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Original Research Article

Production of n-3-rich insects by bioaccumulation of fishery waste

Fernando G. Barroso[a,*], María José Sánchez-Muros[a], Miguel Ángel Rincón[b], María Rodriguez-Rodriguez[c], Dmitri Fabrikov[c], Elvira Morote[d], José Luis Guil-Guerrero[e]

[a] Department of Biology and Geology, CECOUAL, University of Almería, Almería, Spain
[b] Lipid Center, Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile
[c] Department of Biology and Geology, University of Almería, Almería, Spain
[d] Fishery Producers Organization of Almeria (OPP71), Spain
[e] Food Technology Division, University of Almería, Almería, Spain

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ABSTRACT

Black soldier fly (BSF) larvae (*Hermetia illucens*) might be an advantageous option for recycling fish waste for obtaining n-3-fatty acid-rich foods. To investigate the effects of consuming fish waste (an n-3-fatty acid-rich by-product) on the fatty acid (FA) profiles of BSF, larvae were assigned to experimental feeding systems according to the time fish waste was eaten before slaughtering: BSFc-control (without eating fish) and BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d (1, 2, 4, 6, 8, 10 and 12 days eating fish, respectively). The percentage of n-3 polyunsaturated fatty acids (PUFAs) increases significantly ($P < 0.001$) from 4.32 in BSFc to 14.8% in BSF12d. Larval biomass was notably enriched in both eicosapentaenoic (EPA) (up to 7.2%) ($P < 0.001$) and docosahexaenoic (DHA) (up to 4.9%) ($P < 0.001$) fatty acids, while the consumption time of fish waste increased, and the n-6:n-3 ratio and both the atherogenicity (AI) and thrombogenicity (TI) indices were reduced ($P < 0.001$). The maximum percentage of EPA + DHA (12.2% of total fatty acids) ($P < 0.001$) was obtained at 12 days. The recommended daily intake of both n-3 PUFAs for humans could be satisfied with 150 g of a 12-day-feed larval meal.

1. Introduction

The human population faces the challenge of continuing to grow and meet its food requirements. Future food systems must take into account not only production but also their environmental impact (Lang and Barling, 2013). Therefore, it is essential to obtain more sustainable protein. Insects are being evaluated as food for human beings through either direct consumption of the insects or their meal (entomophagy) or indirect use the insects as feed for livestock and/or aquaculture. Insect production is a sustainable alternative to livestock production for the following reasons: high feed conversion efficiency, lower land and water area requirements, lower greenhouse gas and ammonia emissions, and exceptional adaptation to be fed with food by-products (Oonincx et al., 2010; Oonincx and de Boer, 2012; van Huis et al., 2013).

Knowledge about this "novel" food resource, which has been used by humans for thousands of years, is only recently expanding. Consistently, a wide variety of variables need to be investigated before optimising insect production and intake.

However, food waste has negative environmental and socio-economic impacts (Papargyropoulou et al., 2014). It is estimated that at least one-third of the food produced worldwide is wasted and that existing waste management systems are costly, and even environmentally damaging (Surendra et al., 2016). Of all waste, organic waste is the most complex to manage because of its volume and fast degradability (Yin et al., 2014). As a type of organic waste, "fish waste" comprises caught fish that are of no commercial value because they are damaged, small, or simply commercially worthless (Caruso, 2016). The most worrying dimension of this problem is that every year, the waste from fisheries worldwide exceeds 20 million tons, the equivalent of 25% of the total production of marine fishery catches (Kim and Mendis, 2006; Rustad et al., 2011). Such an amount includes “non-target” species, fish processing waste and by-products. According to Mahro and Timm (2007), approximately 5.2 million tons of fish waste are discarded every year in the EU. Unlike other organic waste, fish waste contains significant amounts of n-3 polyunsatturated fatty acids (PUFAs). Therefore, one optimal strategy could be to use fish waste as a substrate for rearing insects that would be used as human food.

In terms of food quality, the composition of fatty acid (FA) plays a fundamental role, as it determines both the nutritional value and

* Corresponding author at: Department of Biology and Geology, CECOUAL, E.S.I. University of Almería, Carretera de Sacramento s/n. 04120, Almería, Spain.
E-mail address: fbarroso@ual.es (F.G. Barroso).

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organoleptic characteristics of fat, while there is evidence that there are FA tasters and nontasters, which could have implications for targeted product development (Tucker and Mattes, 2012). Risk factors for cardiovascular disease, coronary heart disease and cancer can be modified by diet (Willett, 2012). A considerable body of evidence suggests that diets with a high content of saturated fatty acids (SFAs) promote cardiovascular disease and carcinogenesis (de Lorgeril and Salen, 2012). In contrast, foods rich in long-chain PUFAs (LC-PUFAs), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, are fundamental for a healthy life as they are beneficial in preventing diabetes, cancer, coronary heart disease, hypertension, arthritis and other diseases (Gebauer et al., 2006: Lee et al., 2008; Sanhueza et al., 2004; Simopoulos, 2009; Uauy and Valenzuela, 2000).

Black soldier fly (BSF) larvae (*Hermetia illucens*) are one of the most promising candidates of insect species to recycle fish waste. BSF is a detritivorous Diptera species and, as such, is able to transform food waste into high-quality fat and protein-rich foods. Due to its widespread distribution and adaptability to farming (Sheppard et al., 2002), substantial global interest has been shown in the mass production of BSF to obtain protein (Tomberlin et al., 2015). According to Makkar et al. (2014), BSF larvae may consume between 25 mg and 500 mg per day depending on larval development and the environment. Under ideal conditions (food supply, temperature and humidity), BSF larvae can develop into prepupae within 2 weeks, but developmental timing depends heavily on farming conditions (Salomone et al., 2017).

The FA composition of BSF larvae is characterised by the high percentage of lauric acid (LaA, 12:0), which reaches approximately half of the total FA, as well as palmitic (PA, 16:0), myristic (MA, 14:0), oleic (OA, 18:1n9), linoleic (LA, 18:2n6), and α-linolenic (ALA, 18:3n3) acids, with ALA usually in percentages less than 10% of the total FA. Unfortunately, the available data suggest that BSF larvae contain negligible amounts of LC-PUFAs, such as DHA and EPA, which are mainly ingested from marine food sources (Ramos-Bueno et al., 2016; Barroso et al., 2017; Liland et al., 2017; Guil-Guerrero et al., 2018). However, the BSF larvae composition has been modulated by feeding seaweed-enriched media or fish oil waste, leading to an accumulation of EPA and DHA and a nutrient profile more suited for specific feed or food purpose (St-Hilaire et al., 2007; Liland et al., 2017; Barroso et al., 2017).

As pointed out by Salomone et al. (2017), the process of bioconversion through BSF is a very attractive option, as it can potentially solve two problems, namely, organic waste management and global food demand, without competing with food crops for land use. Moreover, improving the percentages of healthy PUFAs in BSF larvae while reusing fish waste is an attractive option for obtaining n-3 functional foods. Thus the aim of this research was to evaluate the FA profiles of BSF larvae reared with fish waste over different time periods.

2. Material and methods

2.1. Experimental design

Hermetia illucens larvae were reared at the ENTOSUR S.L. company. The experiment was performed in an acclimatised environment at 26 ± 1 °C and 65 ± 5% humidity. Commercial feed for broiler (BIONA®, Spain) was used as the control feed (30% feed and 70% distilled water w/v). All larvae were fed the control diet for 3 days. After 3 days of growth, the larvae were divided into groups of 5000 larvae per treatment and separated into three boxes per treatment. Treatments were administered by completely replacing the control feed by discarded fish at different times before slaughter: BSFc-control (without eating fish) and BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d (1, 2, 4, 6, 8, 10 and 12 days eating fish, respectively). The experimental design is described in Fig. 1.

Discarded round sardinella (*Sardinella aurita*) was used to feed the BSF larvae fish waste. This species is of little gastronomic value because it is too large and contains fine bones. The fish was cut into slices of 2 cm (approximately) and offered *ad libitum* to the larvae.

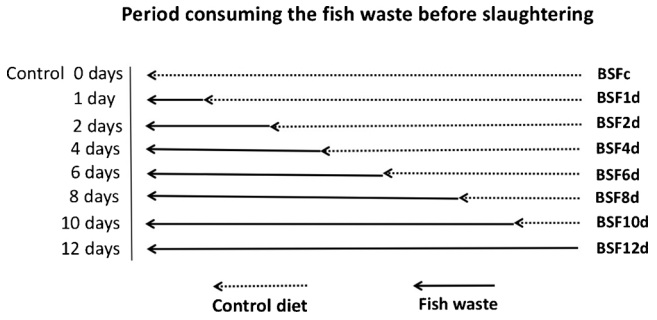

Fig. 1. Experimental design.
(BSF: black soldier fly; BSFc: control without eating fish, BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d: 1, 2, 4, 6, 8, 10 and 12 days eating fish, respectively)

Table 1
Proximate composition (% dry matter) and fatty acid composition (g/100 g total fatty acids) of control diet and fish discard.

	Control diet	Fish discard
Proximate composition		
Ash	7.6	6.47
Crude Protein	22.8	72.7
Ether Extract	5.0	18.6
Fatty acids		
12:0	nd	1.7
14:0	nd	8.2
16:0	15.2	14.5
16:1n7	nd	7.3
17:0	nd	1.1
17:01	nd	1.0
18:0	3.2	5.9
18:1n9	23.8	8.0
18:1n7	0.9	3.8
18:2n6	52.5	1.6
18:3n3	4.5	1.1
18:4n3	nd	2.1
20:1n9	nd	4.0
20:5n3	nd	13.6
22:1n11	nd	2.4
22:5n3	nd	1.7
22:6n3	nd	21.4
24:1n9	nd	1.1
SFA	18.3	31.3
MUFA	24.7	27.7
PUFA	57.0	41.5

SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. nd: not detected (Limit of Detection (LOD) ≈ 0.02 mg/100 g FW).

The ingredients of the control diet were corn, soya, soya oil, monocalcium phosphate, calcium carbonate and sodium chloride. The control diet and fish discard proximate composition and fatty acid profile are shown in Table 1.

2.2. Samples

Hermetia samples were collected before the pre-pupal stage (on approximately day 15). Larvae were frozen for slaughter and stored at -20 °C before being freeze-dried in a freeze-dryer (Cryodos, Ima-Telstar, Terrassa, Spain) and then ground and frozen until analysed. Diets and fish waste were also freeze-dried and ground.

2.3. Oil extraction and transesterification

Simultaneous oil extraction and transesterification were performed according to previous works (Lepage and Roy, 1984; Rodríguez-Ruiz

et al., 1998).

2.4. FA analyses

FA determination in larvae was carried out after direct derivatization to FA methyl esters (FAMEs). For this, 50 mg of freeze-dried larvae was accurately weighed and placed in test tubes and then 0.5 mg of internal standard (nonadecanoic acid, 19:0), 2 mL of a methylating mixture (methanol:acetyl chloride 20:1 v/v) and 1 mL of n-hexane were added. The tubes were then capped and heated at 100 °C for 30 min. After the tubes were cooled to room temperature, 1 mL of distilled water was added to each one, and then centrifuged (2000 x g, 5 min). The hexane layer was collected for GC-FID analysis. FAMEs were analysed in a Focus GC (Thermo Electron. Cambridge. UK) equipped with a flame ionisation detector (FID) and an Omegawax 250 capillary column (30 m x0.25 mm i.d. x 0.25 µm film thickness; Supelco. Bellefonte. USA), as previously described (Guil-Guerrero et al., 2014). The peak area of the internal standard was used as a reference to calculate the mass of each FA in the resulting chromatograms, and the results were computed as g/100 *g* dry weight meal (Table 3). The relative retention factors for each FA as reported by (Cladis et al., 2014) were considered for quantification.

2.5. Quality control

The quality control of FA analyses was performed according to previous protocols (Guil-Guerrero et al., 2013; Griffiths et al., 2010). To determine the limits of detection (LOD) and quantification (LOQ) of the assays, pure EPA and DHA were diluted to 0.001–20 mg mL^{-1} in toluene in triplicate, transmethylated and quantified by GC-FID. Negative controls made without EPA or DHA were also analysed. The LOD was defined as the minimum concentration at which distinct peaks were detected above the baseline noise. The LOQ was defined as the lowest concentration of FA that could be quantified with an accuracy and precision within 15%. The estimated LODs for EPA and DHA were in the 0.4-0.5 µg mL^{-1} range, while LOQs for such molecules were in the 2.0–3.1 µg mL^{-1} range. The recovery was calculated by the formula [$C_{(Exp)}/C_{(Theo)}$ x 100]. Recoveries for EPA were in the 95.8–100% range, those for DHA were in the 99.3–100% range. As a GC quality control, a blank sample (hexane) was run together with the samples in every batch. Control oil samples were analyzed prior and after running samples. Canola oil (46961 SUPELCO, from SIGMA) was used for the control tests. The recoveries for certified FA were between 94.6 (OA) and 103% (PA); and the relative standard deviations were less than 5.8%. As GC quality control, a blank 201 sample (hexane) was run together with the samples in every batch.

2.6. Indices of lipid nutritional quality

In addition to the FA profile of the samples, the nutritional quality of the lipid fraction was assessed by considering the following: the PUFA n-6/PUFA n-3 ratio, and the atherogenicity (AI) and thrombogenicity (TI) indices. The following calculations were performed:

a) Atherogenicity index (Ulbricht and Southgate, 1991).

AI = [12:0 + (4 × 14:0) + 16:0]/(Σn-3 PUFA + Σn-6 PUFA + ΣMUFA)

b) Thrombogenicity index (Ulbricht and Southgate, 1991).

TI = (14:0 + 16:0 + 18:0)/[(0.5 × ΣMUFA) + (0.5 × Σn-6 PUFA) + (3 × Σn-3 PUFA) + (Σn-3 PUFA/Σn-6 PUFA)].

2.7. Proximate composition

The nutritional profile of BSF and fish waste was characterised according to the standard procedures of the Association of Official Analytical Chemists (AOAC, 2005), with specific methods as follows: the crude protein (CP) content was determined by Kjeldahl * 625 (AOAC, 2005; #954.01) (Nx6.25), crude Fat (EE) was determined by ethyl ether extraction (Soxhlet technique) (AOAC, 2005; #920.39), the moisture was determined gravimetrically by drying at 105 ± 0.5 °C (AOAC, 2005; #934.01), and ash was determined gravimetrically after combustion at 500 °C in a muffle furnace (AOAC, 2005; #942.05) to a constant weight. All the analyses were performed in triplicate.

2.8. Statistical analysis

Because the data were not adjusted to the general linear model (ANOVA) requirements such as the normally distributed and homogeneity of variance, generalised linear models (GZLMs) were used to examine the functional relationships between proximate composition and FA data obtained from the larvae (response variable) and the different times of fish discard consumption (explanatory variables). The models were fitted by a maximum quasi-likelihood estimation with the GenLin procedure with gamma errors and the Logarithm link function using the IBM SPSS version 25.0 statistical software package (IBM, 2015). In each trial, the significance of the model was assessed by an Omnibus test (to test whether the explained variance in a data set is significantly greater than the unexplained variance). For each regression effect specified in the model, a Wald statistic (Wald chi^2) was conducted, which is a test based on linearly independent pairwise comparisons among the estimated marginal means. The mean values were then compared pairwise, with significance indicated at P = 0.05 using the same statistical software (IBM, 2015).

3. Results

3.1. Proximate composition

Table 2 summarises the gross compositions of larvae. There was a significant reduction in ash with fish intake (Omnibus test, likelihood ratio chi^2 = 31.4, d.f. = 7; $P < 0.001$) decreasing from 7.91 BSFc to 5.65 for BSF12d. CP sharply decreased in BSF6d, and the values recovered in BFS8d; the CP in BSF10d and BSF12d slightly decreased for BSF8d (likelihood ratio chi^2 = 51.2, d.f. = 7; $P < 0.001$). Crude fat appears to be significantly lower for larvae that eat fish for fewer days (BSF1d and BSF2d) (likelihood ratio chi^2 = 33.7, d.f. = 7; $P < 0.001$), and it appears to recover for larvae that eat fish at least 4 days or more, without differences compared to BSFc.

3.2. FA composition

The FA profiles of the BSF larvae that ate fish waste for different times before slaughter are shown in Table 3.

The larvae fed on fish waste reflected the FA composition of fish by showing large differences with the control-fed reared larvae. The FA

Table 2
Proximate analysis (% dry matter) of *Hermetia illucens* (BSF) larvae. Larvae were fed on discarding fish throughout different time before slaughter.

	Ash	Crude protein	Ether extract
BSFc	7.91 ± 0.30 ^{a}	75.6 ± 1.57 ^{a}	15.4 ± 0.95 ^{abc}
BSF1d	7.66 ± 0.30 ^{a}	77.4 ± 1.60 ^{a}	8.46 ± 0.52 ^{e}
BSF2d	6.73 ± 0.26 ^{b}	78.8 ± 1.63 ^{a}	11.5 ± 0.71 ^{d}
BSF4d	6.21 ± 0.24 ^{bc}	75.4 ± 1.56 ^{ab}	15.5 ± 0.96 ^{abc}
BSF6d	5.47 ± 0.21 ^{d}	50.6 ± 1.05 ^{d}	18.0 ± 1.11 ^{ab}
BSF8d	5.66 ± 0.22 ^{cd}	71.3 ± 1.48 ^{b}	14.9 ± 0.92 ^{c}
BSF10d	5.89 ± 0.23 ^{cd}	61.5 ± 1.27 ^{c}	17.2 ± 1.06 ^{abc}
BSF12d	5.65 ± 0.22 ^{cd}	61.8 ± 1.28 ^{c}	18.2 ± 1.22 ^{a}

Means (± SE) from 3 individual measurements, the same letter within a row are not significantly different from one another (chi^2 of Wald, $P < 0.05$). BSF: black soldier fly; BSFc: control without eating fish; BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d: 1, 2, 4, 6, 8, 10 and 12 days eating fish, respectively.

Table 3
Effect of feeding on discarding fish throughout different time in fatty acid composition (g/100 g total fatty acids) of *Hermetia illucens* (BSF) larvae.

Fatty acid	BSFc	BSF1d	BSF2d	BSF4d	BSF6d	BSF8d	BSF10d	BSF12d
10:0	1.5 ± 0.1^{a}	0.6 ± 0.0^{c}	0.6 ± 0.0^{c}	0.8 ± 0.1^{b}	0.4 ± 0.0^{e}	0.6 ± 0.0^{c}	0.8 ± 0.0 ^{b}	0.5 ± 0.0 ^{d}
12:0	47.1 ± 2.6^{a}	26.7 ± 1.5^{de}	30.6 ± 1.7^{cd}	31.9 ± 1.8^{c}	20.7 ± 1.1^{f}	30.1 ± 1.2^{cd}	38.5 ± 2.1^{b}	24.1 ± 1.3^{ef}
14:0	8.5 ± 0.3^{a}	6.1 ± 0.2^{b}	8.1 ± 0.2^{a}	8.4 ± 0.3^{a}	8.1 ± 0.2^{a}	8.1 ± 0.2^{a}	8.0 ± 0.2^{a}	8.1 ± 0.2^{a}
15:0	0.4 ± 0.0^{ab}	nd	0.3 ± 0.0^{c}	nd	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	nd	0.4 ± 0.0^{b}
16:0	13.1 ± 0.4^{e}	17.8 ± 0.6^{bc}	18.1 ± 0.6^{bc}	16.8 ± 0,5^{c}	21.6 ± 0.7^{a}	18.2 ± 0.4^{b}	14.9 ± 0,5^{d}	19.3 ± 0.6^{b}
16:1n7	3.4 ± 0.2^{d}	2.3 ± 0.1^{e}	7.0 ± 0.3^{c}	8.5 ± 0,4^{ab}	9.4 ± 0.4^{a}	7.5 ± 0.2^{bc}	8.0 ± 0.4^{b}	8.2 ± 0.4^{b}
18:0	2.7 ± 0.2^{e}	6.0 ± 0.5^{a}	4.3 ± 0.4^{bc}	3.9 ± 0.3^{cd}	4.9 ± 0.4^{b}	3.9 ± 0.2^{cd}	3.4 ± 0.2^{d}	4.5 ± 0.4^{bc}
18:1n9	10.3 ± 0.7^{d}	20.5 ± 1.4^{a}	15.8 ± 1.1^{bc}	16.5 ± 1,1^{b}	17.8 ± 1.2^{ab}	15.2 ± 0.7^{bc}	13.2 ± 0,9^{c}	15.4 ± 1.0^{bc}
18:1n7	0.4 ± 0.0^{f}	0.9 ± 0.6^{e}	0.8 ± 0.6^{e}	1.1 ± 0.8^{cd}	1.7 ± 0.1^{a}	1.3 ± 0.1^{b}	1.0 ± 0.1^{de}	1.4 ± 0.1^{b}
18:2n6	7.5 ± 0.4^{b}	10.5 ± 0.5^{a}	3.9 ± 0.2^{c}	3.3 ± 0.2^{d}	2.5 ± 0.1^{e}	1.9 ± 0.1^{f}	1.5 ± 0.1^{g}	2.7 ± 0.1^{e}
18:3n3	0.4 ± 0.1^{c}	0.6 ± 0.1^{bc}	0.5 ± 0.1^{c}	0.5 ± 0.1^{c}	0.8 ± 0.1^{ab}	0.8 ± 0.1^{a}	0.5 ± 0.1^{c}	1.0 ± 0.1^{a}
18:4n3	2.4 ± 0.2^{a}	0.4 ± 0.0^{e}	1.0 ± 0.1^{c}	0.6 ± 0.1^{d}	1.3 ± 0.1^{b}	1.5 ± 0.1^{b}	0.7 ± 0.1^{cd}	1.6 ± 0.2^{b}
20:4n6	nd	0.4 ± 0.0^{d}	0.6 ± 0.0^{c}	0.5 ± 0.0^{d}	0.7 ± 0.0^{a}	0.7 ± 0.0^{ab}	0.6 ± 0.0^{bc}	0.7 ± 0.0^{ab}
20:5n3	nd	3.4 ± 0.2^{e}	4.8 ± 0.3^{cd}	3.5 ± 0.2^{e}	5.7 ± 0.4^{bc}	6.0 ± 0.3^{b}	4.3 ± 0.3^{d}	7.2 ± 0.5^{a}
22:6n3	1.5 ± 0.1^{f}	2.7 ± 0.1^{de}	3.2 ± 0.2^{bc}	2.4 ± 0.1^{e}	3.7 ± 0.2^{b}	3.5 ± 0.1^{b}	2.9 ± 0.2^{cd}	4.9 ± 0.3^{a}
Others*	0.7 ± 0.4^{abc}	1.0 ± 0.5^{ab}	0.2 ± 0.1^{c}	1.7 ± 0.9^{a}	0.3 ± 0.1^{bc}	0.1 ± 0.1^{c}	2.0 ± 1.1^{a}	nd
SFA	73.4 ± 1.5^{a}	57.3 ± 1.2^{d}	62.1 ± 1.2^{bc}	61.4 ± 1.2^{c}	56.2 ± 1.1^{d}	61.5 ± 0.9^{c}	65.6 ± 1.3^{b}	56.9 ± 1.1^{d}
MUFA	14.1 ± 0.8^{d}	23.7 ± 1.3^{bc}	23.7 ± 1.3^{bc}	26.1 ± 1.5^{ab}	28.9 ± 1.6^{a}	24 ± 0.9^{bc}	22.1 ± 1.2^{c}	25.0 ± 1.4^{abc}
PUFA	11.8 ± 0.7^{c}	18 ± 1.0^{a}	14 ± 0.8^{b}	10.8 ± 0.6^{c}	14.7 ± 0.8^{b}	14.4 ± 0.6^{b}	10.3 ± 0.6^{c}	18.1 ± 1.0^{a}

Means (± SE) from 3 individual measurements, with the same letter within a row are not significantly different from one another (chi^2 of Wald, $P < 0.05$). *Proportion of unidentified fatty acids. SFA: Saturated fatty acid; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. BSF: black soldier fly; BSFc: control without eating fish; BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d: 1, 2, 4, 6, 8, 10 and 12 days eating fish discard, respectively. nd: not detected (Limit of Detection (LOD) ≈ 0.02 mg/100 g FW).

composition of all the larvae was dominated by SFAs, which ranged from 73.4% to 56.9% of the total FAs (BSFc and BSF12d, respectively) and decreased with fish intake (Omnibus test, likelihood ratio chi^2 = 39.0, d.f. = 7; $P < 0.001$). Remarkably, the control larvae (BSFc) contained a high proportion (47.1%) of lauric acid (LaA, 12:0), but this proportion significantly decreased (likelihood ratio chi^2 = 40.9, d.f. = 7; $P < 0.001$) in all larvae fed with discarded fish. Nevertheless, the different larvae that ate discarded fish also showed a high proportion of LaA, more than 20%. In contrast, the proportion of palmitic acid (PA, 16:0) was greater in the BSF that had eaten fish waste compared with BSFc (likelihood ratio chi^2 = 41.2, d.f. = 7; $P < 0.001$).

In relation to monounsaturated FAs (MUFAs), a significant increase was observed (likelihood ratio chi^2 = 32.6, d.f. = 7; $P < 0.001$), and there was a significant increase in 16:1n7, which changed from 3.4% in BSFc to a maximum of 9.4% in BSF6d (likelihood ratio chi^2 = 67.0, d.f. = 7; $P < 0.001$).

By using fish waste, the PUFA concentrations in the BSF larvae followed no clear trend over time for all the FA. Nevertheless, the n-3 PUFA percentage increased significantly (likelihood ratio chi^2 = 34.4, d.f. = 7; $P < 0.001$) with the time of eating fish waste, which ranged from 4.32 in BSFc to 14.78% in BSF12d (Fig. 2). Linoleic acid (LA, 18:2n6) (likelihood ratio chi^2 = 79.8, d.f. = 7; $P < 0.001$) and stearidonic acid (SDA, 18:4n3) (likelihood ratio chi^2 = 48.3, d.f. = 7; $P < 0.001$) significantly decreased in the fish-reared larvae, the concentrations of eicosapentaenoic acid (EPA, 20:5n3) (likelihood ratio chi^2 = 34.6, d.f. = 7; $P < 0.001$) and docosahexaenoic acid (DHA, 22:6n3) (likelihood ratio chi^2 = 52.1, d.f. = 7; $P < 0.001$) considerably improved as the fish intake increased: DHA increased from 1.53% in the BSFc larvae to 4.94% in the BSF12d larvae. The most remarkable result was that for EPA, which reached 7.2% in BSF12d but was undetected in BSFc. It was noteworthy that EPA + DHA tripled (likelihood ratio chi^2 = 62.2, d.f. = 7; $P < 0.001$) after only 1 day of eating fish (BSF1d), and the percentage was multiplied by six over 12 days of feeding (BSF12d) (Fig. 2).

To assess the changes in n-3 LC-PUFA in the fish waste-eating larvae, the amount (g per 100 g of product) of LC-PUFA in each larva type was computed and compared with the concentrations reported in various fish (Table 4).

The amounts of EPA + DHA in the 2-day fish-eating larvae (BSF2d) were more than 3-fold greater than those in control-diet reared larvae

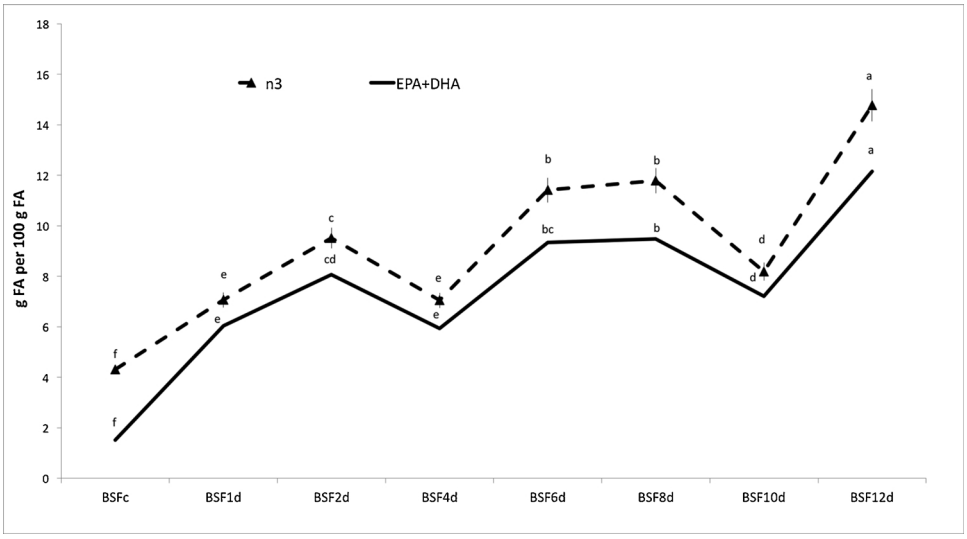

Fig. 2. Dependence of the concentrations of n-3 LC-PUFA classes of *Hermetia illucens* (BSF) larvae on time of fish discard consumption. Means (± SE) from 3 individual measurements, the same letter indicate no significant differences (Wald chi^2, $P < 0.05$).
(LC-PUFA: long-chain polyunsatturated fatty acids; FA: fatty acid; EPA: eicosapentaenoic fatty acid (20:5n3); DHA: docosahexaenoic fatty acid (22:6n3); BSF: black soldier fly; BSFc: control without eating fish, BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d: 1, 2, 4, 6, 8, 10 and 12 days eating fish, respectively).

Table 4
n-3 LC-PUFA content in *Hermetia illucens* (BSF) larvae (g/100 g dry matter meal) and usually consumed fish (g/100 g edible portion).

	EPA	DHA	EPA + DHA
Treatment	g/100 g	g/100 g	g/100 g
BSFc	0.000	0.145	0.145
BSF1d	0.160	0.129	0.289
BSF2d	0.307	0.207	0.514
BSF4d	0.407	0.239	0.645
BSF6d	0.510	0.328	0.838
BSF8d	0.454	0.265	0.719
BSF10d	0.406	0.261	0.667
BSF12d	0.450	0.307	0.757
Fish			
Farmed Atlantic salmon*	0.690	1.46	2.15
Cooked Atlantic salmon*	0.411	1.43	1.84
Cooked Atlantic mackerel*	0.504	0.699	1.203
Cooked rainbow trout*	0.468	0.520	0.988
Cooked swordfish*	0.138	0.681	0.819
Cooked Atlantic halibut*	0.091	0.374	0.465
Cooked catfish*	0.100	0.137	0.237
Cooked Atlantic cod*	0.004	0.154	0.158

* data from FAO (2010). LC-PUFA: long-chain polyunsaturated fatty acids; EPA: eicosapentaenoic fatty acid (20:5n3); DHA: docosahexaenoic fatty acid (22:6n3); BSF: black soldier fly; BSFc: control without eating fish; BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d: 1, 2, 4, 6, 8, 10 and 12 days eating fish discard, respectively.

(BSFc) (0.514 and 0.145 g/100 g dried larvae, respectively) (Table 4). Moreover, n-3 LC-PUFAs reached 0.84 g in BSF6d, which is more than that found in commonly consumed fish (e.g., Atlantic halibut or catfish) and similar to that found in rainbow trout or swordfish.

3.3. Lipid nutritional quality

The quality indices (AI, TI, and the n-6:n-3 ratio) of the different BSFs are reported in Fig. 3. As expected, the n-6:n-3 ratio and the atherogenicity and thrombogenicity indices were significantly higher (likelihood ratio chi^2 = 78.0, d.f. = 7; $P < 0.001$; likelihood ratio chi^2 = 40.1, d.f. = 7; $P < 0.001$; likelihood ratio chi^2 = 50.9, d.f. = 7; $P < 0.001$, respectevely) in the control larvae (BSFc) and lower in the fish waste-reared larvae.

The indices quickly improved when the control feed was replaced with fish waste. It is noteworthy that in only 1 day (BSF1d), these indices (AI and TI) were markedly lower, while the n-6:n-3 ratio dropped on day 2.

4. Discussion

4.1. Proximate composition

In this experiment, BSF showed higher protein and lower ash and fat contents than previously described (Barroso et al., 2017): 36–55% (dw) CP, 9–19% (dw) ash, and 15–22% (dw) EE. Nevertheless, these values correspond to the BSF fed during the experimental time with a mixture of commercial compositions of laying hen feed and fish meal (60:40 W/W), while in the present experiment BSF was fed only fish.

Among the changes observed in the body composition of the larvae when their diet was changed from control to fish, a significant decrease was observed in the fat of the larvae that consumed fish for only 1 day (BSF1d), and a smaller decrease was observed for those that ate for 2 days (BSF2d). This fact may be the result of a decrease in intake due to a certain rejection of the diet. In the following days, the larvae recovered fat to levels similar to the control.

The protein and ashes show a slight decrease, mainly in larvae that consumed fish discard for more than 6 days, compared to BSFc larvae, probably due to the nutritional imbalances that derive from the intake of only fish with higher protein and fat contents and smaller carbohydrates contents than the control diet. Raubenheimer and Simpson (2003) found that the percentage of dietary protein and carbohydrate affects the body composition in *Locusta migratoria* and *Schistocerca gregaria*.

4.2. FA composition

Based on our results, the feeding system clearly influenced the FA profiles of the *H. illucens* larvae. Although several researchers (Barroso et al., 2017; St-Hilaire et al., 2007; Stanley-Samuelson and Dadd, 1983) have already pointed this fact out, the increase in n-3 LC-PUFAs contents in the fish-waste-reared larvae observed herein was exceptional.

In our experiments, the percentage of EPA + DHA obtained in BSF12d (12.1%) was much greater than the values obtained by St-Hilaire et al. (2007), who reared BSF with fish offal (50% fish offal and 50% cow manure) for 21 days to obtain an EPA + DHA enrichment of 2.25%. In their study, the EPA + DHA percentage was 3.1% in the fish offal-reared larvae. However, these authors determined the n-3 PUFA composition of fish offal only for the last 24 h before slaughtering, and the amount of EPA + DHA was not reported. Previously, Barroso et al.

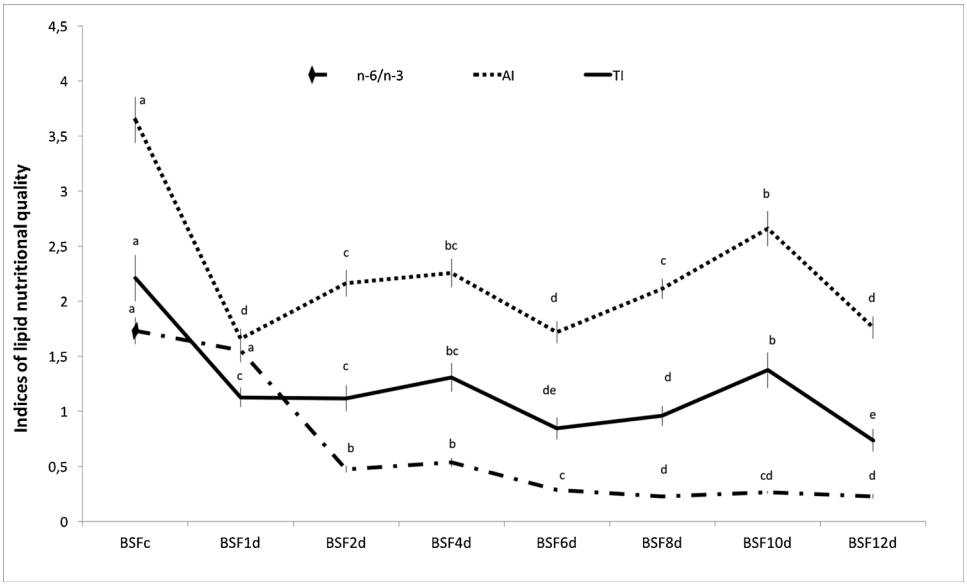

Fig. 3. Dependence of indices of lipid nutritional quality of the *Hermetia illucens* (BSF) larvae on time of fish discard consumption. Means from 3 individual measurements, the same letter indicate no significant differences (Wald chi^2, $P < 0.05$).
(AI: atherogenicity index, TI: thrombogenicity index; BSF: black soldier fly; BSFc: control without eating fish, BSF1d, BSF2d, BSF4d, BSF6d, BSF8d, BSF10d and BSF12d: 1, 2, 4, 6, 8, 10 and 12 days eating fish, respectively).

(2017) obtained BSF containing 3.7% EPA + DHA using an experimental feed containing fishmeal (40 fish meal and 60 control feed, at 9.2% EPA + DHA) for 4 days. Lilan et al. (2017) fed BSF with algae, and EPA reached only 1% in larvae, although the algae contained 6% EPA.

Undoubtedly, the various levels of EPA + DHA in the larvae depend on the amount of the n-3 LC-PUFAs in the food substrate. The fish waste that we used was very rich in EPA (13.6%) and DHA (21.4%) (Table 1). This indicates that despite these larvae being enriched with PUFAs by breeding with n-3-rich waste, the degree of enrichment depends on the PUFA content in the original material.

Additionally, BSF larvae accumulated n-3 LC-PUFASs easily, but they did not bioassimilate them completely. It has been reported that some n-3 leads to energy production, while the remaining FAs accumulates as SFAs and MUFAs (Liland et al., 2017). In our study, the differences noted in DHA were notable, larvae accumulated a maximum of 4.9% (BSF12d), which corresponded to 21.4% of the total FAs in the fish waste DHA. However, the larvae accumulated a maximum of 7.2% EPA (BSF12d), while 13.6% EPA of the total FAs was detected in the fish waste.

According to the results in Fig. 2, interesting questions on production strategies can be raised. Although the maximum EPA + DHA percentage was noted in the 12 day-larvae (12.1% in BSF12d), BSF2d (the 2-day larvae) displayed 8.5% EPA + DHA. However, the use of a rapidly decomposing protein organic waste may cause additional problems (mainly unpleasant odours in the larval rearing areas) for producers. Thus, BSF-producing companies should study whether a higher proportion of n-3 inherent to greater management complexity (BSF12d) is preferable instead of a smaller, but substantial, proportion of n-3 obtained by less complex management (BSF2d).

Both EPA and DHA are molecules that are usually found in BSF in negligible or null amounts. Therefore, their bioaccumulation in larval tissues does not follow the same model as other common metabolites usually found in insect larvae. Consequently, the "peaks" and "valleys" detected in the n-3 LC-PUFA concentration in BSF tissues over time (Fig. 1) might be due to an unstable equilibrium of concentrations between the tissues of the digestive tract and the remaining body compartments. This fact, of course, requires further investigation. In any case, as depicted in Fig. 1, the tendency of the larval tissues is to accumulate n-3 LC-PUFAs from the time of first ingestion. On the other hand, it is necessary to consider that the content of the digestive tract of BSF contains feed with n-3 LC-PUFAs, and therefore, depending on the degree of gastric emptying, there may be fluctuations in the total content of n-3 LC-PUFAs in larvae. This fact can also partially explain the model of bioaccumulation of n-3 LC-PUFAs by BSF.

Several workers (Liland et al., 2017; Renna et al., 2017; Salomone et al., 2017; Surendra et al., 2016) have reported that BSF lacks EPA and DHA. However, we occasionally detected small or trace amounts of DHA in fish-free reared BSF. Whether these marginal DHA amounts come from the ingested feed or are the results of the metabolic activity of BSF larvae, i.e., the desaturation and elongation of C18 n-3 PUFAs to n-3 LC-PUFAs, remains an open question. In this regard, it is not ruled out that the feed we use sometimes contains undetectable amounts of DHA, which could be magnified in the tissues of BSF larvae. Therefore, this subject requires further investigation. Undoubtedly, the ability to synthesize PUFAs is widespread in the invertebrate kingdom, and some information is available on PUFA biosynthesis in insects, especially in older literature. For instance, many insects contain naturally occurring n-3 LC-PUFAs (Thompson, 1973), while twelve species of insects have been identified that can biosynthesize ALA de novo (Cripps et al., 1986). However, all previously cited desaturase activities seem to be very low, and within the various taxa of living beings only some taxa of microalgae can synthesize de novo high amounts of EPA and DHA (Gladyshev et al., 2013).

Several studies have shown that the FA profiles of a diet play a fundamental role in human health. Reduced dietary SFA and increased PUFA intake are recommended (Lefevre et al., 2004). Some terrestrial insects have a high proportion of PUFAs, for example, the mole cricket (*Gryllotalpa africana*) has 1.6 g/100 g, the spur-throated grasshopper (*Chondracris roseapbrunner*) has 2.4 g/100 g, and the ground cricket (*Acheta confirmata*) has 2.9 g/100 g (Yang et al., 2006). These examples include large amounts of LA and ALA but are lacking EPA and DHA. In general, terrestrial insects are clearly deficient in EPA + DHA (Sánchez-Muros et al., 2014). According to Yan et al. (2006), only aquicolous insects contain n-3 LC-PUFAs because they include algae in their diets, which contain small amounts of LC-PUFAs.

Although the BSF larvae used as the control in our experiment contained small amounts of n-3 LC-PUFAs, the EPA + DHA concentration ranged from 0.514 in BSF2d to 0.838 g/100 g DW in BSF6d with fish waste intake (Table 4). It was noteworthy that the EPA + DHA concentration in the BSF meal was similar to that found in cooked rainbow trout or cooked swordfish and was much higher than the values detected in cooked Atlantic halibut, cooked catfish and cooked Atlantic cod (FAO, 2010).

According to the International Society for the Study of Fatty Acids and Lipids (ISSFAL (International Society for the Study of Fatty Acids and Lipids), 2004), the recommended daily intake of EPA and DHA for the primary prevention of coronary heart disease would be 500 mg/day. Based on the results obtained herein, this demand could be covered by a meal composed of 2-day, 100 g fish waste-reared BSF dry larvae.

Barlow (1963) suggested that a high palmitoleic acid level (POA, 16:1n7) is a characteristic of dipterous insects. However, we found that in BSF, the percentages of POA fell within the frequent range noted for other insect orders. Our results show that the most prominent FA percentages of the total FAs in BSFc were those for LaA (47.1%), PA (13.1%) and oleic acid (OA, 18:1n9, 10.3%). Similar results were obtained by Surendra et al. (2016), with 45%, 14%, and 12% for the same FAs, respectively.

Although n-3 LC-PUFAs increased according to the duration of fish waste intake, the largest FA fraction (56–73%) was SFA. Thus, LaA accounted for approximately half of the total FAs. These values agree with previous reports by Liland et al. (2017); Oonincx et al. (2015), and Sealey et al. (2011). In addition to diet, the FA profiles of insects largely depend on their developmental stage and environmental variables. However, knowledge of the role that some FA plays in the insect physiology is lacking. In line with this, Stanley-Samuelson and Loher (1986) observed that black field cricket (*Teleogryllus commodus*) biosynthesised C20 PUFAs to produce prostaglandins, which are needed for reproduction.

4.3. Lipid nutritional quality

The evaluation of the nutritional value of FA is widely assessed by the n-6:n-3 PUFA ratio and by the atherogenic and thrombogenic indices (Popova et al., 2016). The amount of n-6 and n-3 PUFAs in the diet play independent roles in health and disease (Simopoulos and Cleland, 2003). The n-6:n-3 ratio is a widely used index to assess healthy diets (Santos-Silva et al., 2002) because a high proportion of PUFAs in the diet is not healthy if the n-6:n-3 ratio is unbalanced (Simopoulos and Cleland, 2003). A low n-6:n-3 ratio has positive effects on preventing cardiovascular disease and reduces total mortality by lowering the risk of breast cancer and many chronic diseases (Simopoulos, 2002). In this experiment, the n-6:n-3 values were higher in the control larvae (1.7) than in the fish waste-reared larvae, but these values were much lower than the recommended value of 4 (Simopoulus, 2009). This ratio quickly decreased (to 0.2) as larvae were raised on fish waste, while the BSF meal showed a consistent increase and decrease in n-3 and n-6 PUFAs, respectively, when replacing the control fed with fish waste.

To judge the effects of FAs on human health, Ulbricht and Southgate (1991) proposed atherogenic (AI) and thrombogenic (TI) indices. As Popova et al. (2016) point out, these indices assess the probability of

the incidence of pathogenic phenomena increasing, such as atheroma and/or thrombus formation. In relation to these health indices, both AI and TI displayed similar decreasing trends in the BSF meal when the larvae were reared with increasing percentages of fish waste (Fig. 3).

However, the recommended AI values are below 0.5 (Ulbricht and Southgate, 1991), and the levels were higher in the BSF larvae in the present paper. Although LaA was reduced after including fish waste in the diet, the myristic acid (MA, 14:0) concentrations remained high, while PA increased. MA shows a very close relation with coronary heart disease (Bellizzi et al., 1994), and both MA and PA are considered atherogenic, while PA also increases cholesterol. MA also has the potential to increase cholesterol 5–6 times higher than PA (Yu et al., 1995).

5. Conclusion

We conclude that all the BSF larvae reared with fish waste can be enriched in EPA + DHA and total PUFAs. This positive effect is associated with the decrease in the atherogenic and thrombogenic indices and in the n-6:n-3 ratio. These results introduce new perspectives for further research on the rearing practices of BSF to produce larvae with a healthy FA composition. Such investigations should also be aimed at knowing both the bioaccessibility and bioavailability of n-3 LC-PUFAs ingested from insect larvae in various culinary forms, such as cooked and fried foods.

As demonstrated herein, these mass insect breeding systems could include feeding insects on fish by-products to significantly increase their n-3 levels. To make user handling easier to produce BSF larvae meal, this waste could be provided by the 2-day reared larvae (before pupation), which significantly increased the EPA + DHA levels (up to 8%), and requires making only a few modifications in the BSF mass-rearing system.

In this way, insects can act as an excellent tool to solve the environmental problems produced by fish waste, which stems from producing large quantities of EPA + DHA-enriched insect meal. Given the small scale used in our experiments, and in agreement with Halloran et al. (2014), we demonstrate that insect meal can be obtained as local mini-livestock with very little investment made in, for instance, urban areas.

Acknowledgements

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