1.32^{a}	1.52^{bc}	1.46^{ab}	1.62^{c}	0.05
Feeding period from week 5 to week 8						
IW (g/fish)	31.7	33.3	32.7	32.0	31.2	0.45
FW (g/fish)	61.0^{ab}	65.1^{b}	59.2^{ab}	60.3^{ab}	52.4^{a}	0.05
SR (%)	100	96.7	96.7	100	96.7	0.73
WG (%)	92.7^{b}	95.8^{b}	81.7^{b}	88.9^{b}	69.3^{a}	0.05
SGR (%)	2.18^{b}	2.55^{b}	2.16^{ab}	2.31^{b}	1.73^{a}	0.05
FI (g/fish)	37.5	39.4	38.4	37.3	34.5	0.42
FCR	1.35^{ab}	1.19^{a}	1.45^{ab}	1.30^{a}	1.78^{b}	0.05
CF	1.14	1.13	1.13	1.13	1.14	1.00
VSI (%)	11.2	12.4	11.2	11.5	11.2	0.34
HSI (%)	1.79	1.87	1.58	1.77	1.65	0.37

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean; IW, initial weight; FW, final weight; SR, survival rate; WG, weight gain; SGR, specific growth rate; CF, condition factor; FI, feed intake; FCR, feed conversion ratio; VSI, viscera-somatic index; HSI, hepato-somatic index

During the second feeding period (weeks 5–8), SR, CF, VSI, and HSI did not differ among the dietary groups. DBSF10, DBSF30, and FBSF10 had no effect on the growth and feed efficiency of rainbow trout in comparison to the control. However, only fish fed the FBSF30 diet showed a decrease in final weight (FW), WG, and SGR compared to the control. Additionally, the FCR of the FBSF30 group was significantly higher than those of the DBSF10 and FBSF10 groups ($P < 0.05$).

3.2. Proximate composition of carcass and viscera

Proximate composition of the carcass and viscera (including the liver) of the experimental fish are presented in Table 5. There were no significant differences in moisture, crude protein, crude lipid, and ash content. Peroxide values of the carcass and viscera did not differ among the experimental groups.

Table 5
Proximate composition of the carcass and viscera (including the liver) of fish fed experimental diets for 8 weeks.

Group	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
Carcass						
Moisture (%)	70.7	70.1	70.3	70.6	69.4	0.26
Crude protein (%)	17.0	17.5	17.6	17.4	18.3	0.31
Crude lipid (%)	9.64	9.67	9.53	9.41	9.82	0.97
Ash (%)	2.49	2.74	2.53	2.62	2.48	0.15
Peroxide value (meq/kg)	14.5	13.2	14.5	21.9	13.2	0.49
Viscera						
Moisture (%)	61.1	60.6	60.1	60.5	59.7	0.67
Crude protein (%)	11.0	10.8	11.3	11.7	11.7	0.46
Crude lipid (%)	26.5	27.2	27.6	26.4	27.3	0.92
Ash (%)	1.14	1.17	1.10	1.36	1.25	0.55
Peroxide value (meq/kg)	37.4	35.1	59.6	43.8	36.4	0.27

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean

3.3. Fatty acid profiles of experimental diets, carcass and viscera

The fatty acid profiles of the experimental diets are presented in Table 6. The levels of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) did not

differ substantially among the experimental diets. Although no statistical analysis was conducted due to a lack of replication, distinct differences in individual fatty acid profiles were observed among the dietary groups. Lauric (C12:0) and myristic (C14:0) acid levels increased with increasing dietary BSFL inclusion and varied with the type of BSFL (FBSF or DBSF). Notably, lauric acid was not detected in the control diet but sharply increased to 0.73% in the FBSF30 diet. For n-6 PUFA, linoleic acid (C18:2n-6) increased stepwise with BSFL inclusion, rising from 31.5% (control) to 33.5% (FBSF30). Conversely, EPA (C20:5n-3) and DHA (C22:6n-3) levels dropped compared to the control, resulting in a steady decline in the n-3/n-6 ratio from 0.31 in the control down to 0.27 in DBSF30 and 0.26 in FBSF30.

Table 6
Fatty acid profiles of the experimental diets.

Fatty acids as % of total fatty acids	Control	DBSF10	DBSF30	FBSF10	FBSF30
C12:0	ND	0.03	0.09	0.11	0.73
C14:0	1.44	1.53	1.62	1.61	1.71
C15:0	0.11	0.12	0.12	0.12	0.12
C16:0	15.1	14.9	15.4	15.7	14.7
C17:0	0.24	0.21	0.21	0.22	0.23
C18:0	4.66	4.59	4.64	4.70	4.63
C20:0	0.17	0.17	0.18	0.17	0.14
C21:0	0.66	0.65	0.64	0.66	0.64
C22:0	0.27	0.30	0.29	0.29	0.26
C24:0	0.48	0.44	0.41	0.42	0.38
∑ SFA	23.2	23.0	23.6	24.0	23.6
C14:1	0.05	0.04	0.05	0.05	0.07
C15:1	ND*	0.04	0.08	0.08	0.02
C16:1	2.55	2.51	2.35	2.52	2.42
C17:1	0.25	0.25	0.27	0.27	0.29
C18:1n-9	23.8	24.1	23.8	23.8	23.8
C20:1n-9	3.37	3.39	3.21	3.16	2.88
C22:1n-9	0.32	0.34	0.32	0.30	0.30
C24:1n-9	1.07	0.99	0.85	0.93	0.82
∑ MUFA	31.4	31.7	30.9	31.1	30.6
C18:2n-6	31.5	31.9	32.8	31.8	33.5
C18:3n-6	0.51	0.52	0.52	0.50	0.48
C18:3n-3	3.36	3.42	3.57	3.41	3.42
C20:4n-6	2.47	2.43	2.35	2.34	2.18
C20:5n-3	4.14	3.87	3.38	3.79	3.47

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; ND, not detected

Fatty acids as % of total fatty acids	Control	DBSF10	DBSF30	FBSF10	FBSF30
C22:2n-6	0.31	0.29	0.28	0.30	0.25
C22:6n-3	3.17	2.92	2.61	2.78	2.55
∑ PUFA	45.5	45.4	45.5	44.9	45.9
∑ PUFA n-3	10.7	10.2	9.56	9.98	9.44
∑ PUFA n-6	34.8	35.2	36.0	35.0	36.4
∑ MUFA n-9	28.6	28.8	28.2	28.1	27.8
n-3/n-6	0.31	0.29	0.27	0.29	0.26

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; ND, not detected

Fatty acid profiles of the carcass are presented in Table 7. The total SFA content of FBSF30 was significantly higher than that of the other treatments ($P < 0.05$). Specifically, C10:0, C12:0 and C14:0 levels in FBSF30 were higher, whereas C20:0, C21:0, and C24:0 levels were lower than those of the control. Interestingly, C12:0 levels showed a dramatic increase as dietary fishmeal was replaced with BSFL, with levels increasing by 3.4-, 12.2-, 17.4-, and 61.8-fold in DBSF10, DBSF30, FBSF10, and FBSF30, respectively. The total MUFA content did not differ among the treatments. However, C14:1 and C22:1n-9 levels increased, whereas C20:1n-9 and C24:1n-9 levels decreased significantly as dietary fishmeal was replaced with BSFL or as the substitution level increased ($P < 0.05$). The total PUFA content in the FBSF30 group was significantly lower compared to the control ($P < 0.05$). Specifically, total n-3 PUFA, including C18:3n-3, C20:5n-3, and C22:6n-3, decreased significantly as dietary fishmeal was replaced with BSFL or as the substitution level increased ($P < 0.05$). For n-6 PUFA, C20:4n-6 decreased significantly only in the FBSF30 group compared to the control ($P < 0.05$). As a result, the n-3/n-6 fatty acid ratio of the carcass in the BSFL groups was significantly lower than that of the control ($P < 0.05$).

Table 7
Fatty acid profiles of carcass of fish fed experimental diets for 8 weeks.

Fatty acids as % of total fatty acids	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
C10:0	0.02^{a}	0.02^{a}	0.02^{a}	0.01^{a}	0.05^{b}	0.00
C12:0	0.05^{a}	0.17^{a}	0.61^{a}	0.87^{a}	3.09^{b}	0.00
C14:0	2.27^{b}	2.13^{a}	2.08^{a}	2.27^{b}	2.41^{c}	0.00
C15:0	0.15	0.15	0.15	0.16	0.15	0.00
C16:0	13.3	13.7	13.4	13.4	13.4	0.25
C17:0	0.21	0.19	0.20	0.26	0.26	0.24
C18:0	3.67	3.76	3.74	3.80	3.66	0.28
C20:0	0.29^{b}	0.28^{ab}	0.27^{ab}	0.28^{ab}	0.26^{a}	0.05
C21:0	0.68^{b}	0.61^{ab}	0.69^{b}	0.63^{ab}	0.56^{a}	0.03
C22:0	1.55	1.61	1.57	1.61	1.63	0.72
C24:0	0.54^{b}	0.49^{ab}	0.45^{a}	0.51^{b}	0.45^{a}	0.01
∑SFA	23.3^{a}	23.7^{a}	24.2^{a}	23.6^{a}	26.8^{b}	0.00
C14:1	0.05^{a}	0.05^{a}	0.05^{a}	0.07^{b}	0.09^{c}	0.00
C16:1	3.48	3.59	3.41	3.55	3.63	0.65
C17:1	0.38	0.35	0.33	0.37	0.35	0.21
C18:1n-9	27.3	27.7	27.2	27.2	27.2	0.45
C20:1n-9	3.24^{c}	3.28^{c}	3.09^{b}	3.17^{bc}	2.82^{a}	0.00
C22:1n-9	0.81^{a}	0.89^{ab}	1.02^{b}	0.92^{ab}	0.94^{ab}	0.05
C24:1n-9	0.92^{c}	0.81^{b}	0.70^{a}	0.75^{ab}	0.69^{a}	0.00
∑MUFA	36.2	36.7	35.8	36.1	35.8	0.20
C18:2n-6	27.2^{ab}	26.9^{a}	28.1^{b}	26.9^{a}	26.1^{a}	0.03
C18:3n-6	0.69^{a}	0.73^{a}	0.83^{b}	0.72^{a}	0.66^{a}	0.00

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Fatty acids as % of total fatty acids	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
C18:3n-3	2.98^{b}	2.89^{b}	2.97^{b}	2.88^{ab}	2.72^{a}	0.03
C20:2n-6	0.49	0.46	0.43	0.48	0.46	0.39
C20:3n-6	0.04	0.04	0.04	0.04	0.04	0.74
C20:3n-3	0.04	0.03	0.03	0.03	0.03	0.50
C20:4n-6	2.11^{b}	1.98^{b}	2.01^{b}	2.00^{b}	1.73^{a}	0.03
C20:5n-3	1.77^{d}	1.58^{c}	1.43^{b}	1.58^{c}	1.27^{a}	0.00
C22:2n-6	0.39	0.40	0.43	0.40	0.37	0.84
C22:6n-3	5.45^{bc}	5.22^{bc}	4.84^{a}	5.14^{b}	4.84^{a}	0.00
∑PUFA	41.1^{b}	40.2^{b}	41.1^{b}	40.2^{b}	38.2^{a}	0.00
∑ PUFA n-3	9.80^{c}	8.88^{b}	8.36^{ab}	8.73^{b}	7.96^{a}	0.00
∑ PUFA n-6	30.8^{ab}	30.9^{ab}	31.5^{b}	31.2^{ab}	29.6^{a}	0.05
∑ MUFA n-9	32.6^{b}	32.6^{ab}	32.1^{ab}	32.7^{b}	31.9^{a}	0.05
n-3/n-6	0.32^{b}	0.29^{a}	0.27^{a}	0.28^{a}	0.27^{a}	0.00

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Fatty acid profiles of the viscera (including liver) are presented in Table 8. The total SFA content did not differ significantly among the treatments. However, C12:0 and C24:0 levels increased and decreased, respectively, as dietary fishmeal was replaced with BSFL or as the substitution level increased ($P < 0.05$). Similar to the results observed in the carcass, C12:0 levels showed a dramatic increase as dietary fishmeal was replaced with BSFL, with levels increasing by 4.5-, 24.5-, 40.8-, and 51.3-fold in DBSF10, DBSF30, FBSF10, and FBSF30, respectively. C20:0 content decreased significantly only in FBSF30 compared to the control ($P < 0.05$). The total MUFA content did not differ significantly among the treatments. However, the C14:1 content increased significantly in FBSF10 and FBSF30 compared to the control, while C20:1n-9 content decreased significantly only in FBSF30. Additionally, the C24:1n-9 content decreased as dietary fishmeal was replaced with BSFL or as the substitution level increased ($P < 0.05$). The total PUFA content, including C20:4n-6 and C22:6n-3, did not differ significantly among the treatments. However, a significant decrease in C20:5n-3 levels was observed in the BSFL groups compared to the control. The n-3/n-6 fatty acid ratio of the viscera in the BSFL groups showed a decreasing trend compared to the control ($P < 0.05$).

Table 8
Fatty acid profiles of the viscera (including the liver) in fish fed experimental diets for 8 weeks.

Fatty acids as % of total fatty acids	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
C12:0	0.04[a]	0.18[a]	0.98[ab]	1.63[ab]	2.05[b]	0.05
C14:0	2.16	2.15	2.14	2.30	2.33	0.23
C15:0	0.12	0.12	0.13	0.13	0.13	0.84
C16:0	13.7	14.0	13.9	13.2	14.1	0.31
C17:0	0.21[b]	0.20[ab]	0.19[a]	0.20[ab]	0.20[ab]	0.04
C18:0	3.98	4.00	3.85	3.93	4.14	0.59
C20:0	0.28[ab]	0.29[b]	0.26[ab]	0.26[ab]	0.25[a]	0.05
C21:0	0.61	0.60	0.60	0.57	0.53	0.45
C22:0	1.73	1.72	1.70	1.76	1.69	0.95
C24:0	0.52[c]	0.48[bc]	0.38[a]	0.44[ab]	0.44[ab]	0.01
∑SFA	23.3	23.7	24.2	24.4	25.9	0.24
C14:1	0.05[ab]	0.05[a]	0.06[ab]	0.07[c]	0.07[c]	0.05
C16:1	3.51	3.67	3.46	3.32	3.48	0.11
C17:1	0.35[b]	0.34[ab]	0.31[a]	0.34[ab]	0.32[ab]	0.00
C18:1n-9	27.4	27.5	27.4	27.5	27.4	0.69
C20:1n-9	3.48[b]	3.48[b]	3.21[ab]	3.33[ab]	3.13[a]	0.00
C22:1n-9	0.85	0.89	0.94	0.91	0.98	0.24
C24:1n-9	0.86[c]	0.74[b]	0.59[a]	0.69[ab]	0.65[ab]	0.00
∑MUFA	36.5	36.7	36.0	36.6	35.7	0.15
C18:2n-6	27.2	27.0	27.9	27.3	26.4	0.18
C18:3n-6	0.65	0.72	0.74	0.70	0.67	0.43
C18:3n-3	2.88	2.83	2.82	2.75	2.64	0.08
C20:2n-6	0.47[b]	0.43[ab]	0.41[a]	0.44[ab]	0.43[ab]	0.05

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

Fatty acids as % of total fatty acids	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
C20:3n-6	0.03	0.04	0.05	0.03	0.03	0.60
C20:4n-6	2.14	2.14	2.04	2.09	1.98	0.87
C20:5n-3	1.53^{b}	1.37^{ab}	1.23^{a}	1.29^{a}	1.21^{a}	0.05
C22:2n-6	0.37	0.35	0.35	0.34	0.37	0.80
C22:6n-3	4.86	4.64	4.28	4.43	4.33	0.20
∑PUFA	40.1	39.5	39.8	39.9	37.5	0.05
∑ PUFA n-3	9.27^{b}	8.88^{ab}	8.36^{a}	8.50^{ab}	8.19^{a}	0.05
∑ PUFA n-6	30.8	30.7	31.5	30.8	29.9	0.09
∑ MUFA n-9	32.6	32.6	32.1	32.4	32.2	0.27
n-3/n-6	0.30^{b}	0.29^{ab}	0.27^{a}	0.28^{ab}	0.27^{a}	0.05

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

3.4. Fatty acid regression and principal component analysis of fish and diets

Linear regressions between dietary and tissue fatty acid levels are shown in Fig. 1. Carcass C12:0 (R^2 = 0.984, $P < 0.001$), C20:5n-3 (R^2 = 0.833, P = 0.031), and C22:6n-3 (R^2 = 0.969, P = 0.002) were positively related to their dietary levels, as were viscera C20:5n-3 (R^2 = 0.890, P = 0.016) and C22:6n-3 (R^2 = 0.959, P = 0.004). Viscera C12:0 showed a positive but non-significant trend (R^2 = 0.607, P = 0.120). No relationship was detected for C18:3n-3 in either tissue ($R^2 \leq 0.043$, $P > 0.7$), reflecting the narrow range of dietary C18:3n-3 (3.36–3.57% of total fatty acids). Carcass fatty acid profiles differed across dietary treatments according to PCA (Fig. 2). The control and DBSF10 groups clustered closely and were positively influenced by C22:1n-9. FBSF10 was positioned near the PCA origin, indicating limited alteration of fatty acid profiles. DBSF30 exhibited an intermediate but distinct profile associated with moderate increases in SFA and decreases in PUFA. FBSF30 showed a strong association with C12:0, C14:0, C16:1, and other SFA vectors, indicating a shift toward saturated lipid accumulation. These results reinforce that FBSF at high inclusion markedly modifies tissue fatty acid composition, driven by its intrinsic lipid profile.

3.5. Serum antioxidant and immune indices

The results of cortisol, GPx, SOD, IgM, and lysozyme analyzed in the serum of rainbow trout are presented in Table 9. Cortisol showed no differences among all the experimental groups. GPx and SOD

values, indicators of antioxidant activity, also showed no significant differences among the experimental groups. Similarly, no differences were observed in lysozyme and IgM, which are indicators of immune activity, among the experimental groups.

Table 9
Serum biochemical levels of cortisol, GPx, SOD, IgM, and lysozyme of fish fed experimental diets for 8 weeks.

Biochemicals	Control	DBSF10	DBSF30	FBSF10	FBSF30	Pooled SEM
Cortisol (ng/ml)	3.40	3.14	4.19	4.33	4.17	0.08
GPx (Ug/ml)	1.27	1.09	1.27	1.44	1.31	0.83
SOD (µg/ml)	2.22	2.40	2.21	1.96	1.76	0.63
IgM (µg/ml)	22.6	19.2	11.5	28.6	32.8	0.21
Lysozyme (ng/ml)	416	418	330	484	520	0.38

Abbreviation: DBSF, defatted black soldier fly larvae meal; FBSF, full-fat black soldier fly larvae meal; SEM, pooled standard error of the mean; GPx, glutathione peroxidase; SOD, superoxide dismutase; IgM, immunoglobulin M

4. Discussion

4.1. Changes of nutritional composition in black soldier fly larvae meals

Insects have gained attention as an alternative protein source in aquaculture (Nogales-Mérida et al. 2019), because the production of conventional protein sources has placed significant pressure on land use and natural resources and negatively affects the environmental footprint (Cottrell et al. 2025; Stamer 2015). Within this scope, BSFL has been reported as a suitable ingredient, as their nutrient composition allows for the partial replacement of fishmeal in various fish species (Barroso et al. 2014; Belghit et al. 2018; Caimi et al. 2020; Nairuti et al. 2021). However, the feasible levels of replacement depend on the nutritional status of BSFL, which varies based on forage sources and processing methods (Hoc et al. 2021; Hurtado-Ribeira et al. 2023; Liu et al. 2017; Spranghers et al. 2017). These factors can either enhance or diminish its nutritional value as well as affect market price. Previous studies have shown that elevated levels of essential fatty acids can be achieved in BSFL through targeted feeding strategies (Ewald et al. 2020). Additionally, increased concentrations of crude protein, amino acids, ash, and chitin have been observed on a dry matter basis following fat removal (Ko et al. 2025; Weththasinghe et al. 2021). Likewise, the current study has shown that the defatting process of BSFL results in a significant shift in the nutritional profile, affecting both the fatty acid profile (Table 1) and the proximate composition (Table 2). According to Ko et al. (2025), the mean proportion of SFA in BSFL slightly decreased by 2.9% after defatting, while MUFA and PUFA increased by 3.3% and 0.5%, respectively.

However, the current study revealed an opposite trend, with an increase in total SFA up to 3.4%, primarily driven by lauric acid, and decreases in total MUFA and total PUFA up to 2.3% and 1.2%, respectively, mainly due to reductions in oleic acid and EPA. Notably, EPA and DHA contents in both studies were either decreased or entirely eliminated following the defatting process. These discrepancies suggest that lipid content and fatty acid composition are influenced not only by the baseline status of FBSF and their rearing substrate (Barragán-Fonseca et al. 2021; Fitriana et al. 2022), but also by the specific mechanics of the defatting process itself. Hurtado-Ribeira et al. (2023) reported an interrelated effect of slaughtering, drying and defatting methods on the lipid profiles and their characteristics of BSFL larval fat. Notably, the authors observed that non-thermal slaughtering and drying methods induced lipid degradation, with free fatty acids accounting for 18.6% to 23.5% of the total lipid content. This plasticity in BSFL nutrient composition underscores its potential as a targeted vector for specific nutrients. However, the precise techniques required to optimize nutrient fortification through forage management and processing methods remain incomplete. Thus, further research is warranted to optimize the use of BSFL in functional feed formulations.

4.2. Comparison of dietary defatted versus full-fat black soldier fly larvae meals on growth of fish

There are few comparative studies directly evaluating the growth performance of fish fed DBSF versus FBSF in Nile tilapia, turbot, Pacific white shrimp, and Atlantic salmon (Ko et al. 2025; Sangsawang et al. 2024; Weththasinghe et al. 2021; Zhao et al. 2023). Regarding rainbow trout, direct comparisons between dietary DBSF and FBSF forms have been reported for fish weighing approximately 30 g and 90 g (Borland et al. 2024; Riesenbach et al. 2026). Riesenbach et al. (2026) observed a trend where diets containing up to 10% BSFL slightly compromised growth at an early life stage (ca. 30 g), whereas this trend disappeared at a later life stage (ca. 90 g). Similarly, Borland et al. (2024) reported comparable growth at the juvenile stage (initial weight approximately 90 g) when fish were fed diets containing 10% BSFL. Likewise, in the present study, partial replacement of fishmeal with BSFL revealed that only fish fed the FBSF30 diet (10.8% FBSF in the diet) showed compromised growth and feed efficiency, whereas DBSF at the same inclusion level did not. This indicates that a dietary inclusion level of roughly 10% FBSF may negatively affect nutrient utilization and growth in rainbow trout at an early life stage (ca. 12 g), particularly over extended feeding durations (8 weeks).

4.3. Insect meal inclusion caused changes in nutrient utilization and growth

Although the availability of chitin may have varying effects depending on fish species, its antinutritional effects have been reported in several studies (Eggink et al. 2022; Karlsen et al. 2017). Ozturk et al. (2023) noted that residual chitin might compromise intestinal health and lead to growth retardation in fish fed dried BSFL. Similarly, dietary inclusion of roughly 2% insect-derived chitin has been shown to lower protein digestibility and retard growth in salmon (Weththasinghe et al. 2021; Willora et al. 2025). Furthermore, Dumas et al. (2018) demonstrated that trout diets containing DBSF chitin at levels between

0.18% and 0.72% exhibited a linear decrease in thermal growth coefficient and an increase in FCR. These findings suggest that a threshold level for dietary chitin inclusion may exist, particularly in salmonids, since the current study observed no differences in growth performance even in the DBSF30 group, which contained the highest chitin content (1.64%). However, whether chitin directly impairs growth remains unclear, as an increase in dietary insect meal supplementation may overestimate crude protein levels when crude protein is calculated using the standard conversion factors (Kp) of 6.25 (Jonas-Levi and Martinez 2017). A lower Kp value of 4.43–4.76 has been proposed for BSFL owing to the presence of non-protein nitrogen (16–26%), which mainly comprises chitin, ammonia, and uric acid (Janssen et al. 2017; Smets et al. 2021). Therefore, although BSFL has a relatively well-balanced nutritional profile (Nogales-Mérida et al. 2019), the specific effects of dietary chitin must be carefully investigated without confounding variations in crude protein levels.

4.4. Dietary fatty acids cause changes in feed utilization

The essential fatty acid requirement for rainbow trout is 0.7 to 1.0% for C18:3n-3 and 0.4 to 0.5% for C20:5n-3 and C22:6n-3 (NRC 2011). The experimental diets met these requirements, containing 3.4%, 3.7%, and 2.8%, respectively. Replacing fishmeal with BSFL slightly altered these essential fatty acid levels. Over the course of the 8-week feeding trial, although both similarities and differences were observed between the fatty acid profiles of the carcass and viscera, the overall tissue composition correlated with the dietary fatty acid composition (Fig. 1). The tight correspondence between dietary and tissue fatty acid profiles is common in aquatic species due to their limited capacity for desaturation and elongation (Alvanou et al. 2023; Hong et al. 2022; Hu et al. 2023; Moutinho et al. 2024). Notably, lauric acid levels increased dramatically with higher BSFL inclusion, accumulating up to 61.8- and 51.3-fold in the carcass and viscera, respectively, compared to the control. Significant changes in essential fatty acids in the carcass reflected dietary levels, whereas in the viscera, significant differences were observed only for C20:5n-3. Consistently, the regression slopes of tissue on dietary C12:0, C20:5n-3, and C22:6n-3 were steeper in the carcass (4.04, 0.553, and 1.03) than in the viscera (2.25, 0.397, and 0.941; Fig. 1). In rainbow trout, it was suggested that muscle tissue is likely to respond to anabolic hormone action by storing lipids and inhibiting oxidative pathways, whereas liver tissue may stimulate lipolytic processes (Polakof et al. 2010). This highlights that the impact of BSFL-derived lipids on tissue fatty acid composition is distinct in the carcass compared to the viscera, suggesting that the carcass profile could serve as a sensitive indicator of dietary lipid intake.

The PCA in the present study clearly illustrated the tight fatty acid correspondence between dietary composition and tissue deposition in juvenile rainbow trout. Increasing the dietary inclusion of BSFL, particularly FBSF, resulted in a progressive reduction of EPA and DHA and a marked increase in SFA retention, most notably C12:0. These findings align with Turchini and Francis (2009), who demonstrated that rainbow trout can readily accrete SFA and MUFA through *de novo* fatty acid synthesis. They further reported that fatty acid digestibility and retention are influenced by both dietary fatty acid composition and inclusion level, with long-chain SFA (C14:0, C16:0, C18:0, C20:0, C22:0) showing substantially reduced retention when supplied in excess. In our study, the high proportion of SFA, fatty acids that trout

can synthesize *de novo*, accumulated in the tissues of fish fed the FBSF30 diet, potentially creating an imbalanced fatty acid profile and reducing the efficiency of lipid utilization. This suggests that excessive inclusion of FBSF may impose a nutritionally wasteful lipid deposition pattern. Therefore, as emphasized by Turchini and Francis (2009), the dietary balance between SFA and essential long-chain n-3 PUFA such as DHA warrants further investigation to optimize fatty acid metabolism, improve feed efficiency, and support cost-effective formulation strategies using BSFL-based ingredients.

In the current study, diet containing 10.8% FBSF resulted in growth retardation and poor FCR, whereas the corresponding DBSF diet did not. This indicates that high lipid content originating from BSFL may compromise diet utilization and consequently reduce growth. Similar results have been reported in salmonids, in which final body weight, FCR, and apparent digestibility were compromised as the dietary inclusion level of BSFL increased (Dumas et al. 2018; Weththasinghe et al. 2021). Lower lipid retention was observed in fish fed 10–12.5% BSFL compared to the control (Weththasinghe et al. 2021; Willora et al. 2025), which was attributed to lauric acid, the dominant fatty acid in BSFL (Dumas et al. 2018). BSFL lipids feature a high level of saturation, with C12:0 content particularly high at approximately 38% (Ushakova et al. 2016). SFA generally solidifies at room temperature. In this experiment, the rearing water temperature was maintained at approximately 16.5°C throughout the 8-week period. As BSFL replacement levels increased, the content of SFA in both the carcass and viscera also increased. The higher presence of SFAs in an emulsion or solid state within the intestine may influence nutrient absorption, potentially acting as a factor that inhibits growth (Doreau and Chilliard 1997; Guo et al. 2017; Steingoetter et al. 2015). This suggests that while BSFL inclusion can significantly alter fatty acid profiles, the increase in SFA content may require careful consideration to optimize fish growth and health.

4.5. Serum cortisol, GPx, SOD, IgM and lysozyme of fish fed black soldier fly larvae meal

Previous studies have reported that replacing fishmeal with plant-based ingredients can hinder digestion in rainbow trout, leading to increased plasma cortisol levels (Seibel et al. 2022). However, in this study, there was no significant difference in serum cortisol levels when 30% of the fishmeal was replaced with BSFL. This result indicates that rainbow trout did not experience stress when digesting diets containing BSFL, suggesting that BSFL is a digestible and non-stressful alternative protein source for rainbow trout.

GPx and SOD are key antioxidant enzymes that play a crucial role in cellular homeostasis by protecting against oxidative stress. When fishmeal is replaced with plant protein sources containing anti-nutritional factors that impair digestion, levels of GPx, SOD, and cortisol are typically observed to increase (Hossain et al. 2021). In this study, no significant differences in serum cortisol levels were observed in rainbow trout fed the experimental diets. This suggests that rainbow trout did not experience notable stress when digesting diets containing BSFL, reinforcing its potential as a viable and stress-free alternative protein source. In the current study, peroxide values did not differ among the experimental diets or among the carcass and viscera samples.

It was reported in hybrid grouper that DBSF can reduce fishmeal inclusion to 32% without affecting growth, and can improve antioxidant capacity and immunity, including lysozyme and IgM levels (Huang et al. 2025). Similar to findings from an insect meal replacement with sea trout (Mikołajczak et al. 2020), this study found no significant changes in lysozyme and IgM levels. BSFL is known to contain approximately 50 antibacterial peptide genes (Shelomi et al. 2020). IgM, the first immune antibody to respond to foreign substances, would be expected to increase if antibacterial peptides were recognized as foreign by the immune system. However, in this experiment, serum IgM levels showed no significant changes, suggesting that the antibacterial peptides in BSFL were not perceived as foreign substances by rainbow trout. This lack of an immune response may be influenced by the natural feeding habits of rainbow trout, which include consuming insects in their natural environment during specific seasons when insects are available (Candiotto et al. 2011).

5. Conclusion

BSFL represents a promising alternative protein source to fishmeal in aquafeeds. For rainbow trout juveniles, dietary fishmeal can be replaced with up to 30% DBSF or 10% FBSF. Notably, FBSF may offer a cost-saving advantage over DBSF when used in equal volumes. However, careful consideration of inclusion levels and dietary formulations is essential to optimize growth performance and minimize potential metabolic disturbances in rainbow trout. Future studies should focus on strategies to improve the digestibility and nutrient profile of BSFL. Approaches such as defatting and advanced processing methods could enhance its efficacy and sustainability as an aquafeed ingredient.

Declarations

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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.

Authorship contribution

Seong-Won Han:Investigation, Data curation, Formal analysis, Writing-original draft. **Young Uk Park**: Funding acquisition, Methodology, Conceptualization, Project administration. **Min-Soo Joo**: Investigation, Data curation. **Seokryel Kim**: Methodology, Resources, Validation. **Bong-Joo Lee**: Conceptualization, Writing-review and editing, Supervision.

Ethics approval

All procedures involving animals were reviewed and approved by the Animal Experiment Ethics Committee of Kongju National University, South Korea (approval no. KNU_2023-06), and were performed in accordance with the Animal Protection Act and the Laboratory Animal Act of the Republic of Korea and the relevant institutional guidelines. The study is reported in accordance with the ARRIVE guidelines.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

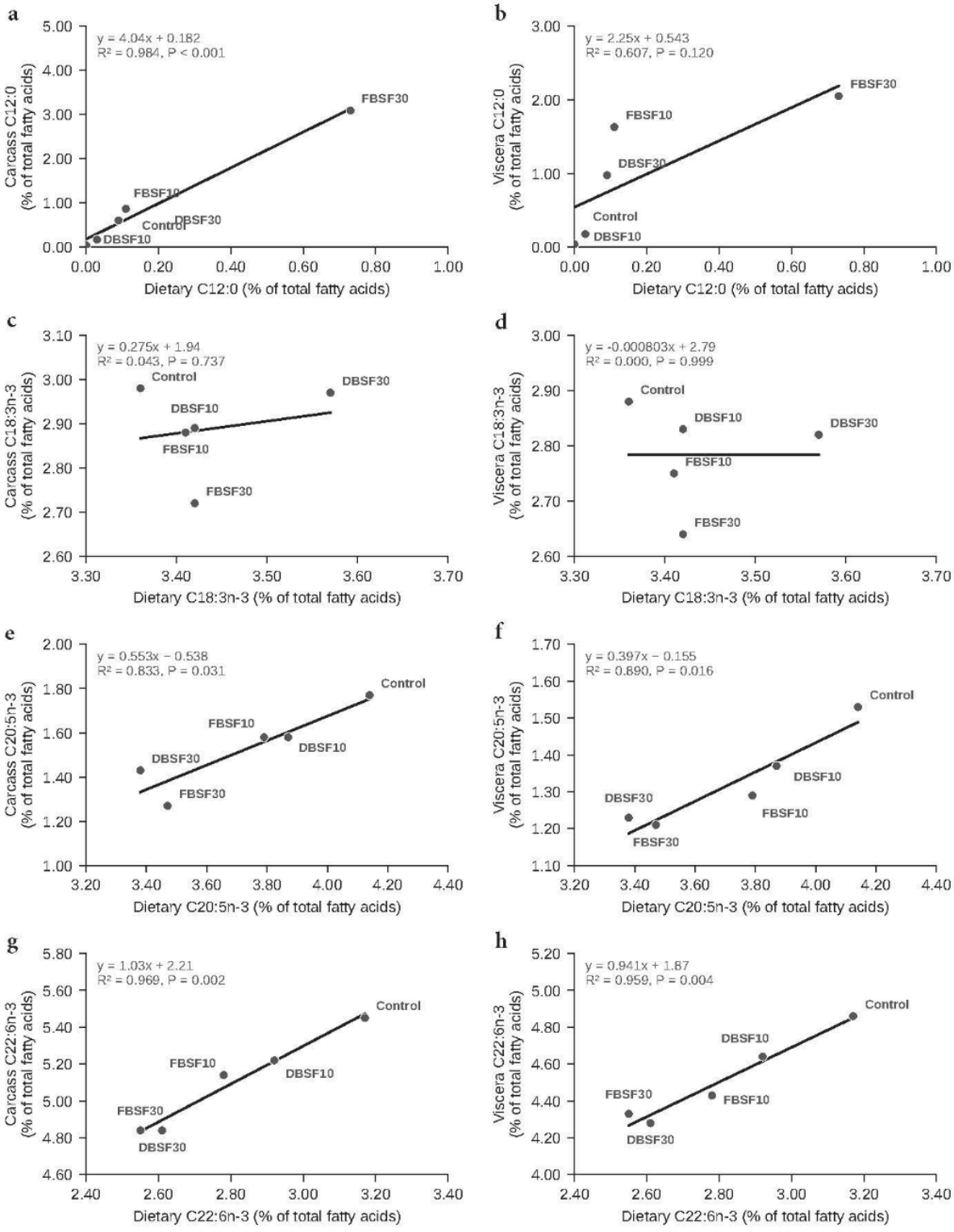


Figure 1

Linear regressions between the dietary levels of C12:0 (A, B), C18:3n-3 (C, D), C20:5n-3 (E, F), and C22:6n-3 (G, H) and their levels in the carcass (left) and viscera (right) of rainbow trout fed the experimental diets (Control, DBSF10, DBSF30, FBSF10, FBSF30) for 8 weeks. Values are expressed as % of total fatty acids. Each point represents the mean of triplicate tanks (n = 3 per dietary treatment); the regression

equation, coefficient of determination (R^2), and P value of the regression (n = 5 diets) are shown in each panel

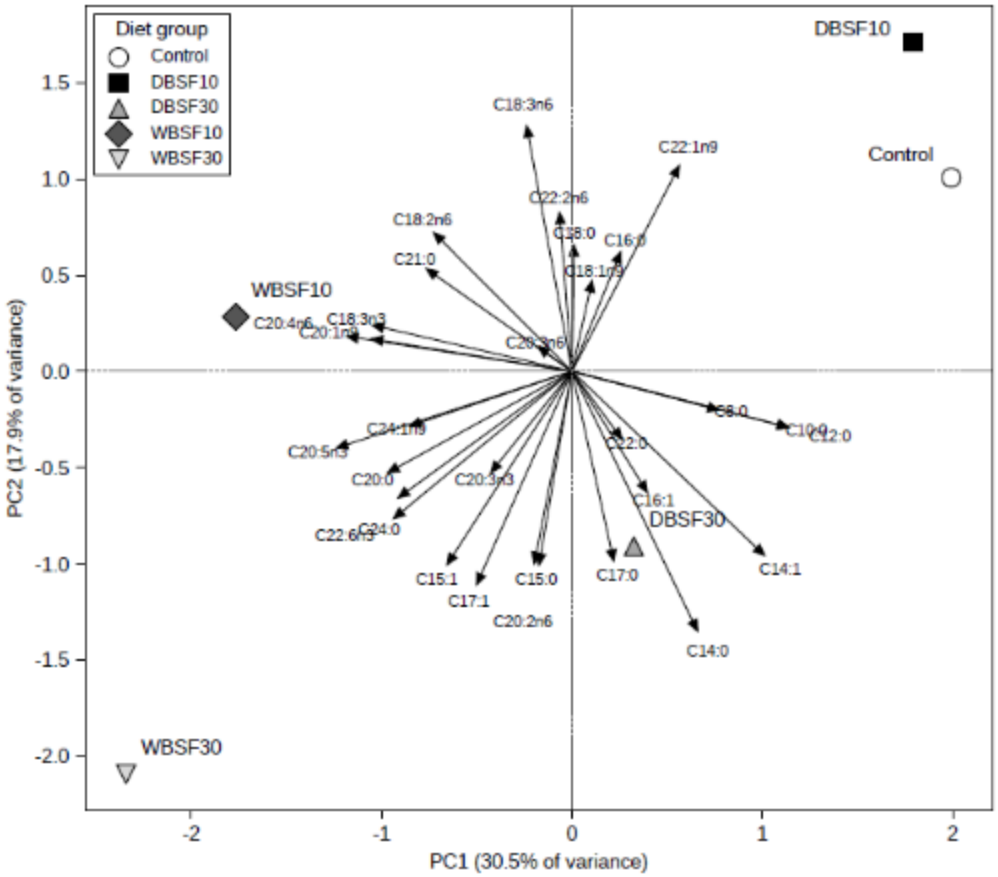


Figure 2

Principal component analysis (PCA) biplot of the carcass fatty acid composition of fish fed the experimental diets (DBSF10, DBSF30, FBSF10, FBSF30) for 8 weeks. Symbols indicate the group mean scores of each dietary group (n = 3), and arrows indicate the loadings of individual fatty acids on PC1 and PC2