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Growth, Health, Survival, and Fillet Quality of Rainbow Trout (*Oncorhynchus mykiss*) Fed Directly with Black Soldier Fly (*Hermetia illucens*) Prepupae

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Abstract

Black soldier fly (BSF; *Hermetia illucens*) has received growing attention as an alternative sustainable ingredient for aquafeeds. This study aimed to investigate the effects of direct BSF feeding on growth, health, and filet quality of rainbow trout. Fish were fed with dried black soldier fly (BSF) prepupae for one, two, or three meals a day. After 90 days of feeding, a substantial reduction in growth was found in dried BSF fed groups as daily dried BSF meal increased. Rejection and reduction in feed intake were only observed in the fish group that was fed with dried BSF 3 times a day. Amino acid content of BSF prepupae was significantly higher than that of commercial diet and some of the fatty acid contents were similar toor higher than commercial diet. When fish were fed with dried BSF 2 or 3 times a day, chitin accumulated in the fish's gut, causing constipation and leading to a decreased or rejected feed intake. A substantial decreasing trend was detected in arginine, histidine, and threonine with the increase in the number of dried BSF meals, while isoleucine, leucine, tryptophan, and alanine levels were considerably increased. Inclusion of one dried BSF meal in a daily feeding resulted in improvements in fish PUFAn-3, PUFAn-6, and DPA fatty acid levels.

Introduction

Aquaculture is the fastest growing animal food-production industry (FAO, 2018). Between 2000 and 2019, global aquaculture production improved from 43 million tons to 120 million tons (FAO, 2021). Fish meal and fish oil are the major marine-derived protein and lipid sources for aquafeed that heavily rely on captured fisheries and put further pressure on wild fish populations. A slight decline in captured fisheries (FAO, 2021) combined with rising demand for fish meal and fish oil over the last few decades, resulted in a sharp decrease in availability and rising prices. Nowadays, feed makes up a significant fraction of fish production costs and is considered one of the key problems restricting the growth and sustainability of the aquaculture sector. Depending on our future efforts, aquaculture may still be a reasonable solution to meet growing demands, or it may result in the depletion of wild fish populations.

Concerns about the use of marine-derived ingredients for aquafeeds were expressed nearly three decades ago, despite an underestimation of the aquaculture sector's growth trend, which predicted possible constraints for certain forms of aquaculture practices and limitations (New & Wijkstrom, 1990; Wijkström & New, 1989). Over the past 25 years, a range of vegetable-derived meals and oils have significantly decreased the amount of fish meal and fish oil in aquafeeds (Kok et al., 2020). Carnivorous fish species, such as salmonids, need 40% to 55% crude protein in their diet (Wilson, 2003). Thus, they require relatively higher levels of marine-derived feed ingredients such as

fish meal and fish oil to satisfy their nutritional demands. Omnivorous species, on the other hand, have a lower protein requirement (35% - 45%) (Wilson, 2003), but their high production rates require the use of a substantial amount of marine-derived ingredients. The shift to plant-based sources might put further pressure on agricultural resources, perhaps having a detrimental economic and environmental impact. Finding commercially effective and sustainable fish meal and fish oil alternatives remains one of the aquaculture industry's most difficult challenges.

Insects are potentially suitable for aquafeed components, according to the circular economy concept, since they may be cultured on waste or by-products in environmentally sustainable and cost-effective farming procedures (Meneguz et al., 2018). As a potential alternative to animal-based components that might substitute marine-derived ingredients in aquafeed since insects are part of the natural diets of both freshwater and marine fish (Henry et al., 2015; Whitley & Bollens, 2014), which are also comparatively more sustainable than fish meal and nutritionally appropriate (Nogales-Mérida et al., 2019; Ravi et al., 2020; Yildirim-Aksoy et al., 2020). Among them, the black soldier fly (*Hermetia illucens*) is a potential insect species because of its essential amino acid profile, which is comparable to fish meals (Devic et al., 2017). BSF is capable of effectively converting different organic wastes into protein and lipid-rich resources. Thus, the nutritional content of BSF can be influenced by the feeding substrate's composition (Barragan-Fonseca et al., 2017; Liland et al., 2017; Scala et al., 2020; Yandi et al., 2022) and could be improved by manipulating it (Spranghers et al., 2017; Tschirner & Simon, 2015). Seaweed-enriched media can improve the essential fatty acid profile of BSF as well (Liland et al., 2017).

In the literature, BSF was added to fish feeds at different rates as a substitute for fish meal (Belghit et al., 2019; Magalhães et al., 2017; Xiao et al., 2018; Yandi, et al., 2022). Based on the species, certain amounts of BSF in the diets had no significant impact on the growth performance (Priyadarshana et al., 2021). A systematic meta-analysis study by Weththasinghe et al. (2022) revealed that BSF is a promising protein source substitution for salmonids. However, when BSF is added to fishmeal, production costs increase as both labor and energy are required. In this context, direct feeding with dried BSF can be evaluated as an alternative for aquaculture sector in undeveloped countries without compromising fish health and growth. To the best of our knowledge, no BSF was given directly to fish in any of the previous studies. It is unknown how direct feeding of BSF to fish several times a day may affect fish growth, carcass quality, and survival rate. The purpose of this study was to assess the influence of dietary replacement rate of aquafeed with dried BSF on growth, fillet quality, and survival aspects of rainbow trout (*Oncorhynchus mykiss*).

Methods

Experimental Diets

The reproduction and culture conditions of Black soldier fly (BSF) larvae were described by Yandi et al. (2022). Accordingly, pine sawdust (8–10 mm in size) was used as the hatching medium to provide ambient humidity and shade throughout the metamorphosis stage. The mature insect was exposed to a 16-hour light and an 8-hour dark photoperiod in a 3 x 2.5 x 2.5 m mating cage. Homogenized vegetable and fruit waste in 35 x 35 x 22.5 cm containers were placed in the mating cage as egg-laying attractants. To create an egg-laying environment, ten 40 x 4.5 x 1 cm hornbeam wood plates were placed on top of each other with 3 mm gaps. The eggs on the wooden plates were gently scraped off using a metal spatula. BSF larvae hatched and developed on a substrate consisting of chicken waste meal (1:1, w:w water added) and fruit and vegetable waste mixture (1:9, w:w). The chicken waste meal (<80 µm in size) was provided by Erpiliç Integrated Poultry Production Company (Bolu, Türkiye) and transferred to the lab through a cold chain (-20°C). The fruit and vegetable wastes were chopped and homogenized in an industrial blender before being stored at -20°C. Substrates were introduced to BSF larvae and their moisture contents were balanced to 70% using sawdust and/or water. The larvae were raised for 16 days at 27±1°C and 65±1.0% relative humidity and kept in complete darkness until harvest. At the prepupae stage, BSF was taken out of the substrate by hand, washed, dried overnight at 50°C, cut by hand into smaller pieces, and stored at -20°C in re-sealable plastic bags.

Experimental Design

Growth experiments were undertaken at the Aquaculture Research Station of Recep Tayyip Erdogan University, Rize/Türkiye. Four experimental groups were generated by incorporating dried BSF (BSF) prepupae into their diet. The experimental feeding trials consisted of groups fed with: i) three times a day with dried BSF (B3C0), ii) two times a day with dried BSF and once a day with commercial feed (B2C1), iii) once a day with dried BSF and two times a day with commercial feed (B1C2), and iv) three times a day with commercial feed (B0C3) (Table 1). Commercial feed used in the present study contained 51% protein and 18.9% lipid.

Prior to experiments, fish were acclimatized to the indoor rearing conditions for 2 weeks and fed with commercial trout feed. Uniformly proportioned 480 juvenile rainbow trout, *Oncorhynchus mykiss*, with an initial body length and weight of 7.50±0.19 cm and 5.19±0.11 g were randomly stocked into twelve 75-L conical fiberglass tanks, supplied with a natural spring water flow-through system, at a density of 40 fish/tank. Experimental diets were randomly allocated to triplicate

tanks and the fish were fed three times a day (09:00, 13:00, and 17:00) with their respective diets in satiation for 90 days. Uneaten dried BSF prepupae and commercial feed were collected, dried, weighed, and deducted from the total daily feeding. Tanks were maintained under artificial light (12 h:12 h, light: dark). During the growth experiment, water quality parameters were monitored and recorded. The average water temperature was 17.1±1.3°C. Average pH and dissolved oxygen were 7.55±0.25 and 7.1±0.5 mg L^{-1}, respectively.

During sampling, fish were anaesthetized by placing them in a benzocaine solution (25 mg L^{-1}). When a decrease in movement of the operculum and loss of equilibrium were observed, the fish were moved out of the solution and individually measured (total length, to the closest mm) and weighed (0.01 g) biweekly to estimate the growth rate. Growth parameter indices: mean length gain ratio (LGR), mean weight gain ratio (WGR), mean length-specific growth rate (SGR_L), mean weight-specific growth rate (SGR_W), and feed conversion rate (FCR) of experimental diet groups were determined according to Altinok et al. (2020).

During the experiment, lesions in the anus area were observed in some of the fish in the B2C1 and B3C0 groups, and some of them died. Dead fish were necropsied for the presence of parasites and bacteria according to Boran et al. (2013) and Kayis et al. (2009). After completing the experiment, the fish were fed with commercial feed for 4 weeks to check whether the fish recovered from the lesion seen in the anus region.

Proximate, Amino Acid and Fatty Acid Composition.

Harvested BSF prepupae and fillets from six fish (2 fish from each tank) per diet groups at the end of the growth experiment (90 days) were allocated, homogenized, and used for analysis. Moisture, ash, and crude lipid content were determined according to the methods 950.46, 920.153, and 2003.05 of the Association of Official Analytical Chemist, respectively (AOAC, 2003). Total nitrogen level was determined according to the Kjeldahl method and crude protein content was estimated by multiplying the nitrogen level by 6.25. Energy content was determined by using Atwater method (Merrill & Watt, 1973).

The amino acid content (arginine, alanine, aspartic acid, glycine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine and valine) was determined using RP-HPLC equipped with a UV-detector, as described by Dimova (2003) and Gheshlagi et al. (2008) with slight modifications using an HPLC system equipped with a UV-detector. Whereas tryptophan content was determined using an HPLC system equipped with a fluorescent detector. The fatty acid content was estimated according to the method described by Tufan and Kose (2021).

Statistical Analysis

The Shapiro-Wilk test was used to evaluate the normal distribution of data. Except for weight and FCR, the data was determined to be normally distributed. One-way ANOVA was used to analyze normally distributed data (length, LGR, WGR, SGR_L, SGR_W, proximate, fatty acids, and amino acids), and differences between groups were assessed using the Holm-Sidak multiple comparison method. Non-normally distributed data, on the other hand, was subjected to the Kruskal-Wallis test and different groups were determined using Dunn's test. A simple linear model was used to depict the growth over time. ANCOVA was used to examine the difference between the slopes derived from the models. To investigate the multivariate difference in the fatty acid and amino acid composition across the experimental groups, principal component analysis (PCA) and PERMANOVA were used. To emphasize the different groups, PERMANOVA was used to do a pairwise comparison. All statistical analyses were performed with a 95% confidence interval using Sigma Plot 14.0 (Systat Software Inc.) and R (ver. 4.1.2). The results were expressed as mean ± standard error of the mean.

Results

Survival and Growth

The survival rate of the fish is influenced by the dried BSF feeding frequency. Survival rates in the control (B0C3) and B1C2 groups were similar (99.2%), but in the B2C1 and B3C0 groups, survival rates dropped to 97.5% and 91.6%, respectively. In dried BSF-fed groups, lesions were first observed in the anus region of fish (Figure 1A), then expanded (Figure 1B), and eventually the anus

Table 1. Experimental feeding trial. The fish were fed three times a day with dried BSF prepupae (B3C0), two times a day with dried BSF prepupae and once a day with commercial feed (B2C1), once a day with dried BSF prepupae and two times a day with commercial feeds (B1C2), and three times a day with commercial feeds (B0C3)

	Groups			
Meals	B3C0	B2C1	B1C2	B0C3 (Control group)
1st (09:00)	dried BSF	commercial feed	commercial feed	commercial feed
2nd (13:00)	dried BSF	dried BSF	commercial feed	commercial feed
3rd (17:00)	dried BSF	dried BSF	dried BSF	commercial feed

entirely vanished, leaving an open wound (Figure 1C). No lesions were observed in the B0C3 and B1C2 groups, whereas lesions were observed in the B2C1 and B3C0 groups. When dead fish were necropsied, it was revealed that chopped BSF particles accumulated in the intestines of the fish. From the lesion, only *Aeromonas hydrophila* was isolated. At the end of the experiment, the lesion in the anus of the surviving fish was determined to be 42.9%, and 50.2% in the B2C1, and B3C0 groups, respectively. After feeding fish with commercial feed for 4 weeks, all fish recovered except the fish that had the lesion described as stage 3 in Figure 1C.

The final body length, weight, and growth parameter indices were significantly different between the experimental groups (Kruskal-Wallis test, $p<0.05$, Table 2). After 90 days of feeding, a significant decrease in growth was detected in the groups of B1C2, B2C1, and B3C0 as the daily BSF meal frequency increased (Figure 2A, B). Similarly, growth parameter indices (SGR_L, SGR_W, LGR, WGR) decreased with an increasing frequency of dried BSF meals (Figure 2E). Feed rejection and a decrease in feed intake were only observed in the B2C1 and B3C0 groups. Overall, the lowest and highest average length and weight were reported in B3C0 and B0C3, respectively, and were significantly different from the other groups throughout the experiment. The final average length (14.57 0.09 cm) and weight (38.69±0.74 g) were the highest in group B0C3 which was significantly different from the other groups ($p<0.05$). The fish in B3C0 group was significantly smaller than the others, of which the final length and weight were 9.41±0.09 cm and 9.79±0.28 g at the end of the experiment. The length and weight gain by time were found to be significantly different between the experimental groups as well (ANCOVA, $p<0.05$) (Figure 2C, D).

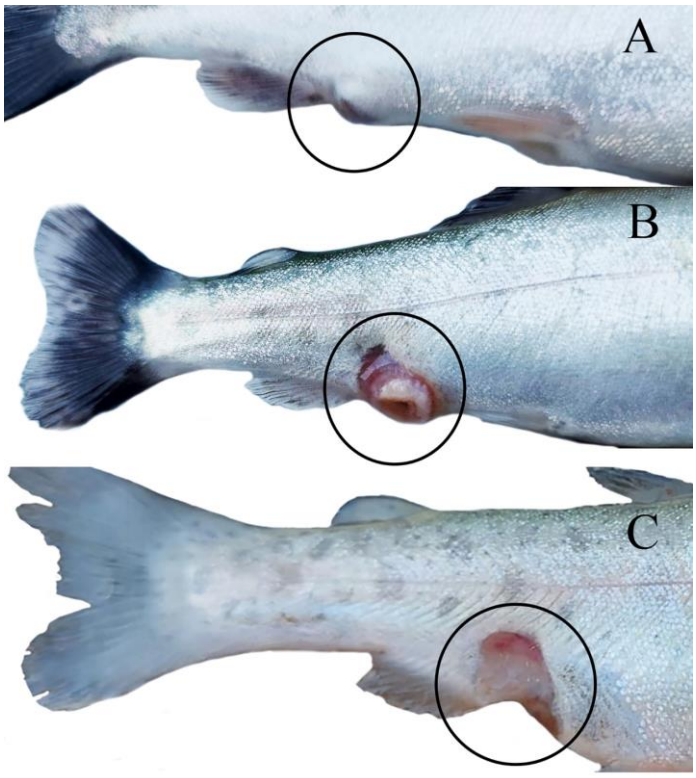


Figure 1. In the dried BSF feed groups, a lesion was visible in the anus region (A, stage 1), and as the lesion process grew around it (Figure 1B, stage 2), the anus vanished completely, leaving an open wound (Figure 1C, stage 3). Stage 2 lesion (B) is reversible after feeding fish with commercial diet.

Table 2. Growth parameter indices and survival of experimental groups. Initial body weight (IBW), final body weight (FBW), mean length gain ratio (LGR), mean weight gain ratio (WGR), mean length-specific growth rate (SGR_L), mean weight-specific growth rate (SGR_W), and feed conversion rate (FCR)

Groups	Growth parameters						
	IBW (g)	FBW (g)	LGR (%)	WGR (%)	SGR_L (% day^{-1})	SGR_W (% day^{-1})	FCR
B0C3	5.18±0.04	38.69±0.74	94.23±1.74	647.7±25.4	0.74±0.01	2.24±0.03	0.79±0.01
B1C2	5.19±0.05	30.63±0.66	80.27±0.34	490.8±8.5	0.66±0.01	2.01±0.01	0.89±0.01
B2C1	5.16±0.07	21.25±0.54	59.47±1.16	312.2±3.8	0.52±0.01	1.61±0.01	1.28±0.02
B3C0	5.21±0.07	9.79±0.28	25.41±0.29	87.9±3.6	0.25±0.01	0.75±0.02	2.72±0.06

Proximate Composition

Ash content of the fillets of the experimental groups was similar. Moisture content showed an increasing trend in B2C1 and B3C0 groups compared to B0C3 and B1C2. However, no significant difference was detected. Crude protein and crude lipid content of B2C1 and B3C0 were significantly lower than B0C3 and B1C2 groups ($p<0.05$). Similarly, energy content of B2C1 and B3C0 were significantly lower than B0C3 and B1C2 groups ($p<0.05$) (Table 3).

Amino Acid Composition

The amino acid composition of dried BSF prepupae, commercial diet, and the fillets of fish fed with experimental diets was reported in Table 4. Amino acid content of BSF prepupa was significantly higher than that of commercial diet. The essential amino acid composition of fish revealed that the most abundant amino acids were lysine, leucine, arginine, and isoleucine. Non-essential amino acids with the highest abundance were glutamic acid, aspartic acid, glycine, and alanine. A significant decreasing trend was detected in arginine, histidine, and threonine with the increase of dried BSF meal frequency ($p<0.05$). On the other hand, a significant increase was observed in isoleucine, leucine, tryptophan, and alanine content with an increase in dried BSF feeding frequency.

The principal component analysis (PCA) plot for amino acid composition data was reported in Figure 3A. The first two components, which represented 92.2% of the variance, were used to construct the PCA plot. PC 1 explained 60.6% of the variance and was closely correlated with alanine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tyrosine, and valine content. Whereas PC 2 accounted for 31.6% of the variation, that was primarily linked to the changes in arginine, histidine, lysine, threonine, and tryptophan content. B3C0 diverged from the other experimental groups on the x axis. On the other hand, group B0C3 was split from B2C1 and B1C2 by differences in threonine, arginine, and histidine levels.

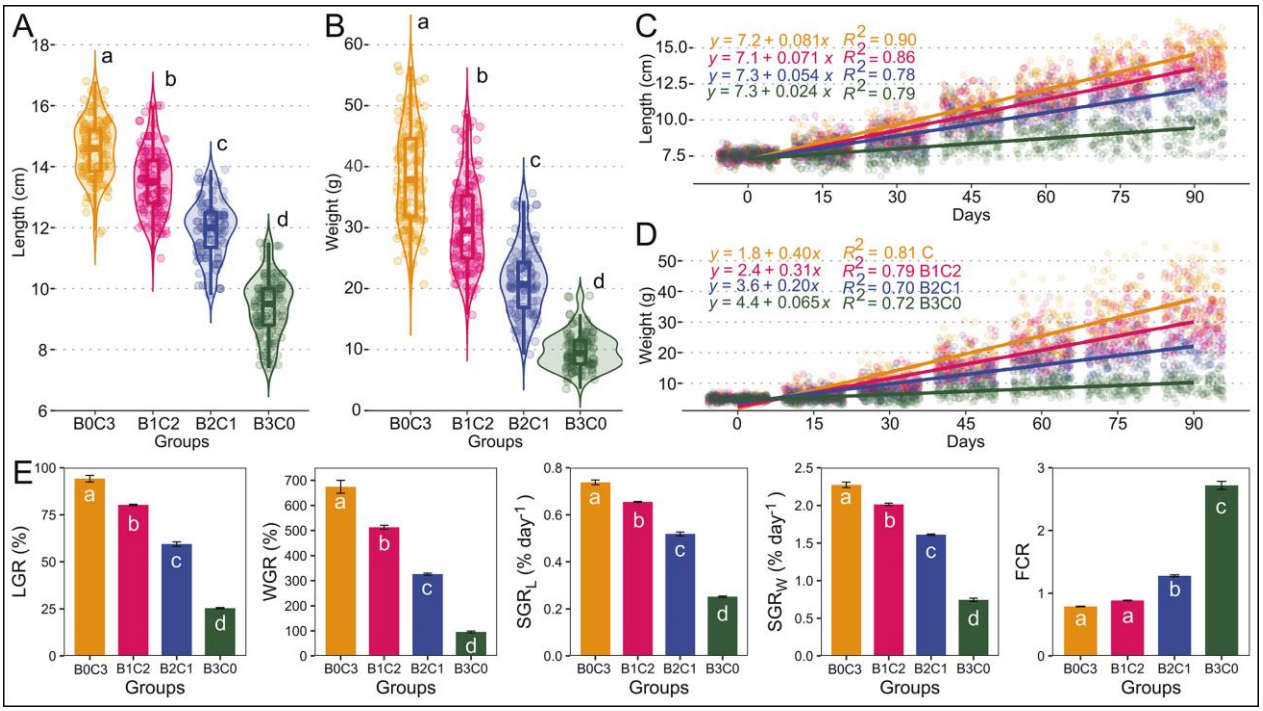


Figure 2. The final length (A) and weight (B) of the experimental groups. Growth by time in terms of length (C) and weight (D). Growth parameter indices (E). Different letters on A, B and E indicate statistically significant differences between the groups as determined Holm-Sidak multiple comparison method and Dunn's test ($p<0.05$).

Table 3. Energy content and proximate composition of the BSF (%) reared on mixture of chicken waste meal and fruit & vegetable waste (1:1, w:w) and rainbow trout muscle tissue fed with three DBSF meals (B3C0), two DBSF meals and one commercial feed meal (B2C1), one DBSF meal, and two commercial feed meals (B1C2), and three commercial feeds (B0C3)

	BSF	B0C3 (Control group)	B1C2	B2C1	B3C0
Energy (kcal/100g)	128.00±4.12	154.54±2.41^{a}	156.18±2.67^{a}	147.13±0.85^{b}	145.3±2.50^{b}
Moisture	70.90±0.12	70.62±0.31^{a}	70.09±0.33^{a}	71.41±0.38^{a}	71.44±0.62^{a}
Ash	4.23±0.31	1.53±0.01^{a}	1.50±0.02^{a}	1.53±0.02^{a}	1.52±0.02^{a}
Crude protein	18.75±0.23	19.44±0.38^{a}	19.78±0.48^{a}	18.92±0.42^{b}	18.82±0.58^{b}
Crude lipid	5.79±0.08	8.41±0.14^{a}	8.63±0.08^{a}	8.14±0.12^{b}	8.22±0.06^{b}

On the same row, different superscripts on groups fed with experimental diets indicate significant differences between groups.

Fatty Acid Composition

The fatty acid content of the BSF prepupae, commercial diet, and fillets of fish fed with experimental diets is presented in Table 5. Some of the fatty acid contents were similar to or higher than in commercial diet. Saturated fatty acid (SFA) content exhibited a declining trend as the number of BSF meals increased, and total SFA content was significantly lower in B3C0. MUFA (g/100g) content increased significantly ($p<0.05$) in the B2C1 group. B2C1 had the highest levels of PUFAn-3 and PUFAn-6. While omega-9 content increased in parallel to the increase in BSF meal frequency, omega-3 and omega-6 levels remained constant (Table 4). The lauric acid, C12:0, content increased significantly in conjunction with the increase in the dried BSF meal frequency. The most abundant MUFA was found as oleic acid (18:1n-9c), which significantly increased with the increase in dried BSF meal frequency. DPA and DHA content were similar among groups.

PCA was performed using twenty-two fatty acids listed in Table 5 (Figure 3B). The first two components, explaining 86.1% of the variation, were used for the plot. PC1 characterized 55.9% of the variation in the fatty acid composition among groups, and had low contributions from C17:0, C21:0, C16:1, C18:1n-9t, and C20:2n-6. PC 2 revealed substantial positive loadings for C17:0, C21:0 and C22:5n-3 (Figure 3B). Overall, the PERMANOVA results showed a statistically significant difference ($p<0.05$) between the fatty acid and amino acid composition of the experimental groups.

Table 4. Amino acid (mg/100 g) profiles of dried black soldier fly (BSF) prepupae and flesh of rainbow trout fed with three DBSF meals (B3C0), two DBSF meals and one commercial feed meal (B2C1), one DBSF meal, and two commercial feed meals (B1C2), and three commercial feeds (B0C3)

Amino acids	BSF	Commercial feed	B0C3 (Control group)	B1C2	B2C1	B3C0
Arginine*	1605.2±4.9	651.71±3.1	896.32±6.9^{a}	847.02±12^{b}	830.04±6.6^{b}	776.88±1.0^{c}
Histidine*	2130.0±4.1	686.72±2.9	768.03±1.7^{a}	639.71±2.3^{c}	629.32±1.2^{b}	591.70±0.7^{d}
Isoleucine*	3081.2±5.5	595.13±3.1	797.30±1.5^{a}	928.32±2.3^{c}	786.71±1.3^{b}	881.31±0.7^{d}
Leucine*	4963.6±9.8	932.22±3.5	1196.3±0.9^{a}	1358.0±3.5^{b}	1195.7±3.0^{a}	1248.7±12^{c}
Lysine*	7581.5±7.3	1785.4±8.7	3778.7±10^{a}	3813.3±29^{a}	3397.0±13^{b}	3431.7±10^{b}
Methionine*	1470.3±4.7	376.84±3.8	508.72±1.2^{a}	567.04±1.5^{b}	496.98±1.1^{c}	503.33±1.2^{a}
Phenylalanine*	3372.1±8.2	608.62±1.7	696.71±6.7^{a}	776.28±3.5^{b}	619.70±2.7^{c}	664.32±1.7^{d}
Threonine*	2186.2±4.8	544.41±2.3	608.34±0.3^{a}	535.04±1.0^{b}	551.30±0.9^{c}	484.71±0.3^{d}
Tryptophan*	592.85±5.2	119.02±0.9	168.73±2.7^{a}	172.03±1.0^{a}	214.85±4.5^{b}	201.74±0.7^{c}
Valine*	4277.0±5.1	611.64±3.1	750.65±4.4^{a}	851.27±3.0^{b}	729.95±2.1^{c}	717.33±0.3^{c}
Alanine	4768.5±5.8	327.32±2.6	905.27±0.3^{a}	1103.3±4.4^{b}	955.88±1.0^{c}	983.71±2.2^{d}
Aspartic acid	2862.4±0.9	686.51±4.9	1510.3±2.6^{a}	1002.0±3.6^{b}	1334.0±2.7^{c}	1122.0±1.5^{d}
Glutamic acid	4246.0±11	2178.1±5.5	2271.3±3.4^{a}	1906.1±4.5^{b}	2286.0±6.0^{a}	2011.3±4.1^{c}
Glycine	3721.7±9.2	388.52±2.4	956.31±1.3^{a}	1146.3±1.2^{b}	1062.0±1.2^{c}	994.79±1.0^{d}
Proline	4216.7±14	1287.6±8.2	641.73±1.8^{a}	815.71±2.3^{b}	696.32±1.0^{c}	699.32±0.9^{c}
Serine	2551.4±8.8	544.93±2.1	604.03±1.5^{a}	535.03±2.0^{b}	606.71±0. 7^{a}	685.74±1.2^{c}
Tyrosine	4142.3±10	521.22±1.6	599.29±0.9^{a}	715.67±1.8^{b}	562.33±0.3^{c}	569.79±0.6^{d}

*-Essential amino acids. On the same row, different superscripts on rainbow trout fed with experimental diets indicate significant differences between groups.

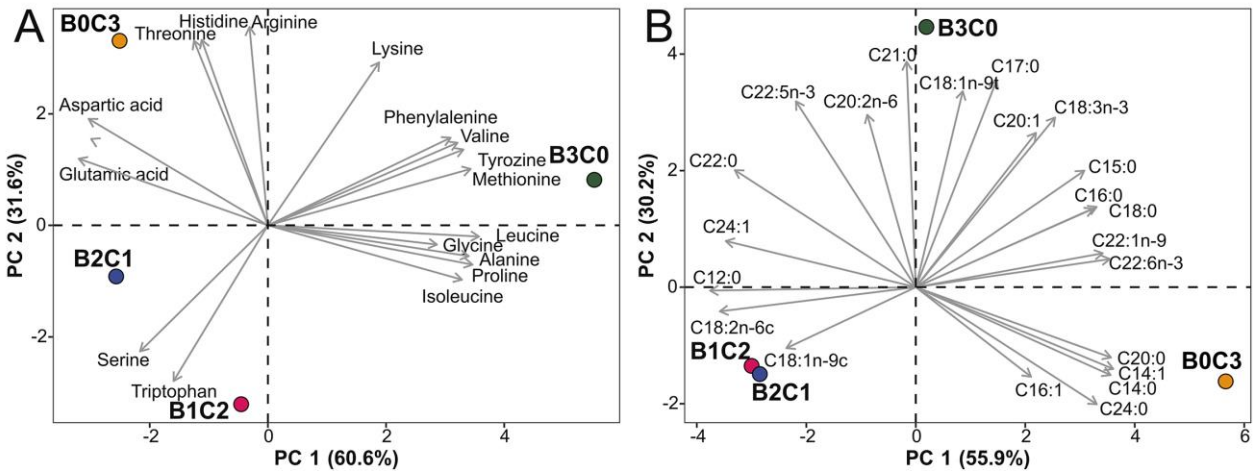


Figure 3. Principal Component Analysis (PCA) plot of amino acid (A) and fatty acid (B) compositions of the experimental groups.

Table 5. Fatty acid (% of the total FAMEs) profiles of dried black soldier fly (BSF) prepupae and flesh of rainbow trout fed with three DBSF meals (B3C0), two DBSF meals and one commercial feed meal (B2C1), one DBSF meal, and two commercial feed meals (B1C2), and three commercial feeds (B0C3)

Fatty acids	BSF	Commercial feed	B3C0 (Control group)	B1C2	B2C1	B3C0
C6:0	N.d.	N.d.	0.10±0.01	0.08±0.01	0.10±0.01	0.09±0.01
C8:0	N.d.	N.d.	0.09±0.01	0.10±0.01	0.11±0.01	0.11±0.01
C10:0	0.93±0.01	N.d.	0.07±0.01	0.07±0.01	0.06±0.01	0.04±0.01
C12:0*	15.2±0.05	N.d.	0.50±0.01	2.34±0.01	3.83±0.03	3.05±0.03
C13:0	0.01±0.00	0.02±0.01	0.05±0.01	0.03±0.01	0.04±0.01	0.05±0.01
C14:0*	0.36±0.01	4.80±0.05	7.20±0.01	6.65±0.02	6.69±0.01	6.65±0.04
C15:0*	0.02±0.01	N.d.	0.72±0.01	0.72±0.01	0.69±0.01	0.67±0.01
C16:0*	18.0±0.13	15.5±0.20	33.0±0.03	31.6±0.06	28.8±0.91	25.0±0.01
C17:0*	0.01±0.00	N.d.	0.62±0.01	0.66±0.02	0.60±0.01	0.60±0.01
C18:0*	2.52±0.09	3.10±0.02	7.93±0.04	7.79±0.01	7.48±0.01	7.15±0.03
C20:0*	0.31±0.01	0.16±0.03.	0.49±0.01	0.42±0.01	0.40±0.01	0.42±0.02
C21:0*	1.78±0.01	0.01±0.00	0.01±0.01	0.08±0.01	0.01±0.01	0.02±0.01
C22:0*	0.32±0.02	0.02±0.01	0.23±0.01	0.25±0.01	0.24±0.01	0.24±0.02
C23:0	0.31±0.03	N.d.	0.04±0.01	0.06±0.01	0.07±0.01	0.04±0.01
C24:0*	0.08±0.00	0.02±0.00	0.11±0.01	0.09±0.01	0.10±0.01	0.10±0.01
C14:1*	0.01±0.00	N.d.	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01
C16:1*	2.98±0.01	4.80±0.21	3.56±0.01	3.47±0.01	3.55±0.01	3.44±0.01
C18:1n-9c*	26.2±0.01	17.2±0.04	26.78±0.01	27.0±0.02	28.3±0.04	27.0±0.03
C18:1n-9t*	0.24±0.01	0.11±0.02	0.09±0.01	0.17±0.01	0.04±0.01	0.10±0.01
C20:1*	4.19±0.04	3.80±0.03	2.03±0.01	2.06±0.01	1.95±0.03	2.00±0.01
C22:1n-9*	0.51±0.01	3.30±0.11	0.29±0.01	0.26±0.01	0.24±0.01	0.20±0.01
C24:1*	0.79±0.01	0.31±0.01	0.30±0.01	0.33±0.01	0.34±0.00	0.32±0.01
C18:3n-3*	3.89±0.02	2.30±0.02	0.06±0.01	0.08±0.01	0.01±0.01	0.02±0.01
C20:3n-3	0.96±0.01	0.01±0.00	N.d.	N.d.	N.d.	N.d.
C20:5n-3 EPA	1.96±0.01	6.60±0.04	N.d.	N.d.	0.01±0.01	N.d.
C22:5n-3 DPA *	0.62±0.01	1.12±0.05	0.14±0.01	0.27±0.01	0.20±0.01	0.21±0.01
C22:6n-3 DHA*	3.21±0.02	8.10±0.12	0.11±0.01	0.10±0.01	0.10±0.01	0.09±0.01
C18:2n-6c*	16.6±0.04	16.1±0.09	0.76±0.01	0.93±0.01	1.14±0.01	1.00±0.01
C20:2n-6*	3.15±0.02	2.12±0.04	0.06±0.01	0.10±0.01	0.08±0.01	0.05±0.01
C18:3n-6	0.56±0.02	0.03±0.01	N.d.	N.d.	N.d.	N.d.
C20:3n-6	0.48±0.02	0.01±0.00	N.d.	N.d.	N.d.	N.d.
C20:4n-6 ARA	0.74±0.03	0.01±0.00	N.d.	N.d.	N.d.	N.d.
C22:2n-6	0.71±0.04	0.01±0.00	N.d.	N.d.	N.d.	N.d.
∑ SFA	39.9±0.26	26.3±0.12	51.1±0.02^{a}	51.0±0.12^{a}	49.2±0.96^{a}	44.4±0.1^{b}
∑ MUFA	34.9±0.25	29.9±0.38	33.1±0.03^{a}	33.3±0.04^{a}	34.4±0.05^{b}	33.1±0.03^{a}
∑ PUFA n-3	10.6±0.03	21.16±0.15	0.30±0.01^{a}	0.45±0.01^{b}	0.32±0.01^{a}	0.31±0.01^{a}
∑ PUFA n-6	22.3±1.00	8.50±0.09	0.82±0.01^{a}	1.02±0.01^{b}	1.22±0.01^{c}	1.06±0.01^{d}
n-3/n-6	0.47±0.01	2.48±0.06	0.37±0.01^{a}	0.44±0.01^{b}	0.26±0.01^{c}	0.30±0.01^{c}
∑ MUFA (g/100g)	2.44±0.03	2.01±0.02	1.64±0.01^{a}	1.86±0.01^{b}	2.08±0.01^{c}	2.16±0.10^{d}
∑ PUFA (g/100g)	1.93±0.02	2.00±0.03	0.05±0.01^{a}	0.08±0.01^{b}	0.09±0.01^{c}	0.07±0.01^{ab}
∑ Omega 3 (g/100g)	1.01±0.01	1.15±0.03	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}
∑ Omega 6 (g/100g)	1.36±0.02	1.02±0.04	0.04±0.01^{a}	0.05±0.01^{ab}	0.07±0.01b	0.05±0.01^{ab}
∑ Omega 9 (g/100g)	4.44±0.04	2.72±0.07	1.34±0.01^{a}	1.52±0.01^{b}	1.72±0.01^{c}	1.70±0.01^{c}

N.d.-Not detected due to very low concentrations.
On the same row, different superscripts on rainbow trout fed with experimental diets indicate significant differences between groups.
The asterisk indicates the fatty acid used in the principal component analysis.
ARA-arachidonic acid; EPA-eicosapentaenoic acid; DPA-docosapentaenoic acid; DHA-dpcpsahexaenoic acid; SFA-saturated fatty acid; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acid

Discussion

Feeding fish directly with dried BSF had a detrimental impact on feed intake and growth performance of fish. While there are numerous researches on substituting fish meal with BSF meal in aquafeeds, the number of studies evaluating the viability of integrating insects directly into fish diets is scarce. Inclusion of whole, chopped, live, and frozen insects including BSF was mainly investigated on diets of different fish species including catfish, tilapia, and carp (Henry et al., 2015) and mostly resulted in suppressed growth compared to commercial diets (Bondari and Sheppard, 1981; Ng et al., 2001) possibly due to being nutritionally unbalanced and high chitin content. Feeding whole or chopped insect larvae to fish results in improved growth performance and FCR in some cases (Ebenson & Udo, 2003; Oyelese, 2007). On the other hand, the successful utilization of partially substituted fish meal with BSF meal in carnivorous species, including salmonids, has been demonstrated in numerous studies. The Black soldier fly is considered a nutritionally

appropriate protein source for fish with a potential to replace or partially substitute marine-derived proteins used in aquafeeds as it contains the indispensable amino acid profile comparable with fish (Abdel-Tawwab et al., 2020; Egerton et al., 2020; Henry et al., 2015). Substitution at a certain level (Cardinaletti et al., 2019; Fawole et al., 2020; Weththasinghe et al., 2021; Xu et al., 2020; Yandi, et al., 2022) or total replacement of fish meal (Belghit et al., 2019) showed that fish can effectively utilize BSF without impairing welfare and growth. Yet, in some cases, negative effects on the digestive system, especially at high dietary substitution rates of fish meal with BSF, were recorded in aquafeeds (Cardinaletti et al., 2019; Chen et al., 2021).

The exoskeleton of BSF contains chitin, and the chitin content varies at different lifecycle stages (Soetemans et al., 2020). Therefore, the prepupae stage of BSF was used in the presence study since the level of chitin was low at this stage. Chitin is considered a growth-limiting factor in the fish diet (Sánchez-Muros et al., 2014). The reduction in growth observed in dried BSF-included diets could be attributed to the prepupae's chitin content and digestion constraints. Ideally, chitin content could be removed from insects using alkaline extraction (Sánchez-Muros et al., 2014) and chitinolytic bacteria could be incorporated into the diet (Henry et al., 2015), or chitin content might be degraded via chemical and enzymatic procedures (Lin et al., 2012). However, the feasibility and cost efficiency of these operations remain matters of controversy.

When fish were fed with dried BSF 2 or 3 times a day, the chopped BSF accumulated in the fish's intestine. The buildup of chitin in the intestines of the fish may have induced fish constipation, resulting in a decreased or rejected feed intake. In addition, BSF chitin particles accumulating in the intestine cause the anus to rupture when being evacuated by the fish. Then, *Aeromonas hydrophila* infiltrated the ruptured anus and caused the anus to completely disappear. However, if the anus has not completely disappeared, the lesion is reversible when fish are fed with a commercial diet. Therefore, chopped BSF is not appropriate for small rainbow trout. However, it may be suitable for bigger fish.

The amino acid composition of the fish was affected by dried BSF feeding frequency. In the present study, the essential amino acid composition of fish tissues revealed that lysine, leucine, arginine, and isoleucine were the most prevalent amino acids. The most abundant non-essential amino acids were glutamic acid, aspartic acid, glycine, and alanine. With increased BSF feeding frequency, there was a significant decline in arginine, histidine, and threonine levels. Although arginine content of the BSF is 3.7 times less than that of fish meal, histidine, and threonine contents were the same (Yandi et al., 2022). In contrast, increase in dried BSF feeding frequency resulted in a significant increase in isoleucine, leucine, tryptophan, and alanine content. The same was also the case for tryptophan and leucine. Despite the fact that both of these amino acids are 50% less in BSF than in fishmeal, the ratios of isoleucine and threonine are the same in both. Therefore, inclusion of dried BSF in fish diet one or more times a day, negatively impacts the arginine, histidine, threonine, isoleucine, leucine, tryptophan, and alanine amino acid composition of fish.

When fish were fed with dried BSF three times daily, the total fatty acid content decreased by 7%. However, no detrimental effects of BSF feeding on total fatty acid content were seen when fish were fed a commercial diet once (B2C1) or twice (B1C2) daily. Feeding fish with dried BSF more than once a day, on the other hand, may result in a decrease in growth rate due to a general reduction in lipid digestibility or retention. Lauric acid (12:0) is readily oxidized and has been shown to reduce fat accumulation in both mammals and fish (Smith et al., 2005; St-Onge et al., 2008). Lauric acid (12:0) is a major fatty acid in BSF larvae, accounting for 15.19% of total fatty acids (Yandi, et al., 2022). In B1C2, B2C1, and B3C0 groups, lauric acid content in fish muscle increased by 468, 766, and 610%, respectively, compared to the control group (B0C3). Dietary lauric acid could thus explain some of the lower fat accumulation in the dried BSF fed fish, although it is unlikely to be the sole cause. Liland (2017) reported that enriching the BSF growth medium with PUFA-rich seaweed might improve the fatty acid profile of the BSF. The inclusion of PUFA-rich purslane weed and parsley in the BSF growth substrate increased the PUFA content of the fish muscle in BSF fed groups in this study.

Conclusion

The results of this study revealed that inclusion of dried BSF in rainbow trout meals reduces the growth performance of fish. The inclusion of dried BSF meals beyond a single meal also resulted in a reduction in feed intake and necrosis in anus due to the inability to digest the dried BSF. Even though the growth performance of dried BSF-fed groups was suppressed, the amino acid content of BSF prepupa was significantly higher than that of commercial diet and some of the FAs contents were similar to or higher than commercial diet. Increasing the number of BSF prepupae meals slowed the reduction in feed intake and fish growth due to the accumulation of chitin in the gut. Yet, inclusion of one dried BSF meal in a daily feeding resulted in improvements in PUFAn-3, PUFAn-6, and DPA fatty acid content.

Ethical Statement

The experiments were conducted under the Local Ethics Committee for Animal Experiments of the Recep Tayyip Erdogan University, Rize, Turkey (Decision no: 2019/41, Date: 20.12.2019).

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Author Contribution

Rafet Cagri Ozturk: Investigation, Writing-Original Draft, Formal Analysis, Project administration; Ilhan Yandi: Investigation, Resources, Project administration, Funding acquisition; Yahya Terzi: Investigation; Writing-Original Draft, Formal Analysis, Visualization; Ilhan Altinok: Conceptualization, supervision, Writing-Review & Editing.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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