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Fisheries and aquaculture by-products modulate growth, body composition, and omega-3 polyunsaturated fatty acid content in black soldier fly (*Hermetia illucens*) larvae

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The black soldier fly (BSF) (*Hermetia illucens* L.) is one of the most promising species for the production of ingredients, mainly protein, useful for animal feed formulation, owing to its ability to convert organic waste into biomass with a high nutritional value. However, the low percentage of n-3 series polyunsaturated fatty acids (PUFAs) in its fatty acid profile is a limiting factor for the utilization of BSF in fish feed. Recent studies have highlighted that wastes derived from different agro-food value chains could affect the nutritional composition of BSF larvae, depending on the composition of the wastes used as larvae-growing substrate. Due to the significant amount of n-3 PUFA in marine products, both in edible produce and in by-products, in this study, different sources of fish by-products were included in experimental diets for BSF to evaluate the effect of its addition on the final nutritional profile of BSF pre-pupae. One control diet and five experimental diets were prepared to feed the BSF larvae: wheat bran as the control diet (diet B), bycatch from Mediterranean trawl fisheries (diet F), *Parapenaeus longirostris* processing by-products (diet S), aquaculture processing by-products (diet R), *Thunnus albacares* processing by-products (diet T), and *Engraulis encrasicolus* processing by-products (diet A). In this study, the effects of the different diets were analyzed on the growth, body composition, and fatty acid profile of BSF larvae and pre-pupae. The obtained results showed that the different experimental diets affected total lipids content and fatty acids composition, when compared with the control. A significant increase in eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) in BSF larvae and pre-pupae fed with all fish by-products was observed when compared with those fed with diet B, in particular in larvae and pre-pupae fed

with diet A, demonstrating that the utilization of fish processing by-products is a suitable solution for improving the nutritional value of insects as ingredients in aqua feeds. The reuse of marine by-products can contribute to the industry's "zero waste" goal, increasing the sustainability of the fishery value chain and the formulation of new valuable products.

KEYWORDS

Hermetia illucens, black soldier fly, fatty acids, marine by-products, PUFA, EPA, DHA

1 Introduction

Global fisheries and aquaculture production increased in 2020 due to the constant expansion of aquaculture (FAO, 2022), which reached the value of 49.2% in 2020 on the global production of aquatic animals (FAO, 2022). The growth of global aquaculture production has consequently led to an increase in the demand for fishmeal (FM) and fish oil (FO), which are indispensable ingredients for satisfying fish nutritional requirements. As the production of FM and FO fluctuates according to catches, the decrease in catches reported over the past 3 years (–4.0%) (FAO, 2022) has led to a gradual decrease in the supply of FM and FO, with a consequent increase in price and availability. This situation has stimulated the need to find additional or alternative sources of these ingredients for the sustainable growth of aquaculture (Ayoola, 2010; Barroso et al., 2014; Liland et al., 2017; Belghit et al., 2018; Belghit et al., 2019; FAO, 2020; Maiolo et al., 2020; Hoc et al., 2021).

In recent years, insects have received growing attention as a food source, owing to their potential as a sustainable, alternative source of proteins in FM for commercial aqua feed formulations and to their relatively easy cultivation (Ng et al., 2001; Osimani et al., 2017; Purschke et al., 2018; Govorushko, 2019; Huang et al., 2019; Alfiko et al., 2022).

Insects are good sources of proteins, fats, minerals, vitamins, and energy (Rumpold and Schlüter, 2013; Gasco et al., 2020). Traditionally used as a food in different countries, their cultivation and commercialization has recently become of global interest as a source of protein and fat for human food and animal food (Starčević et al., 2017).

Since the 1970s, insects have been studied as a protein source in animal feed, mainly due to their ability to convert food waste (e.g., vegetable, fruit, factory, and animal tissue waste) into high-quality protein (Calvert et al., 1969; Ichhponani and Malik, 1971; Teotia and Miller, 1974; Phelps et al., 1975; Newton et al., 1977; Belghit et al., 2019; Oonincx et al., 2019). An important group of insects, able to consume different decaying organic substrates for their larval development, are known as bioconverter species. Among bioconverter insects, the most promising species for industrial production and animal feed in the world is *Hermetia illucens* (order Diptera, family Stratiomyidae), better known as the black soldier fly (BSF) (Surendra et al., 2016; Müller et al., 2017). The BSF life cycle, which lasts from 6 to 7 weeks (Maroušek et al., 2022), can be divided into four stages: egg, larva, pre-pupa, and adult (Li et al., 2011; Liu et al., 2017). From the pre-pupa stage, the organism stops feeding in preparation for metamorphosis and uses the nutrients stored from the larval stage (Sheppard et al., 2002).

BSF larvae under optimal growth conditions (27°C temperature and 70% humidity) are able to bioconvert different organic substrates (of both animal and vegetable origin) into larval biomass in just 14 days (Franco et al., 2021). Among the large variety of organic materials that BSF is able to feed on, agro-food waste is receiving increasing attention (Nguyen et al., 2015; Jucker et al., 2017). Through the consumption of organic waste material, the insect gains energy and nutrients useful for its growth and then converts itself into a biomass that can be used as feed for other livestock and human food (Franco et al., 2021). Thus, the BSF can be reared as a feed ingredient [approved by the Association of American Feed Control Officials and by the European Food Safety Authority (EFSA)] using approved feed-grade materials, including food waste and by-products of food production (Lähteenmäki-Uutela et al., 2021; Maroušek et al., 2022). Several EU member countries have requested a food safety evaluation by the EFSA of new substrates to be used for BSF feeding (Lähteenmäki-Uutela et al., 2021).

In recent years, the use of insect-based meal in aquaculture has grown exponentially, due to its nutritional value, including as a protein source (Ogunji et al., 2006; Belghit et al., 2018; Belghit et al., 2019; Guerreiro et al., 2020; Karapanagiotidis et al., 2023). Several studies have reported on the importance of BSF as a source of protein (Chaklader et al., 2020; Maiolo et al., 2020; Chaklader et al., 2021); however, few studies have reported on the possibility of exploiting BSF as a source of fatty acids (Barroso et al., 2019; Hossain et al., 2023). The low levels of n-3 series polyunsaturated fatty acids (PUFAs), such as the two PUFAs essentials for fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the lipids of BSF is one of the limiting factors for its utilization in diets for farmed fish (Oonincx et al., 2020). In fact, this nutritional gap could compromise the growth, health, and quality of farmed fish (Sealey et al., 2011; Kroeckel et al., 2012; Karapanagiotidis et al., 2014; Guerreiro et al., 2020).

Recent studies have shown that the fatty acid composition of BSF larvae can be modified through the use of different feeding substrates, acquiring a new value (Surendra et al., 2016; Liland et al., 2017; Spranghers et al., 2017; Barroso et al., 2019; Bava et al., 2019;

Starcevic et al., 2019; Lopes et al., 2020; Franco et al., 2021; Hoc et al., 2021). In view of this, the possibility to enrich the concentration of essential PUFAs in BSF larvae with marine-based diets is an attractive option for obtaining n-3-enriched larvae (Barroso et al., 2019). Thus, the interest in insects is related not only to their intrinsic nutritional composition (Ramos-Elorduy et al., 2002; Wang et al., 2007; Hwangbo et al., 2009), but also to the possibility of enriching them with other nutrients and micronutrients (St-Hilaire et al., 2007; Mlcek et al., 2014; Hoc et al., 2021; Ferrari et al., 2022).

The process of bioconversion *via* BSF is a very attractive option, as it could potentially solve two problems: organic waste management and the ability to satisfy global food demand without competing with food crops for land use (Surendra et al., 2016; Salomone et al., 2017; Chavez, 2021).

Under this framework, the use of marine by-products generated from a diverse supply chain could be an important resource for high-nutritional-value components, such as n-3 PUFAs and proteins of high biological value (Caruso et al., 2020; Messina et al., 2021a; Messina et al., 2021b; Ucak et al., 2021; Messina et al., 2022). The use of these by-products and their valorization could lead to a reduction in food losses and waste, ensuring the achievement of the industry's "zero-waste" goal (Alfio et al., 2021; Coppola et al., 2021; Kee et al., 2023).

Recently, some researchers have highlighted the possibility of rearing BSF on a diet including marine wastes and by-products such as aquaculture waste (Lopes et al., 2020), fish trimmings (Magee et al., 2021), carp mince (Chaklader et al., 2021), fish protein hydrolysates (from yellowtail kingfish, carp, and tuna) (Chaklader et al., 2020), and discarded round sardinella (*Sardinella aurita*) (Barroso et al., 2019). These diets improved process performance in BSF larvae composting and improved larval quality, which was characterized by high levels of fat and protein, suitable for farmed animal feeds including pig, chicken, and fish feeds (Barroso et al., 2019; Lopes et al., 2020; Chaklader et al., 2021; Magee et al., 2021).

Owing to the increasing interest in the farming of this insect for diverse purposes in the sector of food and feed, and the increase in interventions aimed at recycling by-products from marine sectors to reach zero waste, in this present work we studied the effects of different fish by-products, obtained from the fishery and aquaculture value chain, on growth, body composition, and modulation of fatty acid profile in BSF larvae and pre-pupae. Our aim was to determine if this solution could be a reasonable method for implementing n-3 components in the body composition of these insects, which are intended as new ingredients for aqua feeds, promoting circular economy pathways at a regional level in accordance with the European Green Deal.

2 Materials and methods

2.1 Larvae rearing and experimental diets

H. illucens larvae were purchased alive from Smart Bugs s.s. (Ponzano Veneto, Italy). The experiment was performed in an acclimatized environment at 26 ± 1°C and 65 ± 5% humidity.

Larvae were kept in transparent containers (28 cm × 20 cm × 14 cm) fitted with lids, a drainage system, and a collection system for the pre-pupae.

Air circulation was enabled through holes in the lower (1 mm diameter) and upper (2–2.5 mm diameter) sections of the boxes.

The experiment was set up to administer one control wheat bran-based diet (B) and five experimental diets, including diverse sources of fish by-products (25%) collected from fisheries, aquaculture facilities, and processing plants located in the region of Trapani (western Sicily, Italy). Each treatment was performed in three replicates. Four hundred 6-day-old BSF larvae were allowed in each container (1,200 larvae for each treatment), as described in the literature (Grossule and Lavagnolo, 2020); substrates were weighed and distributed among the feeding containers in order to provide a daily ration equal to 100 mg/larva/day (Diener et al., 2009) (Table 1). The BSF larvae were fed for 26 days and were sampled at 6, 13, 21, and 26 days.

The five experimental diets were as follows: diet F, comprising fishery bycatch from the Mediterranean trawler fishing species *Trachurus trachurus* (Atlantic horse mackerel), *Merluccius merluccius* (European hake), *Spicara flexuosa* (Mediterranean picarel), *Munida* sp. (squat-lobsters), *Helicolenus dactylopterus* (blackbelly rosefish), *Phycys blennoides* (greater forkbeard), *Galeus melastomus* (blackmouth catshark), *Gadiculus argenteus* (silvery

TABLE 1 Percentage of inclusion of fish by-products in the experimental diets provided to BSF larvae.

Diets	B (%)	F (%)	S (%)	R (%)	T (%)	A (%)
Diet P (inert component)	75	75	75	75	75	75
Diet B	25	0	0	0	0	0
Diet F	0	25	0	0	0	0
Diet S	0	0	25	0	0	0
Diet R	0	0	0	25	0	0
Diet T	0	0	0	0	25	0
Diet A	0	0	0	0	0	25

P: peat; B: wheat bran; F: by-catch from sea trawling fishery; S: *Parapenaeus longirostris* processing by-products; R: aquaculture processing by-products; T: *Thunnus albacares* processing by-products; A: *Engraulis encrasicolus* processing by-products.

pout); diet S, comprising *Parapenaeus longirostris* (deep-water rose shrimp) processing by-products; diet R, comprising aquaculture by-products (from sea bass and sea bream filleting); diet T, made from *Thunnus albacares* (yellowfin tuna) processing by-products; and diet A, comprising *Engraulis encrasicolus* (European anchovy) by-products (heads and viscera) from specimens collected at the Trapani fish market. *P. longirostris* and *T. albacares* processing by-products from companies in the province of Trapani. All the materials were oven dried at 60°C for 48 hours and then ground. The obtained meal was stored under vacuum at −20°C. Different by-products and wheat bran were mixed into the peat in the ratios indicated in Table 1. The water content was increased to 75% (Diener et al., 2009). The choice of peat as an inert component was suggested owing to its easy availability, homogeneity of composition, and high degree of sustainability. The proximate composition and fatty acid profile of the experimental diets are shown in Tables 2, 3.

Diet aliquots were prepared to be added to the substrate at start-up and then every 2 days as a supplement to the diet until the end of the trial (Diener et al., 2009; Parra Paz et al., 2015).

2.2 BSF growth performance

In order to determine the growth rate, 10 larvae at 2, 6, and 13 days and 10 pre-pupae at 21 and 26 days from each replicate were randomly sampled and weighed individually. The "pre-pupae" stage was defined as the mobile, non-feeding, final larval stage of BSF, as defined in the literature (Myers et al., 2008; Tomberlin et al., 2009; Martínez-Sánchez et al., 2011; Harnden and Tomberlin, 2016). Pre-pupae were identified by a dark brown–black dorsal and ventral integument and reduced, darkened mouth parts (May, 1961; Harnden and Tomberlin, 2016). Larvae and pre-pupae were immediately frozen at −20°C for subsequent chemical analysis.

2.3 Proximate composition

The proximate composition of both diets and of BSF was measured as follows: the total lipids (TL) were determined according to Folch et al. (1957); moisture and ash content was determined using the AOAC method (AOAC, 1990); total nitrogen was determined using the Kjeldahl method (AOAC, 1992); and crude protein (P) and chitin (Q) content were determined by applying the equations:

$$P = \frac{(Nt * Cq + k - 100) * Cp}{Cq - Cp} \quad (1)$$

$$Q = \frac{(Nt * Cp + k - 100) * Cq}{Cp - Cq} \quad (2)$$

used by Díaz-Rojas et al. (2006), where *Nt* is the total nitrogen content, *K* is the sum of total lipid, moisture and ash, and *Cp* and *Cq* are the conversion coefficients that relate to the mass fraction of nitrogen with protein and chitin (Díaz-Rojas et al. 2006). The protein content in the literature is mainly based on nitrogen content, using the nitrogen-to-protein conversion factor (Cp) of 6.25 (Gnaiger and Bitterlich, 1984; Woyewoda et al., 1986), while the value of Cq is 14.5 (Díaz-Rojas et al., 2006; Messina et al., 2019).

Carbohydrate content was determined following the method described by Dubois et al. (1956).

Caloric content was measured as total energy content using an isoperibolic oxygen bomb calorimeter (model 6200, Parr Instrument Company, USA).

The composition of experimental diets varies considerably according to the species used and the current fishing season (Kim and Mendis, 2006).

2.4 Fatty acid profile

Total fatty acid (FA) methyl esters were determined from the total lipid content, following the method described by Lepage and Roy (1984) and analyzed using the conditions described by Messina et al. (2013), employing a Perkin Elmer (Waltham, MA, USA) CLARUS 580 instrument equipped using a silica capillary column (30 m × 0.32 mm, df 0.25 µm, Omegawax 320, Supelco, Bellefonte, PA, USA). Individual FAME were measured by the comparison of known standards (a mix of PUFA 1, PUFA 2, and PUFA 3 oil, Supelco).

TABLE 2 Proximate composition (g/100 g wet), chitin (g/100 g wet), and energy content (kJ/100 g) of experimental diets provided to BSF larvae.

Parameters	Diet B	Diet F	Diet S	Diet R	Diet T	Diet A
Total lipid	2.48 ± 0.17	9.55 ± 0.17	5.72 ± 0.45	27.97 ± 0.15	9.32 ± 0.59	12.87 ± 0.64
Moisture	9.54 ± 0.01	4.89 ± 0.17	6.89 ± 0.11	2.64 ± 0.04	2.32 ± 0.26	3.45 ± 0.03
Protein	14.50 ± 0.05	69.73 ± 0.02	25.27 ± 0.48	41.60 ± 0.71	73.26 ± 1.67	49.52 ± 0.61
Ash	3.91 ± 0.04	14.84 ± 0.33	34.04 ± 0.31	18.87 ± 0.14	13.18 ± 0.61	27.46 ± 0.37
Carbohydrates	50.00 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.
Chitin	n.d.	n.d.	17.90 ± 0.29	n.d.	n.d.	n.d.
Energy	1,177.90 ± 6.96	1,543.20 ± 6.64	945.55 ± 6.01	1,741.99 ± 12.31	1,590.19 ± 33.10	1,318.08 ± 24.81

Each value represents the mean of three independent determinations. B: wheat bran; F: by-catch from sea trawling fishery; S: *Parapenaeus longirostris* processing by-products; R: aquaculture processing by-products; T: *Thunnus albacares* processing by-products; A: *Engraulis encrasicolus* processing by-products. n.d., not detectable

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TABLE 3 Fatty acid profile (% of total FA) of experimental diets provided to BSF larvae.

Fatty acid	Diet B	Diet F	Diet S	Diet R	Diet T	Diet A
8:0	0.15 ± 0.07	0.04 ± 0.00	0.02 ± 0.01	0.02 ± 0.02	0.05 ± 0.00	0.04 ± 0.04
10:0	n.d.	n.d.	0.03 ± 0.03	n.d.	n.d.	0.03 ± 0.01
11:0	0.01 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.
12:0	0.02 ± 0.00	0.10 ± 0.01	0.31 ± 0.23	0.05 ± 0.01	0.07 ± 0.01	0.31 ± 0.01
13:0	n.d.	0.03 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.13 ± 0.00
14:0	0.15 ± 0.00	2.64 ± 0.04	1.18 ± 0.06	2.23 ± 0.15	2.91 ± 0.09	5.72 ± 0.14
15:0	0.21 ± 0.01	0.61 ± 0.00	0.67 ± 0.00	0.24 ± 0.01	1.24 ± 0.05	1.26 ± 0.03
16:0	13.86 ± 0.67	20.57 ± 0.25	13.96 ± 0.25	12.56 ± 0.16	24.82 ± 0.45	25.81 ± 0.55
16:1n-7	0.25 ± 0.05	4.34 ± 0.05	4.26 ± 0.06	3.66 ± 0.15	4.69 ± 0.07	3.06 ± 0.08
16:2n-4	0.06 ± 0.01	0.74 ± 0.09	0.37 ± 0.01	0.33 ± 0.01	1.10 ± 0.13	0.91 ± 0.03
17:0	0.18 ± 0.01	0.75 ± 0.01	1.09 ± 0.04	0.25 ± 0.01	1.78 ± 0.09	1.52 ± 0.04
16:3n-4	0.06 ± 0.01	0.72 ± 0.00	0.95 ± 0.01	0.38 ± 0.03	0.97 ± 0.04	0.68 ± 0.02
18:0	1.39 ± 0.03	6.24 ± 0.02	5.64 ± 0.18	3.14 ± 0.14	8.19 ± 0.08	6.33 ± 0.14
18:1n-9	19.56 ± 0.30	22.81 ± 0.43	16.98 ± 0.66	36.40 ± 0.45	18.04 ± 0.49	17.29 ± 0.19
18:1n-7	1.19 ± 0.10	3.29 ± 0.02	4.72 ± 0.18	3.00 ± 0.04	3.06 ± 0.04	2.92 ± 0.00
18:2n-6	55.88 ± 0.29	1.41 ± 0.11	1.87 ± 0.20	19.76 ± 0.08	1.72 ± 0.25	3.08 ± 0.29
18:3n-6	n.d.	0.17 ± 0.01	0.36 ± 0.01	0.05 ± 0.01	0.56 ± 0.20	0.24 ± 0.01
18:3n-4	n.d.	0.23 ± 0.01	n.d.	0.04 ± 0.05	0.23 ± 0.06	0.26 ± 0.01
18:3n-3	3.63 ± 0.07	0.60 ± 0.01	0.27 ± 0.06	3.77 ± 0.06	0.41 ± 0.06	1.00 ± 0.06
18:4n-3	n.d.	0.40 ± 0.00	0.21 ± 0.00	0.52 ± 0.01	0.33 ± 0.01	0.78 ± 0.00
20:1n-9	0.97 ± 0.09	2.43 ± 0.06	1.89 ± 0.51	2.65 ± 0.08	1.41 ± 0.00	1.00 ± 0.02
20:4n-6	n.d.	1.46 ± 0.02	6.07 ± 0.08	0.38 ± 0.01	1.74 ± 0.05	1.51 ± 0.01
20:4n-3	0.20 ± 0.28	0.79 ± 0.00	0.31 ± 0.01	0.46 ± 0.00	0.30 ± 0.01	0.36 ± 0.01
20:5n-3	0.38 ± 0.05	5.11 ± 0.05	12.69 ± 0.18	2.19 ± 0.00	2.99 ± 0.13	6.05 ± 0.07
22:1n-11	n.d.	0.41 ± 0.03	0.55 ± 0.39	1.09 ± 0.06	0.15 ± 0.03	0.29 ± 0.01
22:1n-9	n.d.	0.21 ± 0.24	0.26 ± 0.04	0.40 ± 0.03	0.10 ± 0.00	0.13 ± 0.01
22:4n-6	n.d.	0.35 ± 0.01	0.79 ± 0.00	0.12 ± 0.04	0.34 ± 0.05	0.16 ± 0.03
22:5n-6	n.d.	0.89 ± 0.04	1.24 ± 0.03	0.13 ± 0.02	2.02 ± 0.04	0.81 ± 0.00
22:5n-3	0.34 ± 0.08	1.85 ± 0.33	1.37 ± 0.01	1.48 ± 0.02	1.13 ± 0.03	0.81 ± 0.09
22:6n-3	n.d.	20.83 ± 0.58	21.93 ± 0.75	4.71 ± 0.02	19.60 ± 0.20	17.54 ± 0.15
SFA	15.98 ± 0.70	30.96 ± 0.23	22.92 ± 0.19	18.49 ± 0.14	39.11 ± 0.77	41.14 ± 0.88
MUFA	21.97 ± 0.54	33.49 ± 0.70	28.66 ± 0.33	47.21 ± 0.33	27.46 ± 0.49	24.68 ± 0.27
PUFA	60.55 ± 0.06	35.55 ± 0.47	48.42 ± 0.51	34.30 ± 0.18	33.43 ± 0.28	34.17 ± 0.61
Tot n-3	4.55 ± 0.24	29.57 ± 0.21	36.78 ± 0.62	13.12 ± 0.10	24.76 ± 0.26	26.54 ± 0.35
Tot n-6	55.88 ± 0.29	4.28 ± 0.18	10.33 ± 0.10	20.43 ± 0.01	6.38 ± 0.39	5.79 ± 0.26
n-6/n-3	12.30 ± 0.71	0.28 ± 0.01	0.28 ± 0.01	1.56 ± 0.01	0.26 ± 0.02	0.22 ± 0.01
unknown	1.51 ± 0.11					
DHA/EPA	0.00 ± 0.00	4.08 ± 0.16	1.73 ± 0.08	2.15 ± 0.02	6.56 ± 0.22	2.90 ± 0.01

Each value represents the mean of three independent determinations. B: wheat bran; F: by-catch from sea trawling fishery; S: *Parapenaeus longirostris* processing by-products; R: aquaculture processing by-products; T: *Thunnus albacares* processing by-products; A: *Engraulis encrasicolus* processing by-products. SFA saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids. n.d., not detectable.

2.5 Statistical analysis

Statistical differences were evaluated for each parameter at each time point by diet using analysis of variance (ANOVA). The differences among the mean values were assessed using the Student–Newman–Keuls test. The degree of heterogeneity was measured by the Cochran test (Underwood, 1997). A principal component analysis (PCA) was performed to highlight the differences in fatty acid profiles in BSF larvae and pre-pupae fed different diets. The ANOVA and PCA were performed using STATISTICA (version 8.0, Statsoft Inc. USA).

3 Results

3.1 Effects of the experimental diets on BSF growth performance

The growth performance of BSF larvae during the experiment is shown in Figure 1. Larvae fed diet S died after a few days and did not develop into pre-pupae (data not shown). The best growth performance was recorded in larvae fed the control diet (diet B), which weighed significantly more than the other larvae and pre-pupae ($p< 0.05$) fed marine by-products. The larvae and pre-pupae fed the experimental diets (diet F, diet R, diet T, and diet A) did not differ regarding growth performance (Figure 1).

3.2 Proximate composition of the pre-pupae

The proximate composition of the BSF pre-pupae, analyzed at the end of the experiment, is shown in Table 4. Since larvae fed diet S died after few days into the experiment, their proximate composition is not reported among the pre-pupae results.

The different experimental diets affected the proximate composition of the BSF pre-pupae (Table 4). Total lipid content was highest in pre-pupae fed diet R (P26-R, 28.05 ± 1.90 g/100 g) ($p< 0.05$) (Table 4), which aligns with diet R having the highest energy content of (Table 2). The pre-pupae fed diets B, F, T, and A did not differ significantly in lipid content (ranging between 24.1 and 25.51 g/100 g dw). The highest protein content was observed in pre-pupae fed diet B (P26-B, 39.93 g/100 g) ($p< 0.05$) (Table 4). The pre-pupae fed experimental diets showed a comparable total protein content, ranging from 34.29 to 34.98 g/100 g (Table 4). Ash content was significantly higher ($p< 0.05$) in pre-pupae fed diets T, A, and F than in those fed the control diet (diet B); no significant differences were observed between diet R and diet B. Carbohydrates, despite being absent in all diets except diet B (Table 2), ranged from 22.86 g/100 g (P26-B) to 26.34 g/100 g (P26-F) in the BSF (Table 4). Similarly, the levels of chitin in BSF body composition was comparable regardless of its concentration in diets, ranging between 6 and 8.1 g/100 g (Table 4). Analysis of body energy content showed no significant differences among the pre-pupae fed the experimental diets and control diet (Table 4).

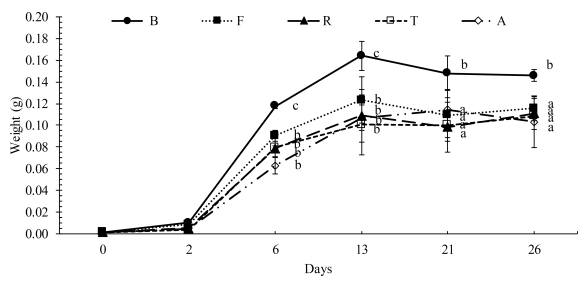

FIGURE 1
Growth performance of BSF fed with B: wheat bran; F: by-catch from sea trawling fishery; S: *Parapenaeus longirostris* processing by-products; R: aquaculture processing by-products; T: *Thunnus albacares* processing by-products; A: *Engraulis encrasicolus* processing by-products. Each value represents the mean of three independent determinations. Different letters within the same day indicate significant differences ($p< 0.05$) within treatments.

3.3 FA profile

The FA profiles of the BSF larvae and pre-pupae fed different diets are shown in Tables 5–10. The larvae fed the experimental diets reflected the FA composition of the diets, showing significant differences ($p< 0.05$) when compared with the larvae fed diet B.

The FA composition of the larvae and pre-pupae was dominated mainly by saturated fatty acids (SFAs) and increased in the pre-pupae (Tables 5–10). In particular, lower values were observed in the larvae and pre-pupae fed fish by-products (diet F, diet R, diet T, and diet A), than in those fed the control diet (diet B) ($p< 0.05$) (Tables 5–10). The predominant fatty acid in the SFA was lauric acid (12:0), which increased significantly in content ($p< 0.05$) during the growth phase in all treatments. In particular, it was observed that larvae and pre-pupae fed control diet (diet B) had the highest value of lauric acid, reaching 46.58 ± 0.19% in P26 (Tables 5, 10). Significantly lower values ($p< 0.05$) were observed in the larvae and pre-pupae fed fish by-products, particularly in those fed diet A. However, after 26 days, the pre-pupae (P26) fed fish by-products showed a lauric acid concentration above 20%. The larvae and pre-pupae fed diet A showed a higher concentration of palmitic acid (16:0) (Tables 9, 10) due to the higher amount present in the diet (Table 2).

Monounsaturated FA (MUFA) represented the second most abundant class of FA in almost all larvae and in all analyzed pre-pupae. L6 showed the highest MUFA content (Tables 5–9). A decrease in MUFAs occurred during the growth phase due to a reduction in oleic acid (18:1n-9), while palmitoleic acid (16:1 n-7) remained almost constant over time (Tables 5–9). Pre-pupae fed the experimental diets had significantly higher MUFA content ($p< 0.05$) than the larvae fed diet B (Table 10).

Similarly, polyunsaturated FA (PUFA) concentrations in the BSF larvae and pre-pupae decreased during growth (Tables 5–9). Linoleic acid (LA, 18:2n6) was the most represented fatty acid in BSF larvae and pre-pupae fed diets B and R (Tables 5, 7). In contrast, pre-pupae fed diets A, T, and F showed significantly lower LA values ($p< 0.05$) (Table 10). These larvae and pre-pupae fed diets A, T, and F also demonstrated an improvement in the

TABLE 4 Proximate composition (g/100 g dw), chitin (g/100 g dw), and energy content (kJ/100 g) of pre-pupae analyzed at the end of the experiment (P26).

	P26-B	P26-F	P26-R	P26-T	P26-A
Total lipid	25.51 ± 1.05^{ab}	25.27 ± 0.83^{ab}	28.05 ± 1.90^{b}	25.23 ± 1.32^{ab}	24.10 ± 1.04^{a}
Protein	39.93 ± 0.13^{b}	34.94 ± 0.22^{a}	34.83 ± 0.23^{a}	34.47 ± 0.43^{a}	34.29 ± 0.65^{a}
Ash	4.23 ± 0.07^{a}	7.25 ± 0.02^{b}	6.16 ± 0.35^{ab}	8.30 ± 3.13^{b}	7.82 ± 1.21^{b}
Carbohydrates	22.86 ± 0.74^{a}	26.44 ± 0.72^{c}	24.73 ± 0.32^{b}	25.92 ± 0.36^{c}	26.37 ± 0.25^{c}
Chitin	7.46 ± 0.25^{b}	8.19 ± 0.15^{c}	6.10 ± 0.11^{a}	6.03 ± 0.19^{a}	5.99 ± 0.14^{a}
Energy	2,011.13 ± 32.57	1,978.20 ± 25.33	2,050.32 ± 78.53	1,960.21 ± 46.28	1,926.64 ± 46.51

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). B: wheat bran; F: by-catch from sea trawling fishery; S: *Parapenaeus longirostris* processing by-products; R: aquaculture processing by-products; T: *Thunnus albacares* processing by-products; A: *Engraulis encrasicolus* processing by-products.

TABLE 5 FA profile (% of total FA) of larvae (L) and pre-pupae (P) of BSF, analyzed at 6, 13, 21, and 26 days, fed diet B.

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
8:0	0.05 ± 0.01^{a}	0.10 ± 0.02^{c}	0.08 ± 0.02^{b}	0.14 ± 0.00^{d}
10:0	0.29 ± 0.08^{a}	0.60 ± 0.03^{b}	0.77 ± 0.03^{c}	0.89 ± 0.08^{d}
11:0	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
12:0	28.23 ± 0.14^{a}	36.47 ± 0.62^{b}	47.14 ± 1.51^{c}	46.58 ± 0.19^{c}
13:0	0.02 ± 0.00^{a}	0.05 ± 0.00^{b}	0.05 ± 0.00^{b}	0.06 ± 0.00^{c}
14:0	6.52 ± 0.43^{a}	7.11 ± 0.19^{b}	8.00 ± 0.13^{c}	7.88 ± 0.03^{c}
15:0	0.32 ± 0.05^{a}	0.46 ± 0.04^{c}	0.38 ± 0.03^{b}	0.38 ± 0.00^{b}
16:0	15.72 ± 0.15^{c}	13.73 ± 0.13^{b}	11.65 ± 0.44^{a}	11.77 ± 0.09^{a}
16:1n-7	3.16 ± 0.60^{a}	3.80 ± 0.26^{b}	3.55 ± 0.18^{ab}	3.90 ± 0.15^{b}
16:2n-4	0.04 ± 0.00^{b}	0.01 ± 0.02^{a}	0.01 ± 0.01^{a}	0.05 ± 0.00^{b}
17:0	0.35 ± 0.01^{b}	0.39 ± 0.02^{c}	0.28 ± 0.02^{a}	0.29 ± 0.00^{a}
16:3n-4	0.21 ± 0.10	0.31 ± 0.08	0.26 ± 0.02	0.25 ± 0.00
18:0	2.65 ± 0.18^{c}	1.84 ± 0.03^{b}	1.23 ± 0.08^{a}	1.12 ± 0.02^{a}
18:1n-9	16.40 ± 0.19^{c}	12.27 ± 0.16^{b}	9.35 ± 0.45^{a}	9.04 ± 0.04^{a}
18:1n-7	1.30 ± 0.02^{c}	1.09 ± 0.07^{b}	0.73 ± 0.05^{a}	0.68 ± 0.06^{a}
18:2n-6	20.21 ± 0.52^{d}	14.69 ± 0.22^{c}	12.86 ± 0.22^{b}	12.09 ± 0.05^{a}
18:3n-6	0.07 ± 0.01^{c}	n.d.^{a}	0.03 ± 0.00^{b}	0.03 ± 0.00b
18:3n-4	n.d.	n.d.	0.01 ± 0.01	n.d.
18:3n-3	1.54 ± 0.09^{d}	1.03 ± 0.03^{c}	0.91 ± 0.02^{b}	0.84 ± 0.00^{a}
18:4n-3	n.d.^{a}	n.d.^{a}	0.19 ± 0.02^{c}	0.12 ± 0.00^{b}
20:1n-9	0.55 ± 0.02^{c}	1.02 ± 0.00^{d}	0.00 ± 0.00^{a}	0.52 ± 0.00^{b}
20:4n-6	n.d.	n.d.	0.02 ± 0.03	0.06 ± 0.00
20:4n-3	n.d.	n.d.	n.d.	n.d.
20:5n-3	n.d.^{a}	0.06 ± 0.02^{b}	0.11 ± 0.03^{c}	0.21 ± 0.00^{d}
22:1n-11	n.d.	n.d.	n.d.	n.d.
22:1n-9	n.d.	n.d.	n.d.	n.d.

(Continued)

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TABLE 5 Continued

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
22:4n-6	n.d.	n.d.	n.d.	n.d.
22:5n-6	n.d.	n.d.	n.d.	n.d.
22:5n-3	n.d.	n.d.	n.d.	n.d.
22:6n-3	n.d.	n.d.	n.d.	n.d.
SFA	54.16 ± 0.20^{a}	60.76 ± 0.34^{b}	69.59 ± 1.08^{c}	69.18 ± 0.08^{c}
MUFA	21.42 ± 0.83^{c}	18.17 ± 0.36^{b}	13.63 ± 0.68^{a}	14.16 ± 0.12^{a}
PUFA	22.07 ± 0.70^{d}	16.11 ± 0.16^{c}	14.42 ± 0.19^{b}	13.67 ± 0.06^{a}
Tot n-3	1.54 ± 0.09^{c}	1.09 ± 0.04^{a}	1.21 ± 0.03^{b}	1.17 ± 0.01^{b}
Tot n-6	20.28 ± 0.51^{d}	14.69 ± 0.22^{c}	12.92 ± 0.19^{b}	12.20 ± 0.05^{a}
n-6/n-3	13.18 ± 0.41^{b}	13.53 ± 0.32^{b}	10.65 ± 0.39^{a}	10.47 ± 0.00^{a}
Unknown	3.04 ± 1.03^{a}	4.97 ± 0.18^{b}	2.36 ± 0.21^{a}	2.99 ± 0.26^{a}
DHA/EPA	n.d.	n.d.	n.d.	n.d.

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). n.d, not detectable.

TABLE 6 FA profile (% of total FA) of larvae (L) and pre-pupae (P) of *BSF*, analyzed at 6, 13, 21, and 26 days, fed diet F.

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
8:0	0.07 ± 0.01^{a}	0.11 ± 0.01^{b}	0.10 ± 0.01^{b}	0.14 ± 0.03^{c}
10:0	0.10 ± 0.00^{a}	0.38 ± 0.02^{b}	0.51 ± 0.13^{c}	0.59 ± 0.04^{c}
11:0	0.04 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02
12:0	12.96 ± 0.14^{a}	25.87 ± 0.69^{b}	33.60 ± 0.64^{c}	38.23 ± 0.75^{d}
13:0	0.05 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	0.07 ± 0.03
14:0	4.19 ± 0.21^{a}	5.89 ± 0.13^{b}	6.85 ± 0.93^{c}	6.91 ± 0.06^{c}
15:0	0.65 ± 0.03^{b}	1.51 ± 0.22^{c}	0.47 ± 0.00^{a}	0.38 ± 0.12^{a}
16:0	19.51 ± 0.26^{c}	16.42 ± 0.17^{b}	15.11 ± 0.21^{a}	14.55 ± 1.02^{a}
16:1n-7	7.38 ± 0.38^{c}	7.48 ± 0.04^{c}	6.81 ± 0.03^{b}	5.63 ± 0.46^{a}
16:2n-4	0.44 ± 0.01^{c}	0.35 ± 0.00^{b}	0.29 ± 0.01^{a}	0.24 ± 0.08^{a}
17:0	0.90 ± 0.03^{d}	0.60 ± 0.03^{c}	0.50 ± 0.02^{b}	0.40 ± 0.13^{a}
16:3n-4	1.10 ± 0.01^{c}	0.93 ± 0.10^{b}	0.85 ± 0.05^{b}	0.55 ± 0.27^{a}
18:0	3.98 ± 0.10^{c}	2.89 ± 0.13^{b}	2.10 ± 0.17^{a}	2.02 ± 0.05^{a}
18:1n-9	28.32 ± 0.14^{d}	24.68 ± 0.35^{c}	21.58 ± 0.61^{b}	19.95 ± 0.38^{a}
18:1n-7	2.92 ± 0.24^{c}	2.06 ± 0.02^{b}	1.43 ± 0.20^{a}	1.39 ± 0.19^{a}
18:2n-6	1.44 ± 0.06^{b}	1.04 ± 0.11^{a}	1.01 ± 0.01^{a}	1.07 ± 0.08^{a}
18:3n-6	0.09 ± 0.00	0.08 ± 0.00	0.07 ± 0.03	0.19 ± 0.15
18:3n-4	0.11 ± 0.01^{b}	0.05 ± 0.02^{a}	0.08 ± 0.03^{a}	0.05 ± 0.04^{a}
18:3n-3	0.46 ± 0.02	0.36 ± 0.04	0.36 ± 0.00	0.44 ± 0.12
18:4n-3	0.54 ± 0.03	0.70 ± 0.22	0.67 ± 0.03	0.59 ± 0.05
20:1n-9	0.91 ± 0.05	0.63 ± 0.01	0.44 ± 0.11	0.83 ± 0.53

(Continued)

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TABLE 6 Continued

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
20:4n-6	0.77 ± 0.03^{c}	0.59 ± 0.06^{b}	0.49 ± 0.08^{ab}	0.39 ± 0.21^{a}
20:4n-3	0.20 ± 0.01^{b}	0.13 ± 0.02^{a}	0.07 ± 0.09^{a}	0.10 ± 0.01^{a}
20:5n-3	4.48 ± 0.26^{b}	3.74 ± 0.26^{ab}	3.43 ± 0.45^{a}	3.03 ± 0.99^{a}
22:1n-11	0.08 ± 0.00^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}
22:1n-9	0.05 ± 0.01^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}
22:4n-6	0.07 ± 0.00^{b}	n.d.^{a}	n.d.^{a}	n.d.^{a}
22:5n-6	0.24 ± 0.01^{c}	0.13 ± 0.02^{b}	n.d.^{a}	n.d.^{a}
22:5n-3	0.34 ± 0.00^{b}	0.31 ± 0.11^{b}	0.08 ± 0.12^{a}	0.15 ± 0.01^{a}
22:6n-3	4.20 ± 0.22^{c}	2.41 ± 0.39^{b}	1.56 ± 0.31^{a}	1.21 ± 0.47^{a}
SFA	42.45 ± 0.07^{a}	53.73 ± 0.67^{b}	59.34 ± 1.77^{c}	63.31 ± 1.50^{d}
MUFA	39.66 ± 0.69^{d}	34.86 ± 0.40^{c}	30.27 ± 0.92^{b}	27.80 ± 0.64^{a}
PUFA	14.48 ± 0.08^{c}	10.82 ± 0.88^{b}	8.96 ± 1.11^{a}	8.00 ± 1.77^{a}
Tot n-3	10.21 ± 0.02^{c}	7.66 ± 0.78^{b}	6.17 ± 0.99^{a}	5.51 ± 1.40^{a}
Tot n-6	2.62 ± 0.03^{c}	1.84 ± 0.19^{b}	1.57 ± 0.12^{a}	1.65 ± 0.02^{a}
n-6/n-3	0.26 ± 0.00	0.24 ± 0.00	0.26 ± 0.02	0.31 ± 0.08
Unknown	3.41 ± 0.70^{c}	0.58 ± 0.19^{a}	1.43 ± 0.26^{b}	0.89 ± 0.37^{ab}
DHA/EPA	0.94 ± 0.10^{b}	0.34 ± 0.49^{a}	0.24 ± 0.34^{a}	0.39 ± 0.03^{a}

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). n.d, not detectable.

TABLE 7 FA profile (% of total FA) of larvae (L) and pre-pupae (P) of *BSF*, analyzed at 6, 13, 21, and 26 days, fed diet R.

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
8:0	0.06 ± 0.02^{a}	0.11 ± 0.06^{ab}	0.10 ± 0.03^{ab}	0.16 ± 0.05^{b}
10:0	0.04 ± 0.02^{a}	0.19 ± 0.06^{ab}	0.31 ± 0.05^{b}	0.53 ± 0.21^{c}
11:0	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
12:0	7.23 ± 0.48^{a}	15.98 ± 0.52^{b}	23.63 ± 0.22^{c}	24.12 ± 0.86^{c}
13:0	0.03 ± 0.01^{a}	0.02 ± 0.00^{a}	0.03 ± 0.00^{a}	0.07 ± 0.04^{b}
14:0	3.11 ± 0.09	3.92 ± 0.50	4.87 ± 0.49	5.22 ± 0.40
15:0	0.40 ± 0.12	0.30 ± 0.05	0.26 ± 0.00	0.37 ± 0.17
16:0	17.72 ± 0.71^{c}	14.35 ± 0.18^{b}	13.57 ± 0.16^{a}	12.89 ± 0.84^{a}
16:1n-7	4.83 ± 0.86	5.09 ± 0.21	4.60 ± 0.15	4.90 ± 0.96
16:2n-4	0.28 ± 0.07^{b}	0.22 ± 0.01^{ab}	0.17 ± 0.00^{a}	0.21 ± 0.05^{ab}
17:0	0.55 ± 0.20^{b}	0.32 ± 0.03^{a}	0.26 ± 0.01^{a}	0.37 ± 0.13^{a}
16:3n-4	0.48 ± 0.17	0.39 ± 0.00	0.33 ± 0.00	0.52 ± 0.32
18:0	3.51 ± 0.04^{c}	2.23 ± 0.16^{b}	1.74 ± 0.07^{a}	1.83 ± 0.13^{a}
18:1n-9	33.99 ± 0.84^{c}	33.36 ± 0.38^{c}	26.96 ± 0.61^{b}	25.74 ± 0.47^{a}
18:1n-7	2.39 ± 0.18^{c}	1.90 ± 0.04^{b}	1.36 ± 0.02^{a}	1.30 ± 0.02^{a}
18:2n-6	14.65 ± 0.37^{c}	13.86 ± 0.13^{b}	13.36 ± 0.16^{a}	14.29 ± 0.50^{bc}
18:3n-6	0.33 ± 0.03^{b}	0.27 ± 0.05^{ab}	0.22 ± 0.03^{ab}	0.17 ± 0.12^{a}

(Continued)

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TABLE 7 Continued

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
18:3n-4	n.d.	n.d.	n.d.	n.d.
18:3n-3	1.98 ± 0.64	2.07 ± 0.02	2.01 ± 0.02	2.05 ± 0.21
18:4n-3	0.40 ± 0.01	0.49 ± 0.03	0.44 ± 0.01	0.53 ± 0.15
20:1n-9	1.45 ± 0.05^{c}	1.36 ± 0.23^{c}	1.08 ± 0.06^{b}	0.41 ± 0.02^{a}
20:4n-6	0.34 ± 0.11	0.19 ± 0.04	0.19 ± 0.01	0.33 ± 0.21
20:4n-3	0.04 ± 0.05^{a}	0.14 ± 0.03^{b}	0.06 ± 0.08^{a}	0.09 ± 0.01^{ab}
20:5n-3	2.09 ± 0.07	2.12 ± 0.04	1.96 ± 0.10	2.06 ± 0.33
22:1n-11	0.05 ± 0.01	0.03 ± 0.05	0.02 ± 0.03	0.02 ± 0.03
22:1n-9	0.03 ± 0.01^{b}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}	0.01 ± 0.01^{a}
22:4n-6	n.d.	n.d.	n.d.	n.d.
22:5n-6	n.d.	n.d.	n.d.	n.d.
22:5n-3	0.25 ± 0.06	0.11 ± 0.16	0.14 ± 0.00	0.15 ± 0.01
22:6n-3	1.16 ± 0.35	0.67 ± 0.05	0.42 ± 0.00	0.93 ± 0.74
SFA	32.66 ± 0.27^{a}	37.44 ± 0.84^{b}	44.81 ± 0.40^{c}	45.59 ± 0.53^{d}
MUFA	42.75 ± 0.13^{d}	41.75 ± 0.50^{c}	34.03 ± 0.39^{b}	32.39 ± 0.41^{a}
PUFA	21.99 ± 0.38^{c}	20.56 ± 0.39^{b}	19.31 ± 0.07^{a}	21.36 ± 0.95^{c}
Tot n-3	5.91 ± 0.34	5.61 ± 0.14	5.04 ± 0.05	5.82 ± 1.00
Tot n-6	15.32 ± 0.28^{d}	14.33 ± 0.21^{b}	13.77 ± 0.12^{a}	14.80 ± 0.41^{c}
n-6/n-3	2.60 ± 0.10	2.56 ± 0.02	2.73 ± 0.05	2.59 ± 0.52
Unknown	2.59 ± 0.03^{c}	0.25 ± 0.05^{a}	1.85 ± 0.06^{b}	0.66 ± 0.83^{a}
DHA/EPA	0.55 ± 0.15^{b}	0.31 ± 0.03^{ab}	0.21 ± 0.01^{a}	0.43 ± 0.29^{ab}

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). n.d, not detectable.

TABLE 8 FA profile (% of total FA) of larvae (L) and pre-pupae (P) of *BSF*, analyzed at 6, 13, 21, and 26 days, fed diet T.

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
8:0	0.07 ± 0.00^{a}	0.12 ± 0.0^{c}	0.09 ± 0.00^{b}	0.14 ± 0.02^{d}
10:0	0.11 ± 0.09^{a}	0.44 ± 0.19^{b}	0.47 ± 0.14^{b}	0.56 ± 0.11^{b}
11:0	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
12:0	18.73 ± 0.77^{a}	23.03 ± 0.87^{b}	33.54 ± 0.38^{c}	35.28 ± 0.42^{d}
13:0	0.05 ± 0.02^{a}	0.08 ± 0.01^{b}	0.09 ± 0.00^{b}	0.10 ± 0.01^{b}
14:0	4.02 ± 0.73^{a}	5.81 ± 0.65^{b}	6.45 ± 0.83^{b}	6.70 ± 0.78^{b}
15:0	0.71 ± 0.22^{a}	0.94 ± 0.00^{b}	0.86 ± 0.06^{ab}	0.88 ± 0.00^{ab}
16:0	19.80 ± 0.24^{b}	19.55 ± 0.07^{b}	17.11 ± 0.17^{a}	16.97 ± 0.29^{a}
16:1n-7	8.41 ± 0.39^{b}	7.90 ± 0.21^{b}	6.97 ± 0.45^{a}	6.69 ± 0.68^{a}
16:2n-4	0.45 ± 0.09^{a}	0.56 ± 0.06^{b}	0.46 ± 0.06^{a}	0.43 ± 0.04^{a}
17:0	1.12 ± 0.42	1.27 ± 0.10	1.01 ± 0.15	0.98 ± 0.14

(Continued)

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TABLE 8 Continued

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
16:3n-4	1.12 ± 0.57	1.54 ± 0.09	1.35 ± 0.01	0.66 ± 0.91
18:0	4.33 ± 0.04^{c}	3.64 ± 0.36^{b}	2.63 ± 0.46^{a}	2.44 ± 0.39^{a}
18:1n-9	23.95 ± 0.86^{c}	21.70 ± 0.60^{b}	18.27 ± 0.41^{a}	18.77 ± 0.03^{a}
18:1n-7	2.76 ± 0.42^{c}	1.98 ± 0.11^{b}	1.40 ± 0.16^{a}	1.28 ± 0.13^{a}
18:2n-6	1.46 ± 0.16^{b}	1.20 ± 0.07^{a}	1.06 ± 0.02^{a}	1.22 ± 0.16^{a}
18:3n-6	0.31 ± 0.08^{b}	0.23 ± 0.02^{a}	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}
18:3n-4	n.d.	n.d.	n.d.	n.d.
18:3n-3	0.41 ± 0.30	0.22 ± 0.00	0.17 ± 0.00	0.22 ± 0.04
18:4n-3	0.45 ± 0.08^{a}	0.55 ± 0.07^{b}	0.58 ± 0.01^{b}	0.60 ± 0.06^{b}
20:1n-9	1.06 ± 0.93	0.34 ± 0.01	0.24 ± 0.03	0.22 ± 0.02
20:4n-6	0.76 ± 0.32	0.93 ± 0.10	0.82 ± 0.06	0.82 ± 0.06
20:4n-3	0.06 ± 0.08	0.03 ± 0.05	0.00 ± 0.00	0.06 ± 0.00
20:5n-3	2.82 ± 0.14^{a}	3.27 ± 0.25^{b}	3.06 ± 0.10^{b}	3.11 ± ^{b}
22:1n-11	0.04 ± 0.06	n.d.	n.d.	n.d.
22:1n-9	0.68 ± 0.92	n.d.	n.d.	n.d.
22:4n-6	n.d.	n.d.	n.d.	n.d.
22:5n-6	1.08 ± 0.96^{a}	0.28 ± 0.04^{b}	n.d.^{a}	n.d.^{a}
22:5n-3	0.04 ± 0.06^{a}	0.17 ± 0.04^{c}	0.10 ± 0.00^{b}	0.11 ± 0.02^{b}
22:6n-3	2.26 ± 0.71^{b}	2.37 ± 0.35^{b}	1.72 ± 0.29^{ab}	1.51 ± 0.23^{a}
SFA	48.97 ± 0.51^{a}	54.89 ± 1.21^{b}	62.28 ± 0.51^{c}	64.08 ± 0.53^{d}
MUFA	36.90 ± 1.73^{c}	31.94 ± 0.48^{b}	26.89 ± 0.16^{a}	26.97 ± 0.56^{a}
PUFA	11.22 ± 0.55^{b}	11.33 ± 1.00^{b}	9.55 ± 0.54^{a}	8.95 ± 1.09^{a}
Tot n-3	6.04 ± 0.43^{ab}	6.61 ± 0.76^{b}	5.64 ± 0.39^{a}	5.61 ± 0.24^{a}
Tot n-6	3.61 ± 0.55^{c}	2.63 ± 0.09^{b}	2.11 ± 0.09^{a}	2.26 ± 0.10^{ab}
n-6/n-3	0.60 ± 0.13^{b}	0.40 ± 0.03^{a}	0.37 ± 0.01^{a}	0.40 ± 0.04^{a}
Unknown	2.91 ± 0.68^{d}	1.84 ± 0.26^{c}	1.27 ± 0.20^{b}	n.d.^{a}
DHA/EPA	0.80 ± 0.21^{b}	0.72 ± 0.05^{b}	0.56 ± 0.07^{a}	0.48 ± 0.06^{a}

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). n.d, not detectable.

concentration of eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) (Tables 6, 8, 9). The most remarkable result was obtained for EPA, which reached 4.33% in P26 fed diet A and was undetected in P26 fed the control diet (diet B) (Table 10). Regarding DHA, the highest value was observed in L6 fed diet F, and levels then decreased during the growth phase. Larvae and pre-pupae fed diet A showed an average DHA content equal to 2% in all experiments (Table 9). DHA was not detected in larvae and pre-pupae fed diet B (Table 5). Larvae and pre-pupae fed diets A, F, and T showed higher Tot n-3 content than Tot n-6 and, consequently, the n-6/n-3 ratio was significantly lower in BSF larvae and pre-pupae ($p< 0.05$) than in BSF larvae and pre-pupae fed the control diet (diet B) (Tables 5–10).

Due to the selection of appropriate biomarkers and quality indicators, PCA allowed us to appreciate the differences among samples. PCA was applied to fatty acid classes (Figure 2). Eight variables (12:0, SFA, MUFA, PUFA, Tot n-3, Tot n-6, DHA/EPA, and n-6/n-3) were considered for the analysis; PCA was used to examine 25 cases consisting of the average fatty acid values of the diets and of the BSF larvae and pre-pupae fed different diets. The analysis generated a small number of linear combinations on the eight variables and three principal components with an eigenvalue greater than one were identified. These three components could explain the 95.51% of the variance of the original variables.

PCA was performed considering the first two principal components (PCs) (Figure 2); this analysis explained 79.73% of

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TABLE 9 FA profile (% of total FA) of larvae (L) and pre-pupae (P) of *BSF*, analyzed at 6, 13, 21, and 26 days, fed diet A.

Fatty acid	Larvae 6 days	Larvae 13 days	Prepupae 21 days	Prepupae 26 days
8:0	0.04 ± 0.01^{a}	0.12 ± 0.00^{c}	0.08 ± 0.00^{b}	0.11 ± 0.02^{c}
10:0	0.02 ± 0.00^{a}	0.15 ± 0.07^{a}	0.07 ± 0.00^{a}	0.33 ± 0.14^{b}
11:0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.01
12:0	2.48 ± 0.59^{a}	10.76 ± 0.74^{b}	10.56 ± 0.25^{b}	20.12 ± 1.03^{c}
13:0	0.11 ± 0.02^{a}	0.13 ± 0.00^{b}	0.14 ± 0.00^{c}	0.16 ± 0.01^{d}
14:0	4.68 ± 0.21^{a}	6.66 ± 0.52^{b}	7.22 ± 0.09^{c}	7.66 ± 0.57^{c}
15:0	1.47 ± 0.09^{b}	1.37 ± 0.04^{b}	1.42 ± 0.07^{b}	1.27 ± 0.04^{a}
16:0	28.85 ± 1.72^{c}	26.46 ± 0.72^{b}	27.03 ± 0.85^{b}	23.74 ± 0.89^{a}
16:1n-7	8.03 ± 0.21	8.09 ± 0.36	8.20 ± 0.87	7.81 ± 0.29
16:2n-4	0.61 ± 0.02^{b}	0.53 ± 0.06^{a}	0.52 ± 0.03^{a}	0.50 ± 0.02^{a}
17:0	1.75 ± 0.14^{c}	1.43 ± 0.03^{b}	1.41 ± 0.08^{b}	1.10 ± 0.08^{a}
16:3n-4	1.63 ± 0.00^{b}	1.56 ± 0.05^{b}	1.43 ± 0.11^{b}	0.67 ± 0.94^{a}
18:0	5.06 ± 0.38^{c}	3.86 ± 0.01^{b}	3.77 ± 0.25^{b}	2.65 ± 0.18^{a}
18:1n-9	27.54 ± 0.25^{c}	23.97 ± 0.27^{b}	24.46 ± 0.61^{b}	21.45 ± 0.61^{a}
18:1n-7	2.69 ± 0.13^{d}	2.29 ± 0.12^{c}	2.05 ± 0.09^{b}	1.47 ± 0.06^{a}
18:2n-6	3.58 ± 0.09^{b}	2.26 ± 0.29^{a}	2.45 ± 0.05^{a}	2.36 ± 0.07^{a}
18:3n-6	0.30 ± 0.01^{b}	0.13 ± 0.01^{a}	0.14 ± 0.00^{a}	0.13 ± 0.01^{a}
18:3n-4	n.d.	n.d.	n.d.	n.d.
18:3n-3	0.69 ± 0.01	0.57 ± 0.04	0.64 ± 0.01	0.40 ± 0.44
18:4n-3	0.31 ± 0.44^{a}	0.67 ± 0.06^{b}	0.73 ± 0.08^{b}	0.80 ± 0.01^{b}
20:1n-9	0.42 ± 0.02^{b}	0.40 ± 0.02^{b}	0.37 ± 0.03^{b}	0.25 ± 0.05^{a}
20:4n-6	0.89 ± 0.04^{b}	0.72 ± 0.09^{a}	0.77 ± 0.01^{a}	0.73 ± 0.05^{a}
20:4n-3	0.08 ± 0.02	0.08 ± 0.02	0.09 ± 0.00	0.09 ± 0.01
20:5n-3	4.48 ± 0.22^{b}	3.92 ± 0.40^{a}	4.21 ± 0.29^{ab}	4.33 ± 0.16^{ab}
22:1n-11	0.03 ± 0.04	n.d.	n.d.	n.d.
22:1n-9	0.04 ± 0.06	n.d.	n.d.	n.d.
22:4n-6	n.d.	n.d.	n.d.	n.d.
22:5n-6	0.09 ± 0.12^{ab}	0.14 ± 0.02^{b}	n.d.^{a}	n.d.^{a}
22:5n-3	0.09 ± 0.11	0.14 ± 0.04	0.13 ± 0.01	0.11 ± 0.02
22:6n-3	1.52 ± 2.15	2.39 ± 0.31	2.07 ± 0.01	1.73 ± 0.30
SFA	44.48 ± 1.97^{a}	50.95 ± 0.53^{b}	51.71 ± 1.58^{b}	57.17 ± 0.59^{c}
MUFA	38.75 ± 0.67^{c}	34.75 ± 0.77^{b}	35.08 ± 1.36^{b}	30.98 ± 0.44^{a}
PUFA	14.27 ± 3.00	13.10 ± 1.24	13.21 ± 0.22	11.85 ± 0.15
Tot n-3	7.18 ± 2.94	7.77 ± 0.83	7.87 ± 0.36	7.46 ± 0.94
Tot n-6	4.86 ± 0.08^{b}	3.25 ± 0.41^{a}	3.39 ± 0.00^{a}	3.22 ± 0.13^{a}
n-6/n-3	0.74 ± 0.29^{b}	0.42 ± 0.01^{a}	0.43 ± 0.02^{a}	0.43 ± 0.04^{a}
Unknown	2.50 ± 0.36^{c}	1.20 ± 0.06^{b}	n.d.^{a}	n.d.^{a}
DHA/EPA	0.33 ± 0.46	0.61 ± 0.02	0.49 ± 0.04	0.40 ± 0.06

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). n.d, not detectable.

TABLE 10 FA profile (% of total FA) of pre-pupae analyzed at the end of the experiment (P26).

Fatty acid	B	F	R	T	A
8:0	0.14 ± 0.00	0.14 ± 0.03	0.16 ± 0.05	0.14 ± 0.02	0.11 ± 0.02
10:0	0.89 ± 0.08^{c}	0.59 ± 0.04^{b}	0.54 ± 0.21^{b}	0.56 ± 0.11^{b}	0.33 ± 0.14^{a}
11:0	0.02 ± 0.00	0.03 ± 0.02	0.02 ± 0.02	0.03 ± 0.01	0.02 ± 0.01
12:0	46.62 ± 0.19^{e}	38.23 ± 0.75^{d}	24.12 ± 0.86^{b}	35.28 ± 0.42^{c}	20.11 ± 1.01^{a}
13:0	0.06 ± 0.00^{a}	0.07 ± 0.03^{a}	0.07 ± 0.04^{a}	0.10 ± 0.01^{a}	0.15 ± 0.01^{b}
14:0	7.89 ± 0.03^{c}	6.91 ± 0.06^{b}	5.22 ± 0.40^{a}	6.70 ± 0.78^{b}	7.66 ± 0.56^{c}
15:0	0.38 ± 0.00^{a}	0.38 ± 0.12^{a}	0.37 ± 0.17^{a}	0.88 ± 0.00^{b}	1.27 ± 0.05^{c}
16:0	11.78 ± 0.09^{a}	14.55 ± 1.02^{c}	12.89 ± 0.84^{b}	16.97 ± 0.29^{d}	23.73 ± 0.92^{e}
16:1n-7	3.91 ± 0.15^{d}	5.63 ± 0.46^{a}	4.90 ± 0.96^{a}	6.69 ± 0.68^{b}	7.81 ± 0.28^{c}
16:2n-4	0.05 ± 0.00^{a}	0.24 ± 0.08^{b}	0.21 ± 0.05^{b}	0.43 ± 0.04^{c}	0.50 ± 0.02^{d}
17:0	0.29 ± 0.00^{a}	0.40 ± 0.13^{a}	0.37 ± 0.13^{a}	0.98 ± 0.14^{b}	1.10 ± 0.08^{b}
16:3n-4	0.25 ± 0.00	0.55 ± 0.27	0.52 ± 0.32	0.66 ± 0.91	0.66 ± 0.94
18:0	1.12 ± 0.02^{a}	2.02 ± 0.05^{b}	1.83 ± 0.13^{b}	2.44 ± 0.39^{c}	2.65 ± 0.18^{c}
18:1n-9	9.05 ± 0.04^{a}	19.95 ± 0.38^{c}	25.75 ± 0.47^{e}	18.77 ± 0.03^{b}	21.43 ± 0.63^{d}
18:1n-7	0.69 ± 0.06^{a}	1.39 ± 0.19^{b}	1.30 ± 0.02^{b}	1.28 ± 0.13^{b}	1.47 ± 0.06^{b}
18:2n-6	12.10 ± 0.05^{d}	1.07 ± 0.08^{a}	14.30 ± 0.50^{c}	1.22 ± 0.16^{a}	2.36 ± 0.07^{b}
18:3n-6	0.03 ± 0.00^{a}	0.19 ± 0.15^{b}	0.17 ± 0.12^{b}	0.22 ± 0.01^{b}	0.13 ± 0.01^{b}
18:3n-4	n.d.	0.05 ± 0.04	n.d.	n.d.	n.d.
18:3n-3	0.84 ± 0.00^{b}	0.44 ± 0.12^{a}	2.05 ± 0.21^{c}	0.22 ± 0.04^{a}	0.40 ± 0.44^{a}
18:4n-3	0.12 ± 0.00^{a}	0.59 ± 0.05^{b}	0.53 ± 0.15^{b}	0.60 ± 0.06^{b}	0.80 ± 0.01^{c}
20:1n-9	0.52 ± 0.00^{ab}	0.83 ± 0.53^{b}	0.41 ± 0.02^{a}	0.22 ± 0.02^{a}	0.25 ± 0.05^{a}
20:4n-6	0.06 ± 0.00^{a}	0.39 ± 0.21^{b}	0.33 ± 0.21^{b}	0.82 ± 0.06^{c}	0.73 ± 0.05^{c}
20:4n-3	n.d.^{a}	0.10 ± 0.01^{c}	0.09 ± 0.01^{c}	0.06 ± 0.00^{b}	0.09 ± 0.01^{c}
20:5n-3	0.21 ± 0.00^{a}	3.03 ± 0.99^{c}	2.06 ± 0.33^{b}	3.11 ± 0.09^{c}	4.33 ± 0.16^{d}
22:1n-11	n.d.	n.d.	n.d.	n.d.	n.d.
22:1n-9	n.d.	n.d.	n.d.	n.d.	n.d.
22:4n-6	n.d.	n.d.	n.d.	n.d.	n.d.
22:5n-6	n.d.	n.d.	n.d.	n.d.	n.d.
22:5n-3	n.d.	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}	0.11 ± 0.02^{a}	0.11 ± 0.02^{a}
22:6n-3	n.d.	1.21 ± 0.47^{ab}	0.93 ± 0.74^{a}	1.51 ± 0.23^{ab}	1.73 ± 0.30^{b}
SFA	69.18 ± 0.08^{d}	63.31 ± 1.50^{c}	45.59 ± 0.53^{a}	64.08 ± 0.53^{c}	57.17 ± 0.59^{b}
MUFA	14.16 ± 0.12^{a}	27.80 ± 0.64^{c}	32.39 ± 0.41^{e}	26.97 ± 0.56^{b}	30.98 ± 0.44^{d}
PUFA	13.67 ± 0.06^{c}	8.00 ± 1.77^{a}	21.36 ± 0.95^{d}	8.95 ± 1.09^{a}	11.85 ± 0.15^{c}
Tot n-3	1.17 ± 0.01^{a}	5.51 ± 1.40^{b}	5.82 ± 1.00^{b}	5.61 ± 0.24^{b}	7.46 ± 0.94^{c}
Tot n-6	12.20 ± 0.05^{d}	1.65 ± 0.02^{a}	14.80 ± 0.41^{e}	2.26 ± 0.10^{b}	3.22 ± 0.13^{c}
n-6/n-3	10.47 ± 0.00^{c}	0.31 ± 0.08^{a}	2.59 ± 0.52^{b}	0.40 ± 0.04^{a}	0.43 ± 0.04^{a}
Unknown	2.99 ± 0.26^{c}	0.89 ± 0.37^{b}	0.66 ± 0.83^{ab}	n.d.^{a}	n.d.^{a}
DHA/EPA	n.d.	0.39 ± 0.03	0.43 ± 0.29	0.48 ± 0.06	0.40 ± 0.06

Each value represents the mean of three independent determinations. Different letters in the same row indicate significant differences ($p< 0.05$). B: wheat bran; F: by-catch from sea trawling fishery; S: *Parapenaeus longirostris* processing by-products; R: aquaculture processing by-products; T: *Thunnus albacares* processing by-products; A: *Engraulis encrasicolus* processing by-products. n.d, not detectable.

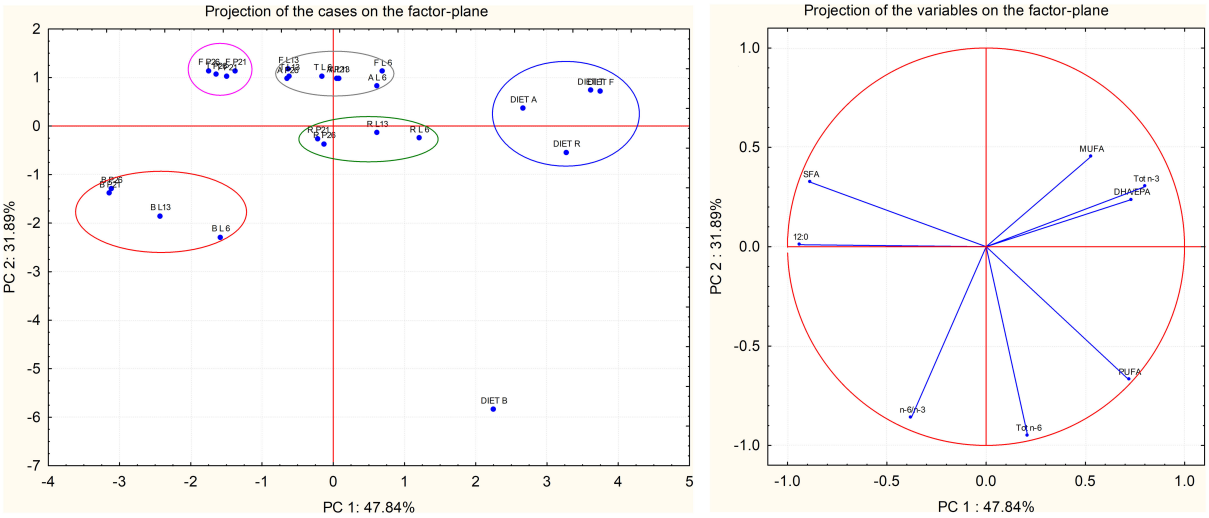

FIGURE 2
Principal component analysis (PCA) obtained from correlation of eight components (12:0, SFA, MUFA, PUFA, Tot n-3, Tot n-6, DHA/EPA, and n6/n-3) and 25 cases determined on larvae and pre-pupae fed with different diets. **(A)** Correlation circle; **(B)** factor-plan. Red circle: larvae and pre-pupae fed diet B; blue circle: experimental diets; green circle: larvae and pre-pupae fed diet R; purple circle: pre-pupae fed diet F and T; gray circle: larvae and pre-pupae fed diet A and larvae fed diets F and T.

the variability in the original data (Figure 2). The first principal component (PC1) explained 47.84% of the combined variance and the second component (PC2) explained 31.89% (Figure 2).

The correlation circle (Figure 2A) showed the correlation of the eight variables in the experimental cases. In particular, a positive correlation was observed between 12:0 and SFA; in fact, 12:0 was the predominant fatty acid in the SFA class. Figure 2B shows the factorial plane of the samples, identifying distinct groups that characterize the different larvae and pre-pupae fed different diets.

It is can be seen that larvae fed diet B were spatially distanced from the other samples (Figure 2B). As shown in the correlation circle (Figure 2A), these samples were mainly influenced by 12:0, which showed the highest values in P26 fed diet B (Table 10).

PCA allowed to discriminate how larvae are influenced by their diet (Figure 2B) since the beginning of feeding. The larvae and pre-pupae fed diet R are grouped together, distinguishing them from larvae and pre-pupae fed other diets; this is influenced by MUFA, as can be observed from the circle of correlations (Figure 2A).

4 Discussion

This study investigated the effects of different fish by-products, characterized by a wide variability in chemical composition, on the development and nutritional composition of BSF larvae and pre-pupae at the laboratory scale. The use of bioconverting insects, such as BSF, allows for the use and valorization of by-products to produce larvae enriched with protein and fat. The obtained results, although not derived from an industrial production process, provide an important insight into the potential sustainable uses for BSF in aquaculture. The use of marine by-products as feed for BSF larvae supports a circular system, enabling the achievement of "zero-waste" goals.

4.1 Effects of the experimental diets on BSF larvae development

Larvae fed the different experimental diets (Figure 1) showed a lower growth rate than larvae fed wheat bran (diet B). As reported by other authors (Grossule et al., 2019; Grossule and Lavagnolo, 2020), these results could reflect the different availability of carbon and degradable nutrients in the feeding substrates employed, thus confirming the predominant role of bran as a source of carbon and nutrients (Grossule et al., 2019; Grossule and Lavagnolo, 2020). Larvae fed diet S died after few days of the trial, probably due to the high level of chitin, which has negative effects on growth, food efficiency, and nutrient (particularly protein) digestibility (Shiau and Yu, 1999; Olsen et al., 2006; Marono et al., 2015; Guerreiro et al., 2020), or due to the lowest energy content of diet S among the diets (Starcevic et al., 2019) (Table 2).

4.2 Proximate composition of the pre-pupae

Recently, interest in the utilization of BSF larvae as a raw material for animal feed has increased, mainly due to its potential as a sustainable source of high-quality protein (Kroeckel et al., 2012; Veldkamp et al., 2012; Liland et al., 2017)

BSF is a scavenger, commonly used to accelerate the composting of organic material, as it can efficiently utilize organic resources, such as fruit, vegetable, and meat waste, (Čičková et al., 2015; Surendra et al., 2016) and, depending on the growing medium, can modulate its own body composition, reaching a high concentration of lipids [(>30% of dry weight (dw)] and proteins (around 40% of dw) (St-Hilaire et al., 2007; Diener et al., 2009).

As reported by Cammack and Tomberlin (2017) diet does not have a significant impact on adult life-history traits; in fact, BSF feeds only in the larval stage. Dietary composition affects larval growth and performance (Nairuti et al., 2022).

The employed by-products in this study demonstrated an influence on the nutrient profiles of the BSF pre-pupae. Obtained results showed the highest lipid content in pre-pupae fed diet R (28.05 ± 1.90 g/100 g dw), which showed the highest lipid content (27.97 ± 0.15 g/100 g wet) of the experimental diets. The lipid content in insects is largely dependent on their diets and stage of development (Stanley-Samuelson and Dadd, 1983) and it is affected by the composition of the rearing substrate (Franco et al., 2021). In fact, the lipid content can vary by 15–49% of the total dry weight (Franco et al., 2021). This confirms that rearing BSF pre-pupae on substrates with a high lipid content resulted in higher lipid and lower protein contents in BSF pre-pupae, as reported by Nairuti et al. (2022).

The pre-pupae fed with experimental diets showed a protein content ranging from 34.29 to 34.94 g/100 g. The highest protein content was observed in pre-pupae fed diet B (P26-B, 39.93 g/100 g), in accordance with the values reported in literature (31.9%–46.3%) (Diener et al., 2009; Barroso et al., 2014; Sánchez-Muros et al., 2014; Surendra et al., 2016).

The varying proximate composition of BSF fed with various diets confirms the entirely generalist nature of this insect and therefore its plasticity in the use of food waste of varying origins and compositions, and confirms its ability to skillfully convert this food waste into nutrients (Cammack and Tomberlin, 2017). Although BSF exhibits high plasticity, some substrates appear to perform less well in the growth processes of reared larvae, as observed in larvae reared on diet S (Shiau and Yu, 1999; Olsen et al., 2006; Marono et al., 2015; Guerreiro et al., 2020). Numerous studies are in progress to define which substrates are most suitable for the optimal growth of this species in qualitative and quantitative terms (Ribeiro et al., 2022).

Our results indicate the high potential of insects as alternative sources of new and renewable animal proteins and fat (Finke, 2002; Gobbi et al., 2013; Rumpold and Schlüter, 2013; Sánchez-Muros et al., 2014; Zielińska et al., 2015).

4.3 FA profile

BSF larvae have been reared mainly for their protein content (Halloran et al., 2016; Nogales-Mérida et al., 2019) and only recently have attracted interest for their lipid content (Spranghers et al., 2017; Barroso et al., 2019; Smetana et al., 2019; Rodrigues et al., 2022; Hossain et al., 2023).

Our results show that the FA profile of the different provided diets influenced the FA profiles of the BSF larvae and partially affected the fatty acid profile of the pre-pupae, as shown in the PCA analysis (Figure 2), which is in agreement with Barroso et al. (2019) and Spranghers et al. (2017). In the pre-pupae the main fatty acid was C12:0, even if this fatty acid was contained in the substrates only in trace amounts. Obtained results, in accordance with Barroso et al. (2019), highlighted the higher C12:0 content in pre-pupae fed the control diet than in pre-pupae fed with fish by-product substrates; indeed, several studies show that larvae fed on substrates containing cereals, fruit, and vegetables show the lauric acid highest content (Rodrigues et al., 2022). High lauric acid content could be due to the bioconversion of carbohydrates to lipids (Guil-Guerrero et al., 2018). Lauric acid represents the main lipid component of BSF and it is converted by animals and humans into monolaurin (antiviral, antibacterial, and antiprotozoal glyceride) (Leong et al., 2015; Ushakova et al., 2016). A high amount of lauric acid enables the larvae to survive in substrates with temperatures above 40°C, as lauric acid gives the larvae a solid consistency that is not subject to the rapid oxidation of fats (the melting point of lauric acid is 43.2°C) (Ushakova et al., 2016).

BSF pre-pupae fed different fish by-products showed higher contents of n-3 fatty acids than the control, in agreement with recent studies (Barroso et al., 2019; Ewald et al., 2020; Rodrigues et al., 2022). In particular, pre-pupae fed diet A had the highest levels of EPA, followed by pre-pupae fed diets T, F, and R (Table 10). Regarding DHA, the pre-pupae accumulated a maximum of 1.73% DHA (P26 diet A), whereas DHA constituting 17.54% of the total FA content was detected in diet A (Table 10). In addition, the n-6/n-3 values decreased in the larvae and pre-pupae fed diets R, A, T, and F (Tables 5–10) when compared with control, as was also reported by Barroso et al. (2019).

Since the essential fatty acids EPA and DHA, which belong to marine environments, are not found in BSF (Barroso et al., 2019), our results, which show an increase of EPA and DHA in BSF larvae and pre-pupae fed fish by-products, confirm that marine sources, in particular fish by-products, are capable of enriching BSF with these fatty acids (St-Hilaire et al., 2007; Sealey et al., 2011; Barroso et al., 2019; Ewald et al., 2020; Rodrigues et al., 2022).

5 Conclusions

The aim of this study was to investigate the effects of different fishery and aquaculture by-product diets on the growth, body composition, and fatty acid profile of BSF larvae and pre-pupae, with the aim of converting organic waste into high-value biomass as part of a "zero-waste" approach. Our results show that *E. encrasicolus* processing by-products, by-catch from a sea trawling fishery, *T. albacares* processing by-products, and aquaculture processing by-products could be interesting feeding substrates for the rearing of *H. illucens*. Using these by-products, it was possible to modulate the lipid and fatty acid profile of BSF larvae, increasing their level of n-3 fatty acids. This possibility represents added value to BSF as an organism capable of modulating its lipid and fatty acid composition for its utilization as an alternative source of ingredients for aquaculture feeds.

Furthermore, the utilization of these by-products could support circular economy pathways, contributing to the reduction of food losses and waste.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

MC and CM contributed to the conception and design of the study. CB and DM provided resources. MC performed the feeding experiment. RA, SM, and EC performed the analyses. RA and SM wrote the first draft of the manuscript. CM and AS revised, and read the submitted version. RA and SM contributed equally to the work. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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