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ω3-enrichment of *Hermetia illucens* (L. 1758) prepupae from oilseed byproducts

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ABSTRACT

To meet the growing demand for fish in human nutrition, aquaculture is developing increasingly from marine fishery products (*i.e.* fish meal and fish oil). New environmentally sustainable protein and lipid resources are recommended for this sector and insects, particularly black soldier fly (BSF), *Hermetia illucens* (L. 1758), are considered as promising candidates for fish feed production. BSF larvae convert organic materials into high-valued protein and lipid, their nutritional composition is related to their diet and could therefore be manipulated. The key issue in fish nutrition will be the fatty acid composition of BSF larvae that are characterized by high saturated fatty acids (SFAs) level (>60%) with lack of essential polyunsaturated fatty acids. This study aimed to adjust the fatty acid profile of BSF larvae by manipulating their food on focusing on diet formulation with local oilseed cakes for essential ω3 fatty acid (ALA: α-linolenic acid, C18:3n3) enrichment. Selected populations (100 individuals/population) of 7 days old BSF larvae were reared on Chicken feed (CF) diets enriched with flax and rape cakes at six incorporation rates (10–20 – 40–60 – 80–100%), the CF was used as the control diet and all diets were tested in triplicate (n = 3). The first prepupae appeared from 15 rearing days on all diets with an average weight of 195 mg excepted for full oil cake diets showing longer prepupal collection time (7 days) and lower average weight (116 mg). Oil cakes incorporation shows an impact on the prepupae fatty acid profiles. The results show that progressive oil cakes diets incorporation decreased saturated fatty acids from 75.86 ± 0.34% to 56.10 ± 0.74%. Simultaneously, rape cake incorporation leads mainly to oleic acid (C18:1n-9) enrichment which not sought in fish nutrition and low ALA rate from 1.16 ± 0.25% to 2.42 ± 0.12% but Flax cake incorporation increase ALA enrichment up till 15.27 ± 0.02% favorable to fish needs allowing a potential increase BSF meal incorporation in fish feed. This research therefore presents a model of progressive prepupae oil enrichment from oilseed coproducts for application in fish feed.

1. Introduction

The growing demand for animal products requires large amounts of resources, including feed, water, energy and land spaces (FAO, 2018). In the context of sustainable development, new feed sources with low ecological footprint and high nutriment quality must be developed (Caparros Megido et al., 2015; Gahukar, 2016). Among the current possibilities (*e.g.* algae or cultured meat), insects rearing development could be an eco-friendly and sustainable solution to provide high-quality food and feed in the future (Govorushko, 2019; Varelas, 2019). Insects are a rich source of proteins and their lipid content could be a source of energy and essential fatty acids for potential feed ingredients (Barroso et al., 2017; Pinotti et al., 2019). Moreover, insects have fast growth rates, show better food conversion ratio to biomass than conventional farmed animals and could be produced locally from organic waste with a small ecological footprint in confined spaces (Premalatha et al., 2011; van Huis, 2020). On this basis, insect production for livestock

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feeding could become an important indirect source of protein for healthy human nutrition (Dicke, 2018). Among potential insect species, *Hermetia illucens* (L.1758), commonly known as black soldier fly (BSF), is a dipteran species of Stratiomyidae family native to tropical and subtropical regions of American continent and whose geographical distribution has extended to all continents (Sheppard et al., 2002, 1994). The extent of its distribution is due to development of human transport means, international trade (Marshall et al., 2015) and its connection with food waste. Moreover, this species is tolerant to wide range of environmental parameters such as temperature, humidity, pH and light quality (Holmes et al., 2012; Meneguz et al., 2018; Tomberlin et al., 2009; Zhang et al., 2010). This polyphagous and scavenger species is a bioconversion agent and can be used in the management of manure from animal rearing of organic waste and of agricultural byproducts (Diener et al., 2011; Gao et al., 2019; Manurung et al., 2016; Nguyen et al., 2015; Oonincx et al., 2015b; Sheppard et al., 1994). The larvae feed numerous organic matters from both plant and animal sources, with a fluctuating impact on their growth rate (Jucker et al., 2017; Lopes et al., 2020; Meneguz et al., 2018; Nguyen et al., 2015). The main applications of BSF larvae are their potential incorporation in animal feed (*e.g.* fish. chicken, duck, pigglet, and rabbit) (Biasato et al., 2019; Caimi et al., 2020; Gariglio et al., 2019; Gasco et al., 2019; Pieterse et al., 2019) or their use as alternative feedstocks for biodiesel production (Li et al., 2011; Li et al., 2015; Wang et al., 2017). The use of BSF meal as feed raw material is notably limited by their high lipid content and their fatty acid profile (Barragan-Fonseca et al., 2017). To bypass this problem, two solutions are potentially available: insect defatting or insect fatty acid profile adjustment by feed modulation (Barroso et al., 2019, 2017; Ewald et al., 2020; Liland et al., 2017; Oonincx et al., 2019; St-Hilaire et al., 2007; Tschirner and Simon, 2015). Different fractioning approaches to separate lipid, protein, and chitin from BSF prepupae are currently developed (Caligiani et al., 2018). Defatting could be done by chemical extraction, aqueous extraction, supercritical CO_2 extraction, or by mechanical pressing (Purschke et al., 2016; Surendra et al., 2016; Tzompa-Sosa et al., 2014). Defatting could improve the transformation process such as extrusion, reduce lipids oxidation during storage (Dumas et al., 2018) and, above all, allow the production of protein-rich flours for feed applications (Dumas et al., 2018; Tschirner and Simon, 2015). Concerning the BSF fatty acid profile modulation, it has been shown that the fatty acid profiles vary according to the insect feed and reflect their diet profiles (Meneguz et al., 2018; Spranghers et al., 2017) . Researches to improve the larval fatty acid profile are mainly conducted with byproducts derived from fish and aimed to increase the levels of long-chain (*i.e.* $\geq$ C20) polyunsaturated fatty acids (ω3 LC-PUFAs), such as eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) as fish and fish oils are the primary sources of ω3 LC-PUFAs in human nutrition (Barroso et al., 2019; Cullere et al., 2019; St-Hilaire et al., 2007; Wang et al., 2006).

These essential ω3 LC-PUFAs are mainly produced by microalgae and are consequently largely accumulated by fishes through the food chain (Dyer et al., 2008). Nevertheless, the fish requirements for essential fatty acids depend on species lipid elongation capacity (Sargent et al., 1999), which is more important in freshwater fishes than in seawater fishes (Tocher, 2003). Salmonid species, such as rainbow trout (*Oncorhynchus mykiss* Walbaum. 1792), are partially able to convert dietary ALA (α-linolenic acid, C18:3n-3) to EPA and DHA via several elongation and desaturation pathways (Cook, 1996; Lazzarotto et al., 2015; Sargent et al., 2002; Thiessen et al., 2004; Vagner and Santigosa, 2011). Yu and Sinnhuber (1972) highlighted that 1% of ALA incorporation in rainbow trout diet results in a better fish growth linked with a quick conversion of ALA into DHA. For these reasons, ALA is considered as an essential precursor of EPA and DHA in rainbow trout (Torno et al., 2017). The bioconversion of ALA into ω3 LC-PUFAs is partially inhibited by dietary linoleic acid (LA, 18:2n-6) and the ratio into these two essentials fatty acid (ALA/LA) plays a major role on fatty acid metabolism (Thanuthong et al., 2011). Therefore, it is important that ω3 PUFAs and ω6 PUFAs intakes are well balanced in the diet of rainbow trout

(Barry and Trushenski, 2020). As an excessive imbalance of essential fatty acids in fish feed formulation can change their fatty acid profile, it is crucial to preserve fish feed ingredients containing these ω3 LC-PUFAs of interest to keep the organoleptic quality and health benefits expected by consumers (Cardinaletti et al., 2019; Fonseca-Madrigal et al., 2005; Seierstad et al., 2005). Currently, the sources of raw materials containing ω3 LC-PUFAs (*i.e.* fishmeal and fish oil) for aquaculture are mainly derived from small pelagic species such as anchovies (Caruso et al., 2013; Lazzarotto et al., 2015). As these resources became increasingly scarce, several studies have highlighted the potential of partial substitution of fish oils by vegetable or other animal oils with low-impact on fish growth (Ballestrazzi et al., 2006). Among potential alternative ingredients in fish nutrition, insects became increasingly popular. Since 2017, European legislation authorizes the use of seven insect species, including BSF (Regulation EU 2017/893). With this new regulation, the insect rearing development for animal nutrition is expanding in European Union (EU) and is based on prevailing rules for conventional farms restricting the use of kitchen waste, manure, and guts as insect feed (Regulation EC 1069/2009). In this legislative context, plant byproducts are considered as the main feed ingredients for BSF mass production (Tschirner and Simon, 2015). Currently, few studies were performed on BSF development on plant byproducts (*e.g.* rice and maize straws, cassava peel or cull potatoes) and were not focused on the fatty acid profile of the produced larvae (Alyokhin et al., 2018; Gao et al., 2019; Manurung et al., 2016; Supriyatna et al., 2016). Nevertheless, within the plant kingdom, only few green vegetables and oil plants could be considered as good source of ω3 (Abbadi et al., 2004; Barroso et al., 2017; Dyer et al., 2008). Flaxseed and rapeseed oils are the major sources of ALA (53% and 12% respectively) of terrestrial origin while more traditional oils as corn, sunflower or olive oils only contain around 1% of ALA (Innis, 2008; Rahimi et al., 2011). As rapeseed and flaxseed are increasingly produced worldwide for their oil, post-extraction cakes containing ALA-rich residual oil should be considered to improve animal health (Çulcuoğlu et al., 2002). As the fatty acid composition of insects can be influenced by their feed (Barroso et al., 2014), the use of the aforementioned cakes may improve the insect fatty acid profile, especially in ALA. This ALA inclusion in prepupae would partially meet the need for essential fatty acids in salmonids and could increase the full-fat insect meal incorporation into their diet. Consequently, this research evaluates the fatty acid profile modifications and the potentiality of an ALA enrichment of BSF prepupae fed with diets enriched from vegetal byproducts.

2. Material and methods

2.1. Experimental design

The experiment was conducted in a controlled dark room (2.4 5 × 2.06 × 2.72 m) with temperature and relative humidity maintained at 27 ± 1 °C and 60 ± 5% (data logger; MCH – 383 SD, Lutron, Taïwan). The BSF larvae used to conduct this experiment came from an experimental rearing in the Functional and Evolutionary Entomology laboratory from Gembloux Agro-Bio Tech (ULiege, Belgium). Populations of 100 larvae, 7 days old (0.008 ± 0.001 g), were manually collected and weighed (STX223, OHAUS Scout, Parsippany, USA) for each replication (n = 3). All populations were reared

in plastic containers (17.20 × 11.50 × 6.00 cm, AVA, Temse, Belgium) covered with a transparent plastic lid with, in the center, a square mosquito net for ventilation (1 × 1 cm). Containers were randomly arranged at half-height on a board in the rearing room.

2.2. Feeding and sampling

Two mechanically extracted oil cakes were selected for their differences in fatty acids composition: rape cake (R) (SCAM. Andenne. Belgium) and flax cake (F) (SCAM. Andenne. Belgium). Oil cakes (*e.g.* rape cake and flax cake) are byproducts from oil production. They are obtained mechanically by trituration, a process of crushing and pressing seeds. The flaxseeds are triturated in two steps: a first cold pressing (*i.e.* ambient temperature) (Modern mechanics, Feuchy, France) followed by a second hot pressing (90–95 °C) (Anderson international, Ohio, United States). These two successive presses produce cold pressing oil, crude oil and residual flax cake (F). Rapeseeds are driven by a worm screw through knives to be crushed and pressed at low (*i.e.* ambient temperature) or high (80–120 °C) temperature (Egon Keller, Remscheid, Germany). These mechanical presses produce cold press oil or crude press oil and residual rape cake (R) which can still undergo solvent extraction process. These oil cakes were incorporated in substitution of chicken feed (CF; Chicken Pellet, AVEVE, Belgium) at 6 rates (10, 20, 40, 60, 80, 100%) and the simple CF was used as the control diet. Thirteen diets have therefore been formulated and tested in triplicate (n = 3). Each diet ingredient (CF, R, F) was ground (Pulverisette 19, Fritsch, Germany) to 0.750 mm particles size. Larvae were fed every three days until the appearance of the first prepupae. Each dry food ration was weighted (Scout STX223, Ohaus, Parsippany, USA), added in the appropriate plastic breeding container, and moistened with a sprayer (Hozelock, Birmingham, England). The daily food rate was set at 100 mg per larva, with 60% moisture content (Diener et al., 2009). When the individuals have reached the prepupal stage, characterized by the appearance of a black coloration (May 1961), they were individually removed from the substrate using forceps (Extra soft stainless steel forceps 03, Cahurel, France). The prepupae of each batch were cleaned with water, dried with paper towels, counted and weighed mainly for growth evaluation. They were then killed by direct freezing and stored at −20 °C for further analyzes.

2.3. Chemical analyzes

All analyses were performed in triplicate for each diet ingredient (CF, R, F) and prepupae batches. A prepupae batch, 100 larvae at the beginning, represent a replication. Before analyses, all diet ingredients (CF, R, F) and prepupae batches have been freeze-dried (Gamma 2–16 LSCPLUS, Martin Christ, Germany) and ground in a blender (IKA® A10, Staufen, Germany). The fatty acid composition was estimated by gas chromatography. Fatty acids of 10.0 mg of lipids were converted into fatty acid methyl esters with boron trifluoride (Sigma-Aldrich, Overijse, Belgium) and methanol (VWR, Oud-Heverlee, Belgium). Fatty acid methyl esters were diluted in 8 ml of hexane (VWR) and analyzed with a Trace GC Ultra gas chromatogram (Thermo Fisher Scientific, Asse, Belgium) equipped with a split/splitless injector (240.0 °C) operating in splitless mode (splitless time: 0.85 min) and a flame ionization detector (250.0 °C). A Stabliwax DA column (Restek Corp, Bellefonte, PA, USA) (30 m × 0.25 μm × 0.25 mm in length × thickness × diameter) was used for the analysis. The temperature program was as follows: hold at 50.0 °C for 1 min, increase to 150.0 °C at 30.0 °C/min, increase to 240.0 °C at 5.0 °C/min, and hold at 240.0 °C for 10 min. Fatty acid methyl esters were identified based on their retention data compared to a reference mixture of 37 components (Supelco® 37 component FAME mix, Sigma-Aldrich, Overijse, Belgium).

The relative percentage of each compound was calculated by comparing individual peak areas with the sum of peak areas of all identified compounds, using Chemstation software (Agilent Technologies, Palo Alto, CA, USA).

2.4. Statistical analyses

All analyses were conducted with Minitab software (version 18 for Windows. State College. PA. USA). The results were presented as the mean and the standard error of the mean (±SE). The accepted level of significance was 5% in all analyses.

Non-parametric Kruskal-Wallis tests (H test) followed by Dunn-Bonferroni's Post-hoc tests were used to evaluate the influence of oil cake incorporation in feed formulation on the growth performance parameters (*i.e.* Total prepupal weight, Individual prepupal weight and Survival rate) and on the fatty acid profiles of prepupae.

Multivariate statistics were used to analyze variations in prepupae fatty acid profiles in relation to diets. The results of the principal component analysis (PCA) were plotted to reduce the variables (*i.e.* fatty acids) using the two new coordinates (PC1 and PC2) which describe the two larger fractions of the variance among the samples. The scores (*i.e.* values of PC1 and PC2) were correlated with the individual fatty acids.

3. Results

3.1. Diet fatty acids profiles

The fatty acid profiles of CF, R and F were contrasted and dominated respectively by linoleic acid (LA, C18:2n6) in CF, by oleic acid (C18:1n9) in R and by α-linolenic acid (ALA, C18:3n3) in F.

The saturated fatty acids (SFAs) levels decreased with increasing incorporations of the oil cakes (R, F). This decrease was mainly due to their lower content in palmitic acid (C16:0) than in the control diet (CF). The monounsaturated fatty acids (MUFAs) levels increased with rape cake (R) incorporation while it decreased with flax cake (F) incorporation due to their contents of oleic acid (C18:1n9). The polyunsaturated fatty acids (PUFAs) rates decreased with rape cake (R) enrichment because of their low LA content while it increased with flax cake (F), rich in ALA. Finally, the ALA/LA ratio (n3-n6) increased with increasing incorporations of the oil cakes (R, F) (Table 1 and 2).

3.2. Growth and survival of the larvae

The first prepupae were observed after 15 rearing days in all diets. The prepupae collection was spread over 7 days for the control diet (CF) and the enriched diets (R, F at 10, 20, 40, 60 and 80% incorporation rates) while it was performed over approximately 15 days for the full oil cakes diets (R100 and F100). The individual prepupal weights for full oil cakes diets (R100 and F100) were lower (110.79 ± 5.94 mg to 126.56 ± 16.05 mg) than for all other diets (CF and 10, 20, 40, 60, 80% of R and F) ranging from180.03 mg to 206.69 ± 0.45 g (H = 33.60; $p = 0.001$). Survival rates did not vary between different diets with a value between 92.33 ± 4.41% to 99.33 ± 0.33% (H = 19.16; p = 0.446) (Table 3).

3.3. Prepupae fatty acids profiles

The fatty acid profiles of prepupae were dominated by SFAs in all diets decreasing with increasing incorporations of oil cakes (Fig. 1) mainly due to their lauric acid (C12:0) content (Table 4 and 5). The MUFAs, dominated by oleic acid (C18:1n-9), increased with increasing incorporation of the two oil cakes (Fig. 1). The PUFAs increase with enrichment of flax cake (F) diets mainly due to their ALA (C18:3n-3) content (Fig. 1, Table 5). Finally, the ALA/

Table 1
Fatty acid profiles (% of total FAMEs) of experimental diets enriched with rape cake.

Diet	CF	R10	R20	R40	R60	R80	R100
C12:0	0.49 ± 0.03	*0.34*	*0.25*	*0.14*	*0.07*	*0.03*	0.00 ± 0.00
C14:0	0.00 ± 0.00	*0.04*	*0.07*	*0.10*	*0.11*	*0.13*	0.13 ± 0.02
C16:0	17.47 ± 0.17	*14.64*	*12.84*	*10.68*	*9.43*	*8.61*	8.04 ± 0.98
C16:1	0.00 ± 0.00	*0.14*	*0.23*	*0.34*	*0.40*	*0.45*	0.47 ± 0.06
C18 :0	3.40 ± 0.03	*2.81*	*2.44*	*1.99*	*1.72*	*1.55*	1.43 ± 0.09
C18:1n-9	20.78 ± 0.06	*31.29*	*37.98*	*46.02*	*50.68*	*53.72*	55.86 ± 2.96
C18:2n-6	53.71 ± 0.09	*45.15*	*39.70*	*33.15*	*29.35*	*26.88*	25.14 ± 1.10
C18 :3n-3	4.15 ± 0.02	*5.58*	*6.49*	*7.59*	*8.22*	*8.63*	8.92 ± 0.74
SFAs	21.36 ± 0.17	*17.84*	*15.59*	*12.90*	*11.34*	*10.32*	9.60 ± 1.07
MUFAs	20.78 ± 0.06	*31.43*	*38.21*	*46.36*	*51.09*	*54.17*	56.34 ± 2.90
PUFAs	57.86 ± 0.11	*50.73*	*46.19*	*40.73*	*37.57*	*35.51*	34.06 ± 1.84
n3-n6	0.08 ± 0.00	*0.12*	*0.16*	*0.23*	*0.28*	*0.32*	0.35 ± 0.01

FAMEs = fatty acid methyl esters; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; CF = chicken feed; R = rape cake. The italics values were mathematically derived from chemical analyses.

Table 2
Fatty acid profiles (% of total FAMEs) of experimental diets enriched with flax cake.

Diet	CF	F10	F20	F40	F60	F80	F100
C12:0	0.49 ± 0.03	*0.39*	*0.31*	*0.19*	*0.11*	*0.05*	0.00 ± 0.00
C14:0	0.00 ± 0.00	*0.03*	*0.05*	*0.08*	*0.10*	*0.11*	0.13 ± 0.01
C16:0	17.47 ± 0.17	*15.74*	*14.36*	*12.31*	*10.85*	*9.77*	8.92 ± 0.29
C16:1	0.00 ± 0.00	*0.02*	*0.03*	*0.06*	*0.07*	*0.09*	0.10 ± 0.01
C18 :0	3.40 ± 0.03	*3.43*	*3.46*	*3.49*	*3.52*	*3.54*	3.55 ± 0.05
C18:1n-9	20.78 ± 0.06	*20.47*	*20.23*	*19.87*	*19.62*	*19.43*	19.28 ± 0.11
C18:2n-6	53.71 ± 0.09	*46.89*	*41.46*	*33.37*	*27.62*	*23.34*	20.01 ± 0.44
C18 :3n-3	4.15 ± 0.02	*13.03*	*20.10*	*30.63*	*38.10*	*43.68*	48.00 ± 0.90
SFAs	21.36 ± 0.17	*19.59*	*18.18*	*16.07*	*14.58*	*13.47*	12.60 ± 0.34
MUFAs	20.78 ± 0.06	*20.49*	*20.27*	*19.93*	*19.69*	*19.52*	19.38 ± 0.12
PUFAs	57.86 ± 0.11	*59.92*	*61.56*	*63.99*	*65.73*	*67.02*	68.02 ± 0.46
n3-n6	0.08 ± 0.00	*0.28*	*0.48*	*0.92*	*1.38*	*1.87*	2.40 ± 0.10

FAMEs = fatty acid methyl esters; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; CF = chicken feed; F = flax cake. The italics values were mathematically derived from chemical analyses.

Table 3
Performance of black soldier fly larvae farmed on substrates with different oil cake types and incorporation levels.

Diet	Total prepupal weight (g)	Individual prepupal weight (mg)	Survival rate (%)
CF	17.20 ± 0.45^{c}	180.64 ± 2.65^{bc}	95.33 ± 3.71
R10	17.46 ± 0.87^{c}	189.21 ± 4.16^{abc}	92.33 ± 4.41
R20	20.53 ± 0.08^{a}	206.69 ± 0.45^{a}	99.33 ± 0.33
R40	19.49 ± 0.46^{abc}	200.91 ± 1.66^{ab}	97.00 ± 1.53
R60	19.04 ± 0.04^{abc}	195.65 ± 2.23^{abc}	97.33 ± 1.33
R80	19.72 ± 0.48^{abc}	199.12 ± 3.20^{abc}	99.00 ± 0.82
R100	12.04 ± 1.81^{d}	126.56 ± 16.05^{d}	95.00 ± 2.31
F10	17.92 ± 0.33^{abc}	186.69 ± 1.22^{abc}	96.00 ± 0.58
F20	19.21 ± 0.92^{abc}	198.70 ± 5.05^{abc}	96.67 ± 1.20
F40	20.13 ± 0.03^{ab}	204.04 ± 0.58^{a}	98.67 ± 0.33
F60	17.95 ± 0.90^{abc}	188.25 ± 3.09^{abc}	95.33 ± 2.33
F80	17.52 ± 0.17^{bc}	180.03 ± 0.63^{c}	97.33 ± 0.67
F100	10.41 ± 0.94^{d}	110.79 ± 5.94^{d}	94.00 ± 0.58
Statistical analyses	H = 32.16; *p = 0.001*	H = 33.60; *p = 0.001*	H = 19.16; p = 0.446

CF = chicken feed; R = rape cake; F = flax cake. Means with different letters significantly differ (Kruskal-Wallis test followed by Dunn-Bonferroni post hoc test; $P < 0.05$).

LA ratio (n3-n6) increased with increasing incorporations of the oil cakes (R, F) (Table 4 and 5).

3.4. Principal component analysis (PCA) of prepupal fatty acid profiles

PCA was used to visually compare the influence of the oil cake incorporation on the fatty acid profile of prepupae oils. This analysis reduces the number of variables (*i.e.* fatty acids) to new ones represented by the principal components (PC). The fatty acids with high values (*i.e.* score > 0.3) for the first two PC were selected and graphically reported (Fig. 2). The first component (PC1. 54.2% of the variance) distributed the data following the percentage of oil cake incorporation while the second component (PC2. 22.9%) distributed them according to the type of oil cake (*i.e.* rape vs flax). The PCA showed that a difference in fatty acid profile could be observed between diets with low (*i.e.* $\leq$ 40%) and high (*i.e.* $\geq$ 60%) oil cake incorporation. Low incorporation rates seem to be characterized by a higher content of SFAs (*i.e.* C10:0. C12:0. C14:00 and C16:00) while high incorporation rates display higher oleic acid (C18:1n-9) contents. Concerning the nature of plant meal, it seems that rape cakes enhanced mainly oleic acid (C18:1n-9) content while flax cake was dominated by ALA (Fig. 2).

4. Discussion

Since the 2017 European agreement on the insect incorporation in fish feed formulation, BSF larvae are increasingly produced. The resulting BSF meals are principally defatted, used to partially replace fish and soy proteins. In order to overcome the issues related to BSF unbalanced fatty acid profile for fish nutrition and allow a larger use of full-fat BSF meals, this study aimed to improve the nutritional value of BSF prepupae by modulating their fatty acid profile using fully plant-based diets. This study investigated the fatty acid profile variations and principally, the α-linolenic acid (ALA, C18:3n-3) enrichment of BSF prepupae fed on enriched diets with rape or flax cakes.

4.1. Larval development

In this study, BSF was collected at the prepupal stage (*i.e.* prepupae) when the prepupae naturally migrate out of the rearing sub-

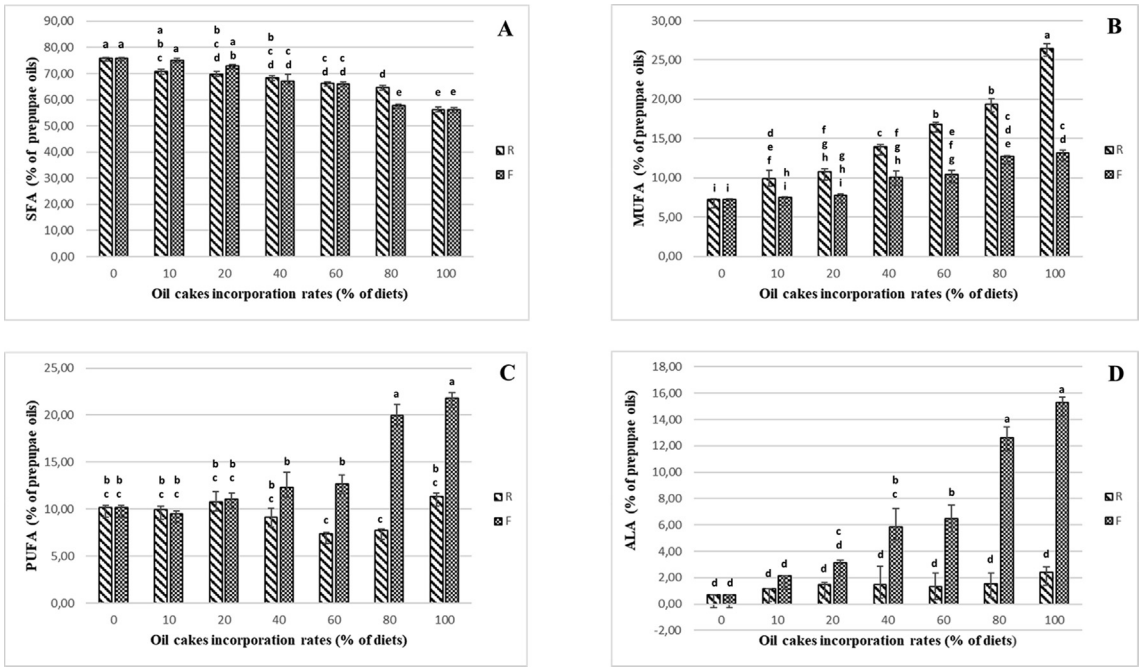

Fig. 1. Changes in saturated fatty acids (SFAs) (A), monounsaturated fatty acids (MUFAs) (B), polyunsaturated fatty acids (PUFAs) (C) and α-linolenic acid (ALA : C18:3n-3) (D) compositions of black soldier fly prepupae oils according to the enrichment of oils cakes (R = rape cake and F = flax cake) in diets. Means with different letters significantly differ (Kruskal-Wallis test followed by Dunn-Bonferroni post hoc test; $P < 0.05$).

Table 4
Fatty acid profiles (% of total FAMEs) of black soldier fly farmed on rape cake enriched diets.

Diet	CF	R10	R20	R40	R60	R80	R100	STA
C10:0	1.63 ± 0.10^{a}	1.62 ± 0.08^{a}	1.45 ± 0.10^{ab}	1.44 ± 0.14^{ab}	1.36 ± 0.02^{b}	1.31 ± 0.04^{b}	1.07 ± 0.02^{c}	H = 16.12;*p = 0.013*
C12:0	55.59 ± 0.48^{a}	52.15 ± 1.10^{ab}	51.42 ± 1.60^{b}	51.23 ± 1.61^{b}	49.77 ± 0.52^{b}	49.22 ± 1.38^{b}	41.97 ± 1.66^{c}	H = 16.71;*p = 0.010*
C14:0	6.90 ± 0.10^{a}	6.36 ± 0.04^{b}	6.51 ± 0.15^{ab}	6.37 ± 0.20^{b}	6.38 ± 0.15^{b}	6.17 ± 0.09^{b}	5.34 ± 0.18^{c}	H = 15.90;*p = 0.014*
C14:1	0.00 ± 0.00^{c}	0.06 ± 0.04^{bc}	0.08 ± 0.05^{abc}	0.11 ± 0.04^{abc}	0.25 ± 0.15^{a}	0.21 ± 0.01^{ab}	0.13 ± 0.02^{abc}	H = 13.80;*p = 0.032*
C15:0	0.10 ± 0.00^{d}	0.12 ± 0.01 ^{cd}	0.14 ± 0.01^{bc}	0.15 ± 0.02^{ab}	0.17 ± 0.00^{a}	0.16 ± 0.00^{ab}	0.16 ± 0.01^{ab}	H = 16.85;*p = 0.010*
C15:1	0.10 ± 0.01^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}	0.08 ± 0.02^{a}	H = 15.03;*p = 0.020*
C16:0	10.03 ± 0.22^{a}	9.01 ± 0.35^{b}	8.80 ± 0.23^{b}	8.01 ± 0.02^{c}	7.49 ± 0.01^{c}	6.89 ± 0.15^{d}	6.77 ± 0.16^{d}	H = 19.15;*p = 0.004*
C16:1	0.24 ± 0.02^{e}	0.54 ± 0.12^{d}	0.54 ± 0.06^{d}	0.68 ± 0.01 ^{cd}	0.79 ± 0.01^{d}	1.13 ± 0.02^{b}	1.82 ± 0.1^{a}	H = 19.06;*p = 0.004*
C17:0	0.15 ± 0.00^{abc}	0.17 ± 0.01^{ab}	0.19 ± 0.02^{a}	0.18 ± 0.02^{a}	0.17 ± 0.01^{ab}	0.14 ± 0.00^{bc}	0.13 ± 0.01^{c}	H = 16.14;*p = 0.013*
C17:1	0.12 ± 0.00^{ab}	0.00 ± 0.00^{c}	0.12 ± 0.02^{ab}	0.16 ± 0.04^{ab}	0.16 ± 0.01^{ab}	0.11 ± 0.05^{b}	0.19 ± 0.02^{a}	H = 15.15;*p = 0.019*
C18:0	1.45 ± 0.05^{a}	1.33 ± 0.05^{a}	1.34 ± 0.12^{a}	1.10 ± 0.07^{b}	0.94 ± 0.02^{bc}	0.82 ± 0.02^{c}	0.87 ± 0.04^{c}	H = 18.82;*p = 0.004*
C18:1n-9	6.73 ± 0.14^{e}	9.34 ± 1.64^{d}	10.02 ± 0.56^{d}	12.98 ± 0.51^{c}	15.55 ± 0.46^{b}	17.82 ± 1.18^{b}	24.22 ± 1.01^{a}	H = 19.32;*p = 0.004*
C18:2n-6	9.43 ± 0.37^{a}	8.78 ± 0.44^{a}	9.33 ± 1.50^{a}	7.67 ± 1.32^{ab}	6.02 ± 0.23^{b}	6.24 ± 0.16^{b}	8.90 ± 0.52^{a}	H = 14.89;*p = 0.021*
C18:3n-3	0.70 ± 0.03^{c}	1.16 ± 0.25^{bc}	1.45 ± 0.39^{b}	1.46 ± 0.29^{b}	1.32 ± 0.05^{b}	1.51 ± 0.04^{b}	2.42 ± 0.12^{a}	H = 14.98;*p = 0.020*
SFAs	75.86 ± 0.34^{a}	70.76 ± 0.89^{b}	69.85 ± 0.84^{b}	68.48 ± 0.74^{bc}	66.28 ± 0.41 ^{cd}	64.70 ± 0.74^{d}	56.29 ± 0.98^{e}	H = 18.72;*p = 0.005*
MUFAs	7.20 ± 0.09^{e}	9.93 ± 1.01^{d}	10.75 ± 0.35^{d}	13.93 ± 0.34^{c}	16.84 ± 0.19^{b}	19.36 ± 0.68^{b}	26.44 ± 0.64^{a}	H = 19.32;*p = 0.004*
PUFAs	10.13 ± 0.40^{ab}	9.94 ± 0.64^{ab}	10.79 ± 1.89^{a}	9.13 ± 1.62^{ab}	7.34 ± 0.28^{b}	7.75 ± 0.20^{b}	11.32 ± 0.64^{a}	H = 14.51;*p = 0.024*
n-3/n-6	0.07 ± 0.00^{e}	0.13 ± 0.01^{d}	0.15 ± 0.01^{d}	0.19 ± 0.00^{c}	0.22 ± 0.00^{bc}	0.24 ± 0.00^{ab}	0.27 ± 0.00^{a}	H = 19.50;*p* = 0.003
Identified FAMEs(%)	93.19 ± 0.48	90.63 ± 0.48	91.39 ± 0.62	91.53 ± 0.55	90.45 ± 0.37	91.80 ± 0.15	94.06 ± 0.07	–

FAMEs = fatty acid methyl esters; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; STA = statistical analyses; CF = chicken feed; R = rape cake. Means with different letters significantly differ (Kruskal-Wallis test followed by Dunn-Bonferroni post hoc test; $P < 0.05$).

strates to find a safe place for pupation allowing us to self-harvest them (Ortiz et al., 2016). Moreover, the prepupal feed seems to have a positive impact on the bacterial abundance and diversity of fish gut microbiota than larval feed leading to a potential improvement of fish health (Huyben et al., 2019; Liu et al., 2017). The first prepupae were observed after 15 rearing days on all diets. This prepupae occurrence timing was already reported for larvae reared on chicken feed (Diener et al., 2009; Gobbi et al., 2013). However, larvae fed on full oil cakes diets took twice as long to reach the prepupal stage. In addition to this growth delay, full oil cake diets also lead to lower prepupal weights. The texture of full oil cakes diets was more compact that all other diets. This physical parameter could reduce larval growth by limiting their movement but also their food intake as shown by Gobbi et al. (2013), who reported an extended growth (*i.e.* 33 days) of larvae fed on an excessively thick meat meal. This decrease in growth parameters should also be explained by the unbalance nutrient composition of mono-diets, known to decrease larval production in BSF (Rehman et al., 2017; Tschirner and Simon, 2015). One hypothesis could be related to the fiber content of flax and rape oil cakes which is known to decrease larval weight gain (Gao et al., 2019). Finally, the survival rate of larvae was high (±95%) and was not influenced by diets. Oonincx et al. (2015a) have also achieved high survival rates (72–86%) with larvae reared on different food by-products.

Table 5
Fatty acid profiles (% of total FAMEs) of black soldier fly farmed on flax cake enriched diets.

Diet	CF	F10	F20	F40	F60	F80	F100	STA
C10:0	1.63 ± 0.10^{a}	1.75 ± 0.05^{a}	1.66 ± 0.93^{a}	1.40 ± 0.05^{b}	1.41 ± 0.04^{b}	1.27 ± 0.03^{bc}	1.12 ± 0.07^{c}	H = 18.53;*p = 0.005*
C12:0	55.59 ± 0.48^{a}	54.54 ± 0.96^{a}	52.91 ± 0.07^{ab}	49.26 ± 3.94^{b}	48.51 ± 0.61^{b}	41.42 ± 1.14^{c}	40.41 ± 1.33^{c}	H = 18.65;*p = 0.005*
C14:0	6.90 ± 0.10^{a}	6.44 ± 0.06^{b}	6.22 ± 0.04^{b}	6.31 ± 0.15^{b}	6.49 ± 0.22^{b}	5.70 ± 0.20^{c}	5.50 ± 0.10^{c}	H = 17.94;*p = 0.006*
C14:1	0.00 ± 0.00^{b}	0.04 ± 0.03^{b}	0.05 ± 0.00^{b}	0.23 ± 0.22^{ab}	0.34 ± 0.25^{ab}	0.46 ± 0.02^{a}	0.28 ± 0.14^{ab}	H = 15.55;*p = 0.016*
C15:0	0.10 ± 0.00	0.12 ± 0.01	0.11 ± 0.05	0.10 ± 0.03	0.12 ± 0.04	0.15 ± 0.00	0.14 ± 0.00	H = 11.13;p = 0.084
C15:1	0.10 ± 0.01^{ab}	0.06 ± 0.06^{b}	0.05 ± 0.12^{b}	0.12 ± 0.03^{ab}	0.11 ± 0.03^{ab}	0.17 ± 0.01^{a}	0.18 ± 0.04^{a}	H = 13.61;*p = 0.034*
C16:0	10.03 ± 0.22^{a}	10.43 ± 0.10^{a}	10.24 ± 0.00^{a}	8.49 ± 0.63^{b}	8.15 ± 0.66^{bc}	7.60 ± 0.05^{bc}	7.24 ± 0.12^{c}	H = 18.67;*p = 0.005*
C16:1	0.24 ± 0.02 ^{cd}	0.25 ± 0.01 ^{cd}	0.20 ± 0.00^{d}	0.39 ± 0.16 ^{cd}	0.47 ± 0.14^{bc}	0.67 ± 0.04^{ab}	0.80 ± 0.00^{a}	H = 19.01;*p = 0.004*
C17:0	0.15 ± 0.00^{c}	0.19 ± 0.01^{a}	0.18 ± 0.00^{ab}	0.14 ± 0.01^{c}	0.14 ± 0.01^{c}	0.16 ± 0.01^{abc}	0.16 ± 0.01^{bc}	H = 16.35;*p = 0.012*
C17:1	0.12 ± 0.00	0.12 ± 0.02	0.10 ± 0.03	0.17 ± 0.03	0.14 ± 0.02	0.32 ± 0.18	0.24 ± 0.10	H = 10.82;p = 0.094
C18:0	1.45 ± 0.05^{bcd}	1.68 ± 0.08^{a}	1.58 ± 0.16^{ab}	1.35 ± 0.05 ^{cd}	1.29 ± 0.11^{d}	1.39 ± 0.05 ^{cd}	1.53 ± 0.06^{abc}	H = 17.04;*p = 0.009*
C18:1n-9	6.73 ± 0.14^{c}	7.00 ± 0.13^{c}	7.40 ± 0.77^{c}	9.13 ± 1.04^{b}	9.32 ± 0.65^{b}	11.11 ± 0.34^{a}	11.67 ± 0.40^{a}	H = 18.91;*p = 0.004*
C18:2n-6	9.43 ± 0.37^{a}	7.41 ± 0.41^{bc}	7.95 ± 0.18^{ab}	6.44 ± 0.68^{c}	6.18 ± 0.10^{c}	7.32 ± 0.71^{bc}	6.50 ± 0.34^{bc}	H = 16.38;*p = 0.012*
C18:3n-3	0.70 ± 0.03^{c}	2.12 ± 0.04^{c}	3.13 ± 0.32^{bc}	5.85 ± 1..38^{b}	6.46 ± 1.03^{b}	12.61 ± 1.44^{a}	15.27 ± 0.02^{a}	H = 19.29;*p = 0.004*
SFAs	75.86 ± 0.34^{a}	75.15 ± 0.60^{a}	72.91 ± 0.57^{a}	67.06 ± 2.70^{b}	66.12 ± 0.74^{b}	57.68 ± 0.74^{c}	56.10 ± 0.74^{c}	H = 18.84;*p = 0.004*
MUFAs	7.20 ± 0.09^{c}	7.47 ± 0.11^{c}	7.80 ± 0.14^{c}	10.04 ± 0.81^{b}	10.38 ± 0.60^{b}	12.73 ± 0.09^{a}	13.18 ± 0.31^{a}	H = 18.86;*p = 0.004*
PUFAs	10.13 ± 0.40^{b}	9.53 ± 0.45^{b}	12.30 ± 1.64^{b}	12.65 ± 1.01^{b}	13.67 ± 0.40^{b}	19.93 ± 2.15^{a}	21.77 ± 1.05^{a}	H = 16.28;*p = 0.012*
n-3/n-6	0.07 ± 0.00^{d}	0.29 ± 0.01^{d}	0.39 ± 0.00^{d}	0.90 ± 0.18^{c}	1.05 ± 0.17^{c}	1.72 ± 0.03^{b}	2.35 ± 0.01^{a}	H = 19.32;*p = 0.004*
IdentifiedFAMEs (%)	93.19 ± 0.48	92.15 ± 0.66	91.79 ± 1.27	89.40 ± 1.45	89.15 ± 1.09	90.34 ± 0.64	91.05 ± 0.34	–

FAMEs = fatty acid methyl esters; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; STA = statistical analyses; CF = chicken feed; F = flax cake. Means with different letters significantly differ (Kruskal-Wallis test followed by Dunn-Bonferroni post hoc test; $P < 0.05$).

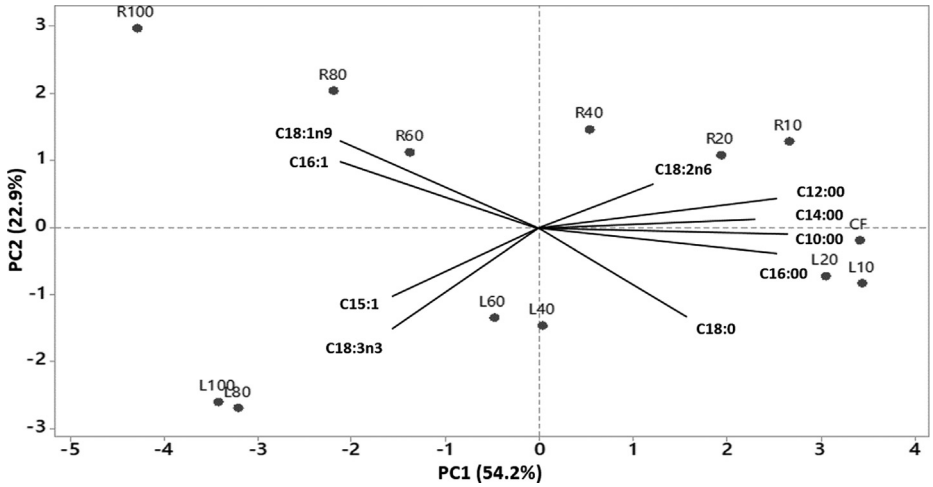

Fig. 2. Spatial distribution of BSF prepupae fatty acid profiles according to the feed source and the contribution of major fatty acids (score > 0.3).

4.2. Prepupae fatty acid profiles

The prepupae fatty acid profiles differed partially according to oil cakes incorporation in diets. Globally, they were mainly composed of SFAs (75.86 ± 0.34: CF), decreasing with the enrichment of oil cake in diets (Fig. 1). Spranghers et al. (2017) also showed in a nutrition experiment on contrasted diets (*i.e.* chicken feed, digestate, vegetable waste and restaurant waste) that prepupal fatty acid profiles are dominated by SFAs (64.8–82.8%). In this study, capric (C10:0), lauric (C12:0) and myristic acids (C14:0) were found in prepupae while they were absent or present in very low amounts in diets. It could be assumed that these fatty acids are synthesized by larvae. Among these SFAs, lauric acid (C12:0) was predominant in prepupal fatty acid profiles for all diets. This result is consistent with previous studies on BSF larval fatty acid profiles demonstrating that lauric acid (C12:0) synthesis is a BSF characteristic. This fatty acid seems partly made from carbohydrates and can increase following organic substrate decomposition (Ewald et al., 2020; Liland et al., 2017; Oonincx et al., 2015a; Rabani et al., 2019; Spranghers et al., 2017; Ushakova et al., 2016). In this study, lauric acid (C12:0) contents of prepupae decreased with oil cakes enrichment in their diets. The lauric acid (C12:0) levels will still be a limitation in attempts to modify the lipid profiles of prepupae (Oonincx et al.. 2015a). As also observed by Oonincx et al. (2019) in their experiments, the level of palmitic (C16:0) acid also decreased with the enrichment in oil cakes. MUFAs as tetradecenoic acid (C14:1), pentadecenoic acid (C15:1) and hepatodecenoic acid (C17:1) are missing in all diets and are mainly found in prepupae rearing on flax-enriched diet. According to Liland et al. (2017), part of MUFAs comes from the metabolism of PUFAs by larvae and the higher rates of MUFAs were obtained for the high - enriched flax cake diets in this study (Table 5. F80 - F100). The addition in BSF diets of rape cake, particularly rich in oleic acid (C18:1n-9), mainly led to an enrichment of this fatty acid in prepupae. This accumulation of C18:1n-9 in BSF from diets rich in this fatty acid was also highlighted by several studies (Liland et al., 2017; Oonincx et al., 2019, 2015a). Generally, the fatty acid composition of feed material used in the formulation of BSF larval diets are low in ω3 and therefore the larval profile also contains few ω3 fatty acid (Finke, 2013; Gao et al., 2019; Meneguz et al., 2018; Oonincx et al., 2015a). As the BSF larval fatty acid profiles can be partially oriented according to their diet, several ω3 enrichment tests have been performed with animal (fish offal or waste, fish meal or mussels) and vegetal (seaweed or flaxseed oil) byproducts and range from 2.99 to 16.5%

of ω3 PUFAs levels in larvae oils (0.74–9.7% of ALA, 0.0–8.2% of EPA and 0.0–4.9% of DHA) (Barroso et al., 2019, 2017; Cullere et al., 2019; Ewald et al., 2020; Liland et al., 2017; Oonincx et al., 2019; St-Hilaire et al., 2007). In this study, flax cake incorporation allows a significant enrichment of prepupae with ALA levels up till 15.2 7 ± 0.02% (F100). In comparison with the ALA levels (*i.e.* ≤ 3.6%) of BSF larvae fed with fish or marine byproducts, the ALA levels found in this study are quite acceptable while following the current legislation on insect rearing (Barroso et al., 2019, 2017; Yu and Sinnhuber, 1972). Moreover, while Oonincx et al. (2019) have shown that the incorporation of flaxseed oil in chicken feed diets could increase ALA levels in larvae up to 9.7%, the use of cakes instead of oil seems to be a more environmentally acceptable solution. A recent study by Giannetto et al. (2020) showed a variation in the fatty acid profile depending on the BSF developmental stage (last instar larvae: ∇5 vs prepupae) with an ALA content more than twice as high at V5 due to activity variations of enzymes involved in the fatty acids metabolism. These results broaden the potential for modulating BSF fatty acid profile depending on harvest stage. Finally, several studies have shown that the degree of larval enrichment in PUFAs generally follows the levels found into diet and that BSF seems not able to *de novo* synthesize PUFAs. It makes sense that EPA and DHA were not found in produced prepupae as these two fatty acids were absent from diets (Barroso et al., 2019, 2017; Ewald et al., 2020; Liland et al., 2017; Oonincx et al., 2019). The main purpose of ω3 enrichment of BSF larvae is a more effective use of their oil in fish nutrition with the lowest impact on fish growth performances and on fish fatty acid composition (Cardinaletti et al., 2019; Gasco et al., 2015). When they are eating fishes, consumers expected to eat products with high ω3 to ω6 ratios for health benefits. Generally, these ω3 are mainly composed of ALA, EPA and DHA while ω6 are mainly composed of LA (Colombo et al., 2018). As the lipid composition of tissue is directly influenced by dietary fatty acids composition in Salmonids, it is important to monitor ω3 and ω6 intakes in their food rations (Bell et al., 2002).

With a dual competition as substrates for desaturases and elongases between ω3 and ω6 PUFAs, the ratio of dietary ALA to LA is important (Betancor et al., 2014). By analyzing several research on long chain-polyunsaturated fatty acids synthesis in salmonids including rainbow trout, Colombo et al. (2018) concluded that an ALA/LA ratio of 1.03/1 is optimal for DHA storage. In this study, the ALA/LA ratio obtained for larvae fed on rape cake enriched diets remained below 1 with maximum value of 0.27 ± 0.00% (RH100). In contrast for the larvae fed on flax cake enriched diets, the ALA/LA ratio was>1 from 60% incorporation (F60: 1.05 ± 0.02%) and reached a maximum of 2.35 ± 0.01% for 100% incorporation (F100). Moreover, while dietary lipids (principally SFAs and MUFAs) favor the overall nutritional balance of fish and decrease the use of protein as an energy resource, too high SFAs feed formulation became poorly digestible and could interfere with polyunsaturated fatty acids digestibility (Caballero et al., 2002; Turchini and Francis, 2009). In this study, the PUFAs profiles of BSF prepupal oils varied according to the type of oil cake (*i.e.* rape and flax) and percentage of incorporation. As a reminder, when considering a BSF production with sound growth rates, the flax cake enriched diets significantly increased the prepupal ALA levels from 2.12 ± 0.04% (F10) to 12.61 ± 1.44% (F80) with a well-balanced ALA/LA ratio from a 60% flax cake incorporation rate. Based on this data, it is therefore reasonable to imagine fish diet formulation containing a proportion of ALA-enriched BSF prepupae. When considering the rainbow trout as fish farmed, feed formulation should not contain>30% of SFAs and should contain a minimum requirements of EPA and DHA of respectively 0.4 and 0.5% in their diet still coming from fish-based material (Caballero et al., 2002; NRC, 2011). Finally, current studies on BSF larvae nutrition use chicken feed as a base to formulate diets (Barragan-Fonseca et al., 2019; Barroso et al., 2017; Oonincx et al., 2019; Woods et al., 2019) and this control does not constitute a low-value food with a low ecological impact. In Europe, plant agricultural byproducts for insect feed are limited. The plant productions are mainly seasonal (*i.e.* fruit, vegetable and food crops) and some of their byproducts (*e.g.* beet pulp or corn residue) are already used in livestock feed. The oilseed byproducts (*i.e.* rape and flax cakes) used in this study showed high larvae growth and biomass production (to 80% of incorporation) and represent potential ingredients for BSF larvae mass production. These byproducts can be stored easily, are available over time and their nutritional composition varies slightly depending on the oil extraction process. In order to increase production profitability and reduce the ecological footprint production, other local plant by-products (*i.e.* spent grain) must be incorporated and tested to replace the value-added food (*i.e.* chicken feed) in larval diets.

5. Conclusion

Currently, the main reasons to promote insect mass rearing are the nutritional value of insects as well as their resource efficiency when converting organic matter into protein for feed or food. Besides focusing on the types of feed that maximize feed conversion and insect growth rate, it is important to select insect feed possessing economic and environmental sustainability. In this study, agricultural byproducts (rape and flax cakes) show a rapid BSF growth and allow a prepupal weight around 180 mg with incorporation rates up to 80% in their diet. The remaining 20% in these formulations consisted of chicken feed and other vegetable byproducts should be considered and tested to further reduce the ecological footprint. By omitting the SFAs that are predominantly synthesized by larvae, the larval fatty acid profiles globally reflect their diet profiles. The oil cakes enrichment in BSF diets reduced significantly their prepupal SFAs rates.

The rape cake incorporation leads mainly to oleic acid (C18:1n-9) enrichment which not sought in fish nutrition and low ALA level. Flax cake incorporation increase significantly ALA enrichment favorable to rainbow trout needs and allowing a potential increase of full-fat prepupae (*i.e.* whole prepupae without a delimitation step) in trout nutrition. Rainbow trout nutritional requirements have been widely studied as one of the earliest domesticated aquaculture species in Occident. The artificial feed formulations for this species are complex and involve both animal and plant-based inputs with complex interactions. The results obtained in the present study can, therefore, constitute a reflection basis for potential trout nutrition trials. Different BSF larval meal characteristics and their implications on fish nutrition (*e.g.* developmental stage, amino acid profiles, chitin content, microbiological risks or toxin bioaccumulation) are currently being studied and have to be included in these thoughts.

This research assesses the potential for progressive modification and enrichment of interest fatty acids in BSF oil prepupae from plant byproducts. In order to anticipate the modification of fatty acid profiles of these insects, it seems interesting to focus researches on the understanding of BSF lipid metabolism by using current genomic, proteomic or fatty acid labeling tools.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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