

RESEARCH ARTICLE

Contrasting effects of maltose and lactose on dietary lipid accumulation and fatty acid profiles in *Hermetia illucens* larvae (Stratiomyidae)

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Received 12 January 2026 | Accepted 11 July 2026 | Published online 30 July 2026

Abstract

The black soldier fly (*Hermetia illucens* L. 1758) is increasingly used for waste bioconversion due to its remarkable nutritional and metabolic plasticity. While substrate composition strongly influences larval lipid content and fatty acid (FA) profiles, the specific role of dietary carbohydrates remains unclear. This study examined the effects of maltose and lactose on lipid recruitment and storage in BSF larvae. Ten isolipidic and isoglucidic diets were formulated using chicken feed as a basal substrate, supplemented with 2% peanut or rapeseed oil and either 4% or 8% maltose or lactose. Larval lipid content increased with sugar inclusion, independently of sugar type. Maltose enhanced lauric acid (C12:0) accumulation. By contrast, lactose-fed larvae displayed similar FA profiles across inclusion levels. Nevertheless, net lipid removal from the substrate was lower in lactose-fed treatments, as reflected by the higher frass lipid content at the end of the experiment. Both sugars activated fatty acid synthesis, yet lactose appeared to additionally elicit delta-9 desaturase activity, increasing oleic acid (C18:1n9) levels. These results highlight how the type of carbohydrate modulates lipid deposition in BSF larvae. Manipulating dietary sugars could be a strategy to optimise larval lipid profiles for industrial applications. The study also outlines new hypotheses regarding carbohydrate-specific metabolic regulation in this species.

Keywords

black soldier fly – disaccharides – edible insect – fats

1 Introduction

Climate change, combined with population growth, compels us to reconsider our production and consumption patterns. In this context, organic by-products and wastes should be valorised to their highest potential –

that is, regarded as valuable sources of nutrients for animal feeding. Over the years, the black soldier fly (BSF), *Hermetia illucens* L. 1758, has been explored for its potential in organic waste management. The principle is simple: young BSF larvae are fed on plant-, animal-, or human-derived substrates, resulting in waste reduc-

tion and the production of mature larvae and frass (i.e. substrate residues mainly composed of insect feces) (Rehman *et al.*, 2023). BSF larvae are primarily composed of protein, lipids and chitin, the proportions of which are strongly influenced by the substrate composition (Spranghers *et al.*, 2017). Studies have revealed the exceptional dietary plasticity of BSF larvae, which can develop on a wide range of organic materials, including flax cake, spent grains, pomace, manure, offal, food leftovers and even microplastics (Abenaim *et al.*, 2025; Miranda *et al.*, 2019; Renna *et al.*, 2024; Spranghers *et al.*, 2017; St-Hilaire *et al.*, 2007). This diversity highlights the broad potential of BSF larvae worldwide and across various industrial sectors. Consequently, the biochemical composition of the larvae determines the industries that may use them as raw materials, including animal feed, cosmetic ingredients and biofuel production (Franco *et al.*, 2021; van Huis, 2020).

BSF must rely entirely on its body reserves to complete metamorphosis and reproduction, as the adult stage does not feed once larval growth is achieved. Lipid storage synthesis is particularly active during larval development, mainly through the digestion and absorption of carbohydrates and fats. Storage lipids are accumulated in an organ common to most insects – the fat body (Arrese and Soulages, 2010). In insects, the processes of nutrient digestion, absorption and fat body development are well documented (Arrese and Soulages, 2010; Canavoso *et al.*, 2001). In the gut, carbohydrases and lipases release monosaccharides, monoacylglycerols and free fatty acids, which are absorbed through the peritrophic membrane. Monoacylglycerols can then be esterified into diacylglycerols by the addition of either dietary fatty acids or newly synthesised fatty acids derived from carbohydrates. Once formed, diacylglycerols are transported to the fat body, where they are further esterified to form triacylglycerols (Canavoso *et al.*, 2001; Delamotte and Montagne, 2024). Although the digestion, absorption and storage of nutrients are well characterised in insect species such as *Drosophila melanogaster* M. 1830, the influence of diet composition on these metabolic pathways – and consequently on the final composition of BSF larvae – remains poorly understood (Carpentier *et al.*, 2024; Delamotte and Montagne, 2024; Hoc *et al.*, 2020). Most studies have focused on increasing the polyunsaturated fatty acid (PUFA) content of BSF larvae by supplementing the diet with PUFA-rich ingredients, demonstrating that the species can bioaccumulate these fatty acids (Hoc *et al.*, 2020; Liland *et al.*, 2017; St-Hilaire *et al.*, 2007). However, when larvae are reared on substrates

not enriched in PUFA, the correlation between dietary and larval fatty acid profiles is often weak, suggesting that other nutrients – such as carbohydrates – may also influence lipid absorption and/or fatty acid synthesis (Danieli *et al.*, 2019; Liu *et al.*, 2023; Meneguz *et al.*, 2018). Previous work (Carpentier *et al.*, 2024) demonstrated that the type of disaccharide modulates both performance and fatty acid composition in BSF larvae. Despite higher mortality and an extended development period, larvae fed lactose exhibited similar lipid content to those fed other soluble carbohydrates. Moreover, their fatty acid profiles were richer in unsaturated fatty acids, suggesting that storage lipids were mainly synthesised from dietary fatty acids. By contrast, maltose induced a fatty acid profile with a higher proportion of saturated fatty acids, primarily due to increased lauric acid synthesis.

In this study, we investigated the roles of lactose and maltose in the recruitment of dietary lipids for storage-lipid synthesis in *Hermetia illucens* larvae. Two main hypotheses were formulated: (1) larvae fed lactose primarily rely on dietary fatty acids for storage lipid synthesis, resulting in a final fatty acid profile like that of the diet; and (2) larvae fed maltose mainly use dietary soluble carbohydrates for lipid storage, leading to a higher proportion of saturated fatty acids. To test these hypotheses, ten isolipidic and isoglucidic diets were formulated. Chicken feed served as the basal diet and was supplemented on an as-fed basis with 2% peanut oil (PNO) or rapeseed oil (RSO), combined with maltose or lactose at inclusion levels of 4% (MAL4 and LAC4) and 8% (MAL8 and LAC8). PNO and RSO were selected for their respective richness in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

2 Materials and methods

Insect rearing

BSF larvae were obtained from the colony of the Functional and Evolutionary Entomology Laboratory of Gembloux Agro-Bio Tech, established in 2017, as described in Hoc *et al.* (2020). For experimental purposes, eggs were rigorously harvested at 24-h intervals and incubated in moistened chicken feed adapted for laying hens (77/420 Chicken pellet, Aveve, Leuven, Belgium) with a moisture content of 70%. After five days, the larvae were sieved (mesh size = 2 mm) from the substrate and counted by hand for experimental purposes.

TABLE 1 Formulation of experimental diets and energy value

Modality	Soluble carbohydrate inclusion rate	Chicken feed (g)	Cellulose (g)	Oil (g)	Soluble carbohydrate (g)	Water (g)	Total metabolisable energy (kcal)
Control	0.00%	37.80	12.60	–	–	75.57	134.64
PNO-CEL	0.00%	37.80	10.08	2.52	–	75.57	157.32
RSO-CEL							
PNO-LAC4	4.00%	37.80	5.04	2.52	5.04	75.57	177.48
PNO-MAL4							
RSO-LAC4							
RSO-MAL4							
PNO-LAC8	8.00%	37.80	–	2.52	10.08	75.57	197.64
PNO-MAL8							
RSO-LAC8							
RSO-MAL8							

CEL, cellulose; LAC, lactose; MAL, maltose; PNO, peanut oil; RSO, rapeseed oil. Diets containing either RSO or PNO combined with MAL or LAC at 4% or 8% inclusion levels. Control diets contained cellulose instead of soluble sugars and oils. For example, RSO-MAL8 denotes a diet containing rapeseed oil and 8% maltose. Quantities correspond to one replicate (100 larvae).

Feeding assays

A total of ten isonitrogenous, isolipidic and isoglucidic diets were prepared (Table 1). Chicken feed for laying hens (77/420 Chicken pellet, Aveve, Leuven, Belgium) was used as the basal ingredient. Carbohydrates were supplied either as microcrystalline cellulose (Sigma-Aldrich, Darmstadt, Germany), considered non-digestible for BSF larvae, or as soluble disaccharides: maltose (maltose monohydrate, Millipore Merck, Darmstadt, Germany) and lactose (lactose monohydrate, AnalaR Normapur, VWR International Europe, Leuven, Belgium) (Barragan-Fonseca *et al.*, 2018; Carpentier *et al.*, 2024). Oils were added as rapeseed oil (Vandemoortele, Ghent, Belgium) or peanut oil (Vandemoortele), chosen for their contrasting fatty acid profiles (PUFA-rich vs MUFA-rich).

Each diet contained 1.26 g of fresh feed per larva, corresponding to 37.80 g of chicken feed per 100 larvae, adjusted with cellulose, oil (2% (w/w) of the fresh diet), soluble sugar (0, 4, or 8% (w/w) of the fresh diet) and autoclaved deionised water (Milli-Q, Merck, Darmstadt, Germany) to reach approximately 63% moisture. Soluble sugars were first dissolved in deionised water, autoclaved and then weighed into the containers. Cellulose was used to balance carbohydrate content and to obtain isoglucidic diets across treatments. Metabolisable energy was estimated using Atwater factors (4 kcal/g for protein and carbohydrates, 9 kcal/g for lipids) based on the manufacturer's composition of the chicken feed, where carbohydrates in dry matter were

calculated as nitrogen-free extract (NFE = 100 – protein – fat – ash – fibre).

For each diet and replicate, substrate was prepared for 110 larvae per 750 ml plastic tray (17.20 × 11.50 × 6.00 cm; AVA, Temse, Belgium). Chicken feed, cellulose and oils were thoroughly mixed the day before larval inoculation to ensure complete absorption of the oils and prevent phase separation. The following day, the autoclaved sugar solutions were homogenised into the dry mixture to achieve the final inclusion levels. Then, a 12.60 g subsample was removed at day 0 for dry matter, total lipid and fatty acid profile analyses. The remaining substrate intended for 100 larvae was smoothed out. Trays were closed with perforated lids (central hole 1 × 1 cm) and covered with plastic mosquito netting (934226, CanDo, Hubo, Wommelgem, Belgium) to ensure aeration and prevent larval escape. The mean dry matter content of the diets was 37.07 ± 0.37% (n = 33) and treatment diets were confirmed to be isolipidic (8.67 ± 0.41% lipids on a dry matter basis). By contrast, the control diet contained 3.50 ± 0.16% lipids (Table A1 in the Appendix). Finally, the PNO diet contained more MUFA than the RSO diet, which contained more PUFA as expected (Table A2 in the Appendix).

Larval performance and conversion efficiency

Five-day-old larvae were obtained from eggs laid in the same rearing cage and collected precisely at 24-hour intervals. At the start of the experiment (D0), 100 larvae were hand-counted for each replicate and the populations were weighed using an STX223 scale (readability:

0.001 g; Ohaus Scout, Parsippany, NJ, USA). The mean initial weight of the larvae was approximately 7 mg and no significant difference was observed between populations at D0 (LM: $F_{10,32} = 0.122$, $p = 0.993$; Table A3 in the Appendix). After eight days (D8), weight measurements were taken. For each measurement, twenty larvae were extracted from the substrate. Larvae were washed using autoclaved water, dried on a paper towel and weighed. After weighing, the larvae were returned to the centre of the substrate. From day 10, the larvae were checked daily until the first prepupae appeared. At this point, trays were weighed; all larvae were collected, counted and weighed as described previously. Growing larvae (white larvae) and prepupae were separated and stored at -18°C for composition analysis. Approximately 10 g of frass samples (i.e. substrate mixed with feces) were also taken at the end of the experiment and stored at -18°C . The number of white larvae and prepupae constituted the survival of the replicate. The total weight of the population constituted the larval biomass. The mean weight of larvae was obtained by dividing the total weight of white larvae by the number of white larvae counted at the end of the experiment.

For each tray, the yield rate was calculated by dividing the larval biomass by the number of days until the appearance of the first prepupae. The reduction rate (RR) was calculated with the formula: $((D_{\text{tot-start}} - R_{\text{tot-end}})/D_{\text{tot-start}}) \times 100\%$ (Bosch *et al.*, 2020), where $D_{\text{tot-start}}$ and $R_{\text{tot-end}}$ are, respectively, the dry weight of substrate at the beginning and at the end of the experiment. The bioconversion efficiency corrected for residue (BER) was calculated by adapting the formula: $((L_{\text{end}} - L_{\text{start}})/(D_{\text{start}} - R_{\text{end}})) \times 100\%$, proposed by Bosch *et al.* (2020), where L_{end} represents the total dry weight of white larvae, L_{start} represents the dry weight of the larvae at the start of the experiment, D_{start} represents the dry weight of substrate at the start of the experiment and, R_{end} represents the dry weight of the residue at the end of the experiment. As L_{end} considered only the white larvae, L_{start} and D_{start} were adjusted to the number of white larvae collected at the end of the experiment. R_{end} was first adjusted to the survival rate and then to the number of white larvae. The dry matter content of L_{end} , L_{start} , D_{start} and R_{end} was estimated by weighing samples before and after freeze-drying.

Lipid content and fatty acid profile

Each frozen white larva was cut in half to prevent heterogeneous drying. The larvae and frass samples were placed in a FreeZone6 freeze-dryer (Labconco Corp., Kansas City, MO, USA) for 72 h and ground (IKA

A10, Staufen, Germany). Total lipids were extracted and quantified from 3 g of powder by Soxhlet extraction with diethyl ether for eight hours (AOAC Method 920.39). The dry matter of each freeze-dried sample was determined in duplicate using an infrared balance (MA 150, Sartorius, Göttingen, Germany) to adjust the total lipid amount.

Concerning the FA's profile, total lipids were extracted at room temperature to prevent degradation of unsaturated fatty acids using a fatty acid extraction kit (MAK114, Sigma-Aldrich) according to the manufacturer's instructions. Then, 1 ml solvent containing the total lipids of the sample was placed in a weighed 2 ml tube and concentrated for 1 h at 45°C (Concentrator 5301, Eppendorf, Hamburg, Germany). The concentrated samples were flushed with nitrogen to remove residual solvent. A lipid/chloroform solution (10 mg/ μL) was prepared. Finally, total lipids were transesterified under acidic conditions, as described by Carpentier *et al.* (2024). Samples were analyzed using a Trace GC Ultra instrument (Thermo Scientific, Waltham, MA, USA) equipped with a split/splitless injector (240°C) in split mode (split flow: 10 ml/min), an ionising flame detector (250°C) and a Stabilwax®-DA column (30 m, 0.25 mm ID, 0.25 μm , Restek., Bellefonte, PA, USA). The temperature program was as follows: 50°C for 1 min, increase to 150°C at $30^{\circ}\text{C}/\text{min}$, increase to 240°C at $4^{\circ}\text{C}/\text{min}$ and 240°C for 5 min. Hexane was used as a blank. A reference standard containing 37 fatty acid methyl esters was used for identification (Supelco 37-component FAME Mix, Sigma-Aldrich, Overijse, Belgium). The unidentified compounds were analysed by gas chromatography coupled to mass spectrometry to accurately detect fatty acids and rule out contaminants (see Table A4 in the Appendix for instrumental details).

Frass lipid mass and net lipid removal from the substrate

To evaluate net lipid removal from the substrate (LR), each rearing box was weighed throughout the experiment. At harvest, frass mass was determined by subtracting the tare weight of the box and the harvested larval biomass from the final box weight. Mean frass quantities are presented in Table A5 in the Appendix. Lipid concentration of the frass was determined on homogenised freeze-dried samples by proximate analysis (see section above). Frass lipid mass was calculated as the product of frass dry mass and lipid concentration. LR was estimated as the difference between the initial lipid mass supplied in the diet and the lipid mass remaining in the frass. To complement this measure-

ment, residual lipid mass in the frass and lipid mass recovered in harvested white larvae were also calculated and are reported in Table A5 in the Appendix. Because larval survival differed among treatments and prepupae were excluded from lipid analyses, these values were expressed as mg per collected white larva, using the number of harvested white larvae as the normalisation factor.

Statistical analysis

Raw results were processed using Excel (Microsoft, Redmond, WA, USA). Statistical analyses were conducted using R Studio (version 2023.12.1+402, Boston, MA, USA) for Windows. Data on larval weights, emergence of the first prepupae, production yields, RR and BER were analysed using a linear model (LM) because they follow a Gaussian distribution. Survival, studied with a binomial model, was evaluated using a General Linear Model (GLM) test. Normality and homoscedasticity were assessed using Shapiro tests on the residuals and the data dispersion (beta dispersion). Significant p -values ($p < 0.05$) from the LM or GLM tests were followed by an EMM test to pinpoint substantial differences between groups.

The complete fatty acid profiles were analysed using a permutational multivariate analysis of variance (i.e. PERMANOVA) using a Euclidean distance matrix and 999 permutations ("adonis2" command, R-package vegan (Oksanen *et al.*, 2020)). To account for repeated biological replicates, permutations were constrained within replicates (strata = Replicate). Marginal effects of dietary factors (oil type, sugar type and sugar inclusion level) were tested independently (by = "margin") to evaluate their individual contributions to fatty acid composition. Sugar type, oil type and sugar level were all found to significantly influence fatty acid profiles, with each factor explaining a distinct portion of the variance. The data were then visualised using principal component analysis (PCA). Significant differences in fatty acid profiles were further analysed using multivariate analysis. Key fatty acids driving these differences were identified by interpreting the correlation circles in Figure A1 in the Appendix. These candidate fatty acids were confirmed using analysis of variance (ANOVA), followed by a Tukey post hoc test with Bonferroni adjustment. Before analysis, normality was assessed using the Shapiro-Wilk test and homoscedasticity was checked using the Bartlett test. If both assumptions were met, comparisons were made using ANOVA and Tukey's test. Otherwise, a Kruskal-Wallis test followed by Dunn's post-hoc test was applied.

3 Results

Larval performance and conversion efficiency

Initial larval weight did not differ among treatments (≈ 7 mg per larva), confirming homogeneous starting conditions across diets (Table A3 in the Appendix). At day 8, control larvae reached 152.0 ± 7.6 mg and the addition of oil alone (PNO-CEL, RSO-CEL) did not significantly increase larval weight compared with the control. By contrast, diets containing 8% maltose (PNO-MAL8, RSO-MAL8) produced significantly heavier larvae than the corresponding 8% lactose diets, whereas 4% sugar inclusion (MAL4, LAC4) resulted in intermediate weights, similar to oil-only treatments. At the end of the growth period, PNO-MAL8 and RSO-MAL8 yielded the heaviest larvae, while PNO-LAC8 and RSO-LAC8 produced larvae with final weights comparable to the control (Table 2).

Survival was generally high ($\geq 90\%$ across all treatments) but lower in the PNO-LAC8 and RSO-LAC8 treatments than in the corresponding oil-only diets (EMM: $p \leq 0.001$; Table 2). The shortest development times (13 days) were observed for control and PNO-CEL larvae. Adding 4% soluble sugar did not significantly affect development time, whereas 8% lactose prolonged larval growth by more than two days. Diets containing 8% maltose increased final larval weight, but also slightly extended development compared with the control.

Total larval biomass per tray was strongly affected by sugar type and inclusion level. Without soluble sugars, only PNO-CEL increased biomass compared with the control. At 8% inclusion, maltose diets (PNO-MAL8, RSO-MAL8) produced more than 22 g of larvae per tray, clearly outperforming the corresponding lactose diets and the control, which remained around 18 g. Yield (g larvae per day) followed similar patterns: PNO-CEL showed the highest yield among sugar-free diets and the addition of 8% maltose markedly improved yield in RSO-based diets, whereas 8% lactose resulted in the lowest yields. RR and BER confirmed these trends, with maltose-based diets achieving higher substrate reduction and more efficient conversion of feed dry matter into larval biomass than lactose-based diets, particularly at the 8% inclusion level (Table 3).

Lipid content of the larvae and the frass

The inclusion of oil and soluble sugars in the diets affected the final lipid content of the larvae (Figure 1). Control larvae contained the lowest lipid levels, with $21.94 \pm 0.78\%$. The addition of 2% oil significantly increased lipid levels by about seven percentage points,

TABLE 2 Larval growth and survival of BSF

Soluble carbohydrate inclusion rate	Condition	Mean weight of larvae $\pm$ SD (mg)		Survival $\pm$ SD (%)	Mean time to first prepupae $\pm$ SD (day)
		Day 8	End of the development		
0%	Control	152.0 $\pm$ 7.6 ab	180.8 $\pm$ 1.8 d	98.25 $\pm$ 0.50 a	13.00 $\pm$ 0.00 b
	PNO-CEL	166.1 $\pm$ 9.7 a	210.5 $\pm$ 3.2 bc	99.25 $\pm$ 0.50 a	13.00 $\pm$ 0.00 b
	RSO-CEL	149.2 $\pm$ 21.9 ab	196.2 $\pm$ 11.5 cd	98.50 $\pm$ 1.29 a	14.50 $\pm$ 1.00 ab
4%	PNO-LAC4	153.2 $\pm$ 33.2 bc	212.3 $\pm$ 7.9 bc	97.00 $\pm$ 2.16 ab	14.75 $\pm$ 0.50 ab
	PNO-MAL4	160.7 $\pm$ 14.4 a	211.5 $\pm$ 4.0 bc	98.50 $\pm$ 1.91 a	15.25 $\pm$ 0.50 ab
	RSO-LAC4	152.7 $\pm$ 33.0 ab	211.0 $\pm$ 12.0 bc	95.25 $\pm$ 3.30 abc	15.25 $\pm$ 0.50 ab
	RSO-MAL4	160.3 $\pm$ 21.0 ab	214.0 $\pm$ 5.1 abc	98.00 $\pm$ 2.65 ab	14.67 $\pm$ 0.58 ab
8%	PNO-LAC8	95.8 $\pm$ 6.3 c	200.9 $\pm$ 6.5 cd	90.00 $\pm$ 2.50 c	16.33 $\pm$ 0.96 a
	PNO-MAL8	179.6 $\pm$ 20.4 a	228.7 $\pm$ 6.8 ab	97.25 $\pm$ 2.22 ab	16.00 $\pm$ 0.00 a
	RSO-LAC8	109.1 $\pm$ 2.2 bc	203.3 $\pm$ 15.4 c	92.25 $\pm$ 5.85 bc	15.75 $\pm$ 0.50 a
	RSO-MAL8	189.6 $\pm$ 10.0 a	235.3 $\pm$ 5.8 a	97.50 $\pm$ 0.58 ab	15.50 $\pm$ 0.58 ab
Statistical analysis		LM: $F_{10,32} = 8.258$ $p < 0.001$	LM: $F_{10,32} = 12.556$ $p < 0.001$	GLM: $\chi^2 = 87.923$, df = 10, $p < 0.001$	K-W: $\chi^2 = 32.261$, df = 10, $p < 0.001$

CEL, cellulose; LAC, lactose; MAL, maltose; PNO, peanut oil; RSO, rapeseed oil. Larvae were fed diets containing either RSO or PNO combined with MAL or LAC at 4% or 8% inclusion levels. Control diets contained cellulose instead of soluble sugars and oils. For example, RSO-MAL8 denotes a diet containing rapeseed oil and 8% maltose. Values are means $\pm$ SD. Within a column, means with different superscript letters differ significantly ($p < 0.001$). End of development corresponds to the day when the first prepupae were observed.

TABLE 3 Larval performance of BSF larvae

Soluble carbohydrate inclusion rate	Condition	Mean larval biomass $\pm$ SD (g)	Mean yield rate $\pm$ SD (g of larvae/day)	RR $\pm$ SD (%)	BER $\pm$ SD (%)
0%	Control	17.63 $\pm$ 0.19 d	1.36 $\pm$ 0.01 abcd	50.14 $\pm$ 0.49 abc	15.71 $\pm$ 0.31 c
	PNO-CEL	20.80 $\pm$ 0.27 abc	1.60 $\pm$ 0.02 a	53.74 $\pm$ 0.31 ab	19.12 $\pm$ 0.18 bc
	RSO-CEL	18.83 $\pm$ 1.40 bcd	1.31 $\pm$ 0.20 bcd	53.78 $\pm$ 1.51 ab	17.41 $\pm$ 0.56 c
4%	PNO-LAC4	20.59 $\pm$ 1.18 abc	1.40 $\pm$ 0.13 abc	46.59 $\pm$ 3.08 bcd	22.77 $\pm$ 0.93 a
	PNO-MAL4	20.97 $\pm$ 0.27 ab	1.38 $\pm$ 0.03 abc	46.94 $\pm$ 2.30 bcd	22.36 $\pm$ 0.38 ab
	RSO-LAC4	20.18 $\pm$ 1.47 abcd	1.33 $\pm$ 0.13 bcd	44.18 $\pm$ 1.48 cd	23.21 $\pm$ 1.63 a
	RSO-MAL4	20.96 $\pm$ 0.86 abc	1.43 $\pm$ 0.09 abc	45.22 $\pm$ 1.41 cd	24.08 $\pm$ 0.34 a
8%	PNO-LAC8	18.04 $\pm$ 0.87 cd	1.11 $\pm$ 0.02 d	43.03 $\pm$ 3.98 cd	24.71 $\pm$ 1.86 a
	PNO-MAL8	22.13 $\pm$ 1.07 a	1.38 $\pm$ 0.07 abc	56.65 $\pm$ 1.22 a	22.20 $\pm$ 0.59 ab
	RSO-LAC8	18.78 $\pm$ 2.07 bcd	1.19 $\pm$ 0.12 cd	41.78 $\pm$ 3.13 d	25.26 $\pm$ 1.80 a
	RSO-MAL8	22.97 $\pm$ 0.40 a	1.48 $\pm$ 0.08 ab	56.23 $\pm$ 1.58 a	22.44 $\pm$ 0.71 ab
Statistical analysis		LM: $F_{10,32} = 9.507$, $p < 0.001$	LM: $F_{10,32} = 6.796$, $p < 0.001$	LM: $F_{10,32} = 13.169$, $p < 0.001$	LM: $F_{10,32} = 19.982$, $p < 0.001$

BER, bioconversion efficiency corrected for residue; CEL, cellulose; LAC, lactose; MAL, maltose; PNO, peanut oil; RR, reduction rate; RSO, rapeseed oil. Larvae were fed diets containing either RSO or PNO combined with MAL or LAC at 4% or 8% inclusion levels. Control diets contained cellulose instead of soluble sugars and oils. For example, RSO-MAL8 denotes a diet containing rapeseed oil and 8% maltose. Values are means $\pm$ SD. Within a column, means with different superscript letters differ significantly ($p < 0.001$). Mean larval biomass at the day when the first prepupae were observed.

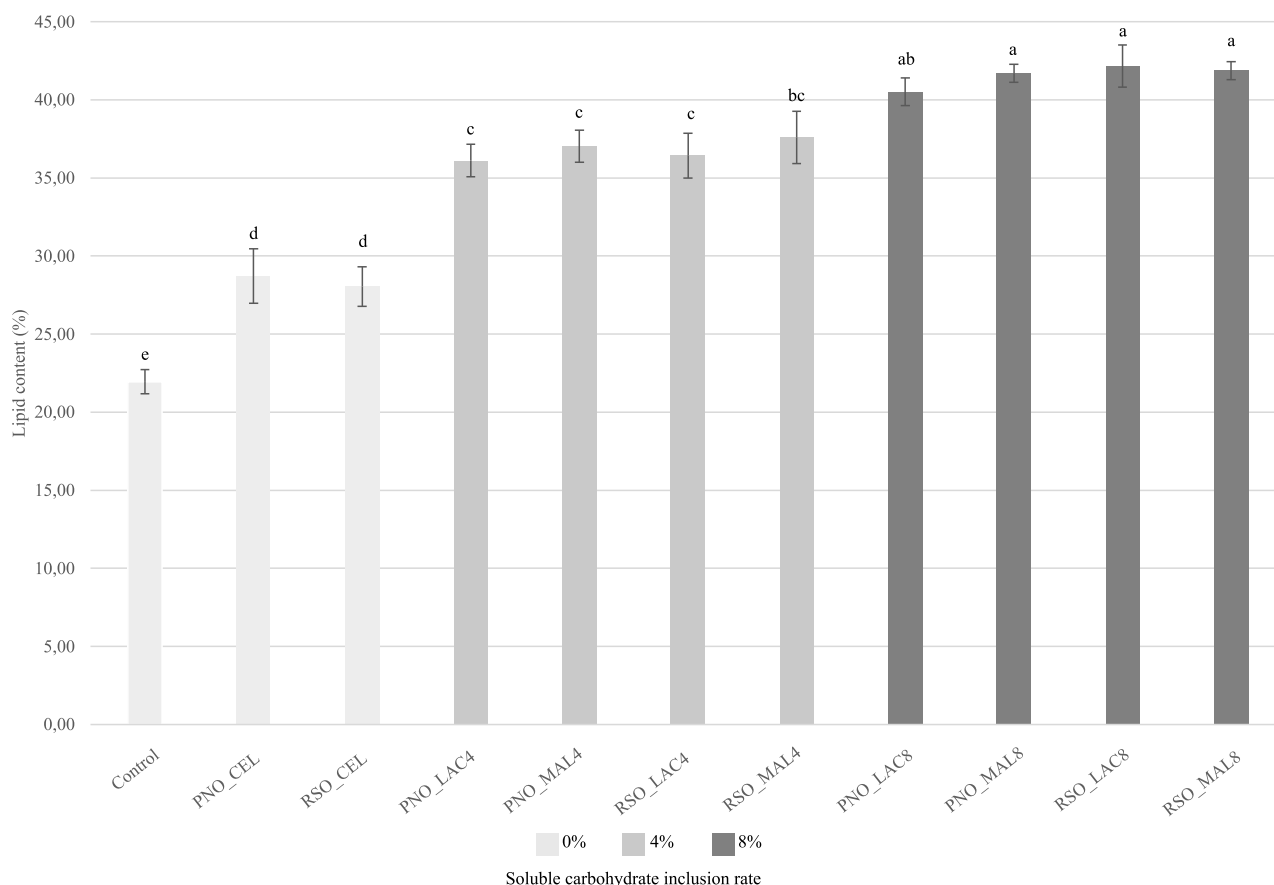


FIGURE 1 Total lipid content of BSF larvae fed maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO-CEL) and peanut oil (PNO-CEL). Soluble sugars and oils were replaced by cellulose in the control substrate. The results are expressed as a percentage of dry matter. Bar plot letters indicate the different groups for total lipid content ($F_{10,32} = 128.97, p < 0.001$).

reaching $28.71 \pm 1.74\%$ and $28.04 \pm 1.27\%$ for PNO and RSO, respectively. The inclusion of soluble sugars also increased larval lipid content, with a similar effect observed in both MAL and LAC. Higher inclusion levels resulted in higher lipid contents, reaching over 35% and 40% for sugar additions of 4% and 8%, respectively. The effect of sugar inclusion rate was statistically significant for all treatments, except for RSO-MAL4 and PNO-LAC8. A similar pattern was observed when larval lipid mass was expressed on a per collected white larva basis (Table A5 in the Appendix). Control larvae contained the lowest lipid mass (12.35 ± 0.51 mg per larva), whereas MAL8 treatments exhibited the highest values (35.33 ± 0.72 and 36.13 ± 1.28 mg per larva for PNO and RSO, respectively).

At the end of larval development, the inclusion of soluble sugars increased frass lipid content (Figure 2). Interestingly, LAC8 treatments (PNO-LAC8 and RSO-LAC8) showed frass lipid content approximately twice that observed in the other sugar-supplemented diets. Based on frass lipid mass, net lipid removal from the

substrate (LR) was estimated as the difference between initial and residual (frass) lipid mass. Oil-only treatments showed the highest LR. Diets supplemented with 4% soluble sugars resulted in the removal of approximately 4 g of lipids, whereas LAC8 treatments resulted in approximately 3 g of LR (Figure 2). Residual lipid mass in the frass, expressed per collected white larva (Table A5 in the Appendix), was significantly higher in LAC8 treatments (18.79 – 19.50 mg per larva) than in all other diets, while the control treatment exhibited the lowest residual lipid mass (1.57 ± 0.54 mg per larva).

Fatty acid profile of the larvae

PNO and RSO and their combinations with MAL and LAC, modulated the fatty acid profiles of BSF larvae (SFA: $F_{10,32} = 27.633, p < 0.001$; MUFA: $F_{10,32} = 28.219, p < 0.001$; PUFA: $F_{10,32} = 37.849, p < 0.001$; Figure 3). SFAs were predominant across all treatments. The lowest SFA levels were observed in the oil-only treatments (PNO-CEL = $56.38 \pm 0.99\%$; PNO-RSO = $54.26 \pm 0.51\%$). The addition of soluble sugars significantly increased

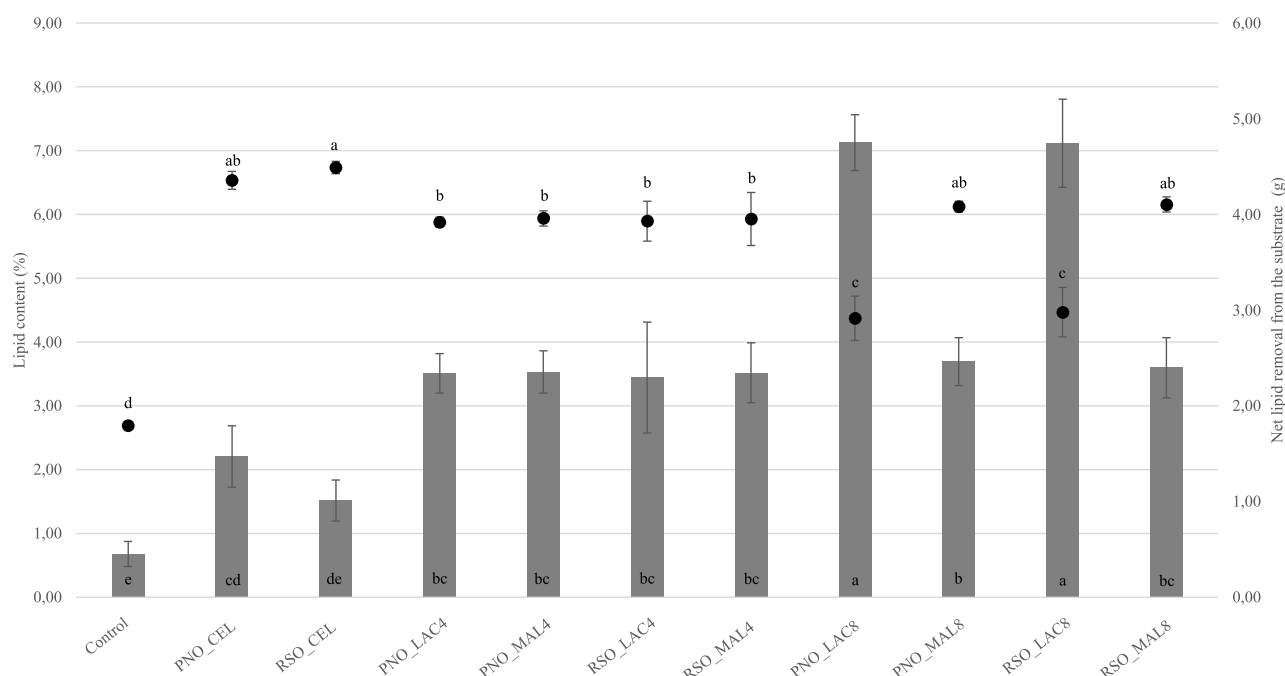


FIGURE 2 Bar plots indicating lipid content (%) of BSF frass of larvae fed maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO-CEL) and peanut oil (PNO-CEL). Soluble sugars and oils were replaced by cellulose in the control substrate. The results are expressed as a percentage of dry matter. Bar plot letters indicate the different groups for total lipid content ($F_{10,32} = 48.78, p < 0.001$). Grey spots indicate the net lipid removal from the substrate (g). Close-up letters indicate differences among groups in consumed lipid ($F_{10,32} = 82.91, p < 0.001$).

SFA content. Regarding inclusion levels, at 8%, MAL resulted in larvae richer in SFAs than those fed LAC. Oil type influenced unsaturated fatty acid proportions: RSO, richer in PUFAs than PNO (Table A2 in the Appendix), led to higher larval PUFA content. For a given sugar, larvae fed RSO consistently showed higher PUFA levels than those fed PNO.

The principal component analysis (PCA) of the fatty acid profiles is presented in Figure 4. The first component accounted for 55.7% of the variance and, in order of importance, included lauric acid (C12:0), myristic acid (C14:0), stearic acid (C18:0), oleic acid (C18:1n9), UND1 and C18:2n6 c10t12 (Figure A1 in the Appendix). Treatments were distributed along this dimension according to the amount of sugar added. PNO-CEL and RSO-CEL were located on the right and opposed the modalities containing soluble sugars.

The second component accounted for 19.3% of the variance and, in order of importance, included α -linolenic acid (C18:3n3) and linoleic acid (C18:2n6) (Figure A1 in the Appendix). Treatments were distributed along the second dimension by oil type. Specifically, the use of RSO increased the C18:3n3 content in the larvae, positioning the samples in the upper part. PNO showed lower C18:3n3 content, with treatments positioned toward the lower end of the graph. These observations were confirmed by comparing C18:3n3 levels,

which were significantly higher in the RSO groups ($F_{10,32} = 87.92, p < 0.001$; Table 4).

PERMANOVA with marginal effects and permutations constrained within replicates revealed that oil type ($R^2 = 0.12, F_{1,34} = 32.71, p < 0.001$), sugar type ($R^2 = 0.07, F_{1,34} = 20.01, p < 0.001$) and sugar inclusion level ($R^2 = 0.07, F_{1,34} = 19.21, p < 0.001$) all significantly influenced larval fatty acid composition. Sugar type explained a smaller proportion of variance when tested independently of oil type, highlighting the independent contribution of each dietary factor. Consistent with these results, treatments without soluble sugars each formed a distinct group. The addition of 4% and 8% maltose (MAL) significantly altered the fatty acid profiles, placing the groups increasingly distant from PNO-CEL and RSO-CEL, primarily due to elevated C12:0 levels. Specifically, PNO-CEL and RSO-CEL treatments had the lowest C12:0 levels (<40%), whereas MAL-supplemented treatments had significantly higher C12:0 levels (45–55%; Table 4). Lactose (LAC) also affected fatty acid profiles; however, these profiles did not differ across inclusion levels or oil types. For a given oil, treatments containing 4% MAL produced profiles similar to those of larvae fed LAC.

Regarding MUFAs, the highest C18:1n9 levels were observed in PNO-CEL and RSO-CEL (>20%, Table 4), whereas the lowest levels occurred in MAL-RSO8

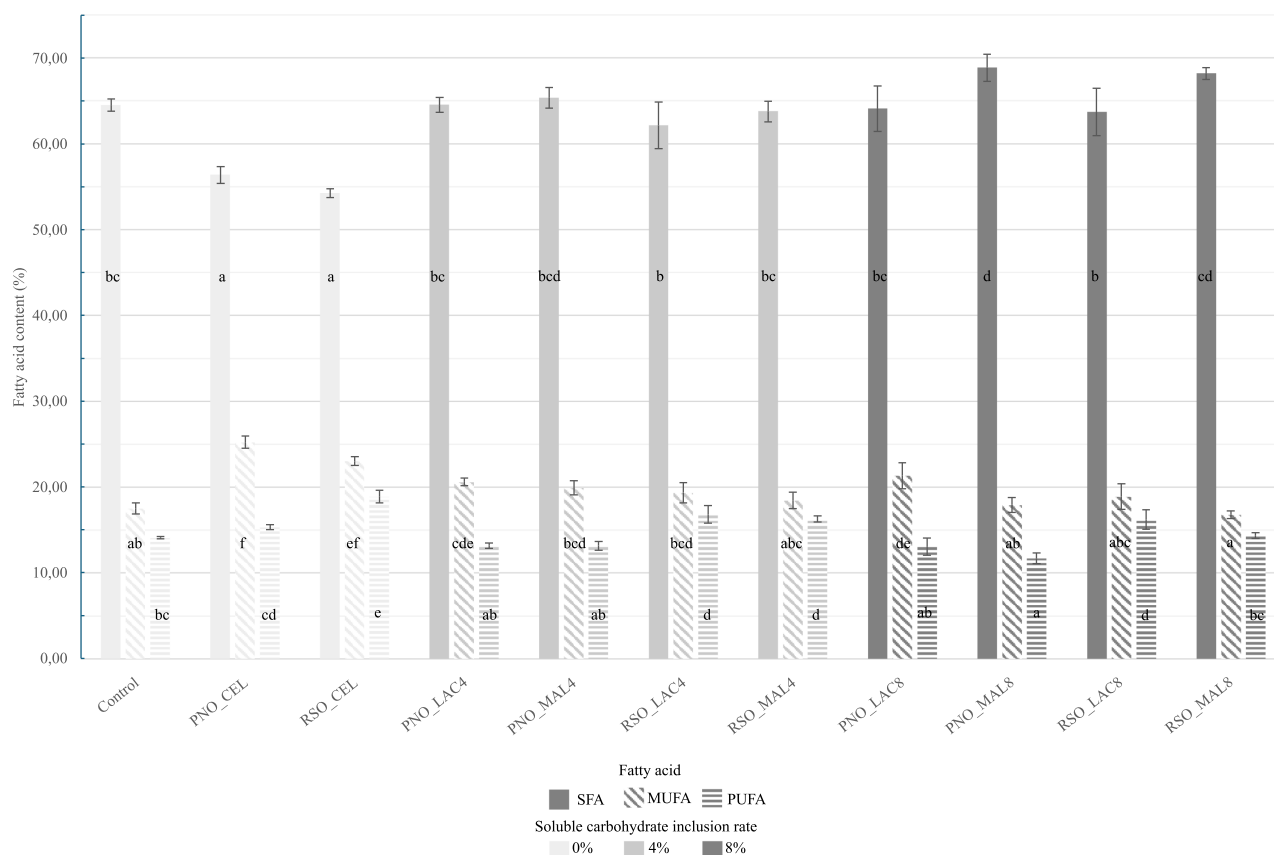


FIGURE 3 Fatty acid composition of BSF fed maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO-CEL) and peanut oil (PNO-CEL). Soluble sugars and oils were replaced by cellulose in the control substrate. The results are expressed as a percentage of total FAME. Bar plot letters indicate the different groups for fatty acid content (saturated fatty acid (SFA): $F_{10,32} = 27.633$, $p < 0.001$; monounsaturated fatty acid (MUFA): $F_{10,32} = 28.219$, $p < 0.001$; polyunsaturated fatty acid (PUFA): $F_{10,32} = 37.849$, $p < 0.001$).

($15.23 \pm 0.38\%$) and the control ($12.57 \pm 0.63\%$). By contrast, the control showed the highest C16:1 content ($4.92 \pm 0.17\%$).

For PUFAs, C18:2n6 levels were only slightly affected by soluble sugar addition. Interestingly, a linoleic acid isomer (C18:2n6 c10t12; Figure A2 in the Appendix) was mainly detected in treatments without soluble sugars. A similar pattern was observed for an unidentified PUFA (UND1).

4 Discussion

In this study, populations of 100 BSF larvae ($n = 4$) were reared on eleven chicken feed-based diets. The main objective was to assess the effects of maltose (MAL) and lactose (LAC) on BSF larval performance and fatty acid composition. Specifically, the influence on dietary fatty acid incorporation was evaluated by combining soluble sugars with peanut oil (PNO) and rapeseed oil (RSO), which are, respectively, rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

MAL and LAC were incorporated at inclusion levels of 4% (4) and 8% (8) on an as-fed basis and the diets were compared with those containing oil combined with cellulose (CEL) instead of soluble sugars. Soluble sugars and oils were entirely replaced by cellulose to produce a control substrate.

Regarding performance, the control diet produced typical results, with a growth period of 13.00 ± 0.00 days, nearly maximal survival and an individual larval weight of 180.8 ± 1.8 mg (Nguyen *et al.*, 2013; Carpentier *et al.*, 2024). A low content of digestible carbohydrates and proteins is known to shorten the development time of BSF larvae (Barragan-Fonseca *et al.*, 2019). In this study, it was observed that the energy value of the diet, when oil was combined with soluble sugars, tended to extend the growth period and produce heavier larvae. Regarding the effects of soluble sugars, our results align with previous studies that investigated the protein: carbohydrate ratio by including starch (Barragan-Fonseca *et al.*, 2019, 2021; Cammack and Tomberlin, 2017). Despite the diverse effects of carbohydrates, yield rates (i.e. larval biomass produced per tray per unit time) remained

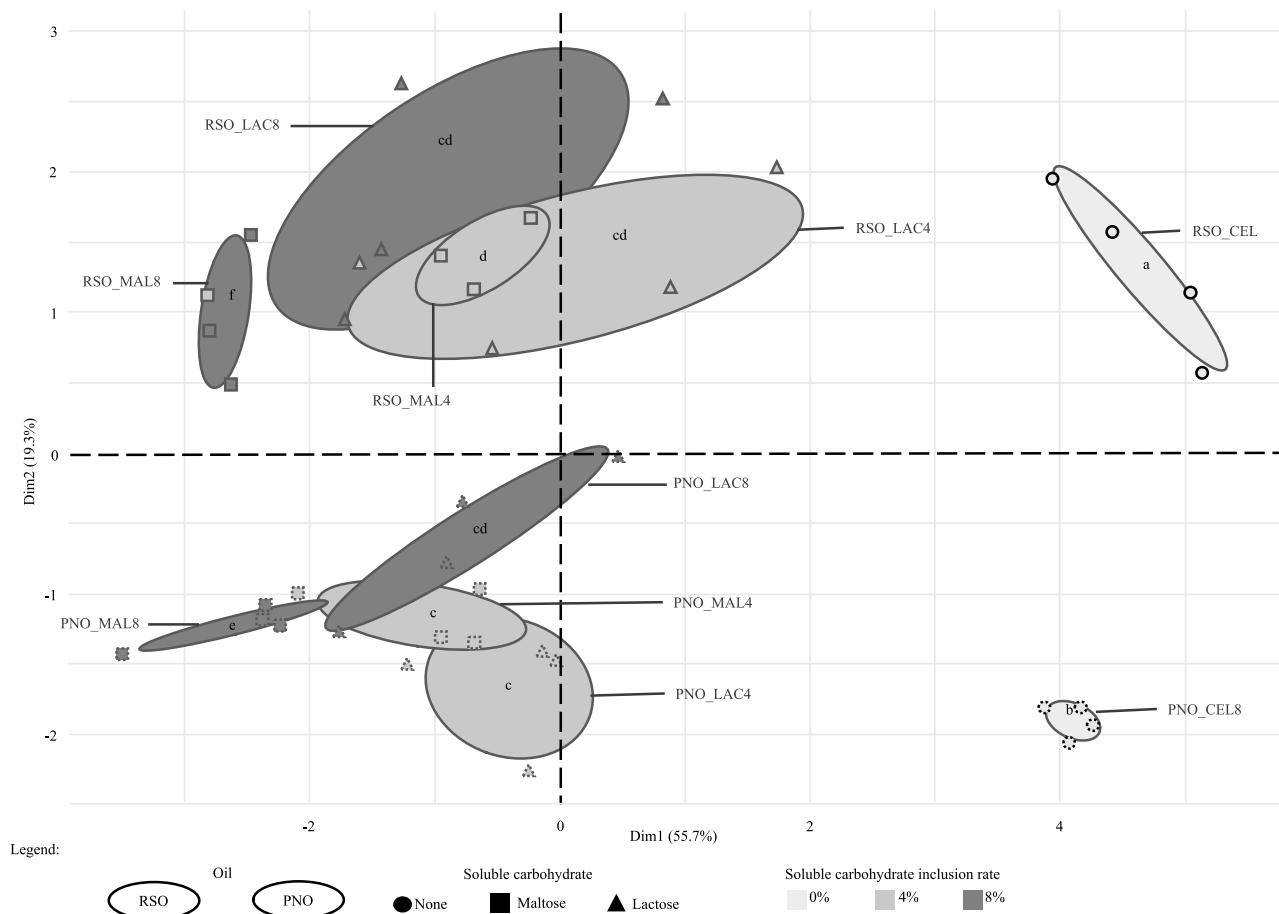


FIGURE 4 PCA score plot for fatty acid profiles of BSF larvae fed maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO-CEL) and peanut oil (PNO-CEL).

similar, as the positive effect on total larval weight per batch compensated for the extended growth period and regarding the impact of lipids, Bellezza Oddon *et al.* (2022) observed that increasing lipid content by including cocoa butter in isoenergetic diets accelerated growth. In our study, the development time of PNO-CEL was similar to that of the control diet, despite an increase in final individual weight and lipid content. However, RSO-CEL did not produce heavier larvae, highlighting that lipid composition is also a key factor in BSF larval development. Interestingly, in the absence of soluble sugars, dietary lipids impacted yield. Larvae-fed peanut oil (PNO-CEL) grew faster and became heavier than those fed rapeseed oil (RSO-CEL), leading to a higher yield. Recently, palmitic acid (C16:0) was found to enhance the growth and final weight of BSF larvae, which aligns with our results, as peanut oil is richer in C16:0 than rapeseed oil (Li *et al.*, 2022).

Regarding conversion efficiency, RR values were similar to those reported in previous studies (Broeckx *et al.*, 2021; Resconi *et al.*, 2024). In the present study, PNO-LAC8 and RSO-LAC8 exhibited the lowest RR. This

result can first be partly attributed to the reduced survival observed in these treatments. In addition, larvae from PNO-LAC8 and RSO-LAC8 were smaller, which is consistent with the slightly higher BER values observed for these modalities. Finally, the reduction in RR may be partly explained by the higher lipid content measured in the frass of these treatments. However, the increase in residual lipid mass accounted for only a fraction of the increase in total residual dry matter. Together with the lower larval biomass production observed in these treatments, this suggests that the utilisation of dietary components other than lipids may also have been impaired under high lactose inclusion. In line with this, Zhang *et al.* (2025) reported that the conversion efficiency in BSF is strongly influenced by the protein-to-energy ratio (mg/kJ), suggesting that an optimal balance can be identified for a given energy source. However, our results indicate that once a certain level of soluble sugar is reached in the diet, the nature of the sugar may become a more decisive factor influencing conversion efficiency than protein-to-energy ratio. Regarding the BER values, the control diet yielded results like those reported by

TABLE 4 Fatty acid profile of BSF larvae

	Control	PNO_CEL	RSO_CEL	PNO_LAC4	PNO_MAL4	RSO_LAC4	RSO_MAL4	PNO_LAC8	PNO_MAL8	RSO_LAC8	RSO_MAL8	Statistical analysis
Cl0:0	1.71 ± 0.19 a	1.54 ± 0.24 ab	1.39 ± 0.16 ab	1.33 ± 0.10 ab	1.28 ± 0.03 ab	1.34 ± 0.15 ab	1.24 ± 0.09 ab	1.21 ± 0.12 b	1.47 ± 0.07 ab	1.25 ± 0.11 ab	1.39 ± 0.19 ab	KW: $\chi^2 = 23.25$, df = 10, $p < 0.05$
Cl2:0	43.20 ± 0.66 c	37.95 ± 1.33 d	36.77 ± 0.56 d	46.26 ± 1.15 c	47.45 ± 1.49 abc	44.67 ± 3.33 c	46.47 ± 1.27 bc	46.59 ± 2.79 c	51.80 ± 1.95 a	46.79 ± 2.89 c	51.54 ± 0.9 ab	$F_{10,32} = 24.45$, $p < 0.001$
Cl4:0	4.84 ± 0.10 c	3.75 ± 0.15 d	3.75 ± 0.16 d	4.88 ± 0.25 bc	5.14 ± 0.18 abc	4.98 ± 0.24 bc	5.17 ± 0.13 abc	5.13 ± 0.09 abc	5.31 ± 0.06 ab	5.33 ± 0.25 ab	5.51 ± 0.19 a	$F_{10,32} = 44.393$, $p < 0.001$
Cl6:0	12.77 ± 0.24 a	11.05 ± 0.53 b	10.29 ± 0.18 bc	10.22 ± 0.34 bc	9.81 ± 0.25 cd	9.35 ± 0.56 de	9.17 ± 0.10 def	9.44 ± 0.28 cde	8.83 ± 0.28 ef	8.82 ± 0.23 ef	8.46 ± 0.32 f	$F_{10,32} = 54.05$, $p < 0.001$
Cl6:1	4.92 ± 0.17 a	1.65 ± 0.07 bc	1.84 ± 0.15 b	1.10 ± 0.10 g	1.21 ± 0.01 fg	1.29 ± 0.12 efg	1.31 ± 0.10 defg	1.54 ± 0.12 bcde	1.46 ± 0.07 cdef	1.64 ± 0.13 bcd	1.53 ± 0.16 cde	$F_{10,32} = 322.39$, $p < 0.001$
Cl8:0	2.00 ± 0.10 bc	2.09 ± 0.07 ab	2.06 ± 0.09 bc	1.86 ± 0.13 bcd	1.69 ± 0.17 cde	1.83 ± 0.25 bcde	1.74 ± 0.11 bcde	1.74 ± 0.19 bcde	1.47 ± 0.10 ef	1.54 ± 0.21 def	1.30 ± 0.02 f	$F_{10,32} = 11.62$, $p < 0.001$
Cl8:1n9	12.57 ± 0.63 g	23.56 ± 0.73 a	21.17 ± 0.58 c	19.48 ± 0.39 bcd	18.68 ± 0.82 cde	18.03 ± 1.09 cde	17.12 ± 0.91 def	19.77 ± 1.43 cd	16.44 ± 0.83 ef	17.23 ± 1.36 def	15.23 ± 0.38 f	$F_{10,32} = 43.59$, $p < 0.001$
UND1	1.24 ± 0.32 ab	1.10 ± 0.32 ab	1.65 ± 0.21 a	0.34 ± 0.07 bc	0.33 ± 0.02 bc	0.71 ± 0.21 abc	0.67 ± 0.04 abc	0.34 ± 0.02 bc	0.22 ± 0.06 c	0.62 ± 0.13 abc	0.42 ± 0.13 abc	KW: $\chi^2 = 37.96$, df = 10, $p < 0.05$
Cl8:2n6	9.66 ± 0.44 e	12.74 ± 0.42 abc	14.02 ± 0.83 a	12.25 ± 0.28 dc	12.32 ± 0.47 cd	13.94 ± 0.61 ab	13.85 ± 0.23 abc	12.28 ± 0.92 cd	11.13 ± 0.60 de	13.40 ± 0.98 abc	12.40 ± 0.34 bcd	$F_{10,32} = 17.52$, $p < 0.001$
Cl8:3n3	0.59 ± 0.08 c	0.71 ± 0.20 c	2.64 ± 0.08 a	0.71 ± 0.14 c	0.64 ± 0.08 c	2.57 ± 0.40 b	2.20 ± 0.13 ab	0.73 ± 0.09 c	0.55 ± 0.05 c	2.45 ± 0.36 a	1.90 ± 0.04 b	$F_{10,32} = 87.92$, $p < 0.001$
Cl8:2n6 c10t12	3.83 ± 0.47 a	1.85 ± 0.20 a	2.21 ± 1.42 a	0.17 ± 0.11 ab	0.17 ± 0.04 ab	0.30 ± 0.03 ab	0.21 ± 0.06 ab	0.00 ± 0.00 b	0.00 ± 0.00 b	0.35 ± 0.21 ab	0.00 ± 0.00 b	KW: $\chi^2 = 37.58$, df = 10, $p < 0.05$
Σ	97.32 ± 0.24 e	97.98 ± 0.37 de	97.79 ± 0.23 e	98.59 ± 0.42 cd	98.71 ± 0.29 bcd	99.00 ± 0.41 abc	99.14 ± 0.13 abc	98.76 ± 0.31 bc	98.68 ± 0.27 bcd	99.39 ± 0.21 ab	99.67 ± 0.26 a	$F_{10,32} = 21.57$, $p < 0.001$

Means ± SD within a column followed by different letters are significantly different ($p < 0.05$). χ^2 are presented for Kruskal-Wallis test. F values are presented for LM test. Unidentified fatty acid is named

UND. CEL, cellulose; LAC, lactose; MAL, maltose; PNO, rapeseed oil; RSO, rapeseed oil. Larvae were fed diets containing either RSO or PNO combined with MAL or LAC at 4% or 8% inclusion levels.

Control diets contained cellulose instead of soluble sugars and oils. For example, RSO-MAL8 denotes a diet containing rapeseed oil and 8

Resconi *et al.* (2024) for the Gainesville diet, another commonly used control diet for BSF. The inclusion of oil, combined with soluble sugar, induced a significant increase in BER compared to the control, indicating that the larvae effectively converted both lipids and soluble carbohydrates. However, BER was not proportional to the sugar inclusion level. This suggests that under our experimental conditions, the maximum metabolisable energy per larva ranged from 1.35 to 1.78 kcal.

To better understand the variations in larval lipid content and fatty acid profiles, it is essential to place these results in the context of lipid digestion and metabolism. Fatty acids in refined plant oils are mainly esterified with glycerol to form triglycerides (TAG), the principal lipid class in both plants and animals. In each TAG molecule, glycerol binds three fatty acids at specific positions, called sn-1, sn-2 and sn-3. For instance, in rapeseed and peanut oils, linoleic acid is predominantly located at the sn-1 or sn-3 position (Dong *et al.*, 2015; Wei *et al.*, 2019). To be absorbed by BSF larvae, fatty acids must first be released from TAGs by lipases (Canavoso *et al.*, 2001; Delamotte and Montagne, 2024; Terra and Ferreira, 2012). Digestion produces diacylglycerides (DAG), monoacylglycerides (MAG) and free fatty acids (FFA). Insect lipases preferentially hydrolyse fatty acids from the sn-1 and sn-3 positions, which allows fatty acids remaining at sn-2 to accumulate (Delamotte and Montagne, 2024; Garrido *et al.*, 2015). FFAs can be degraded or isomerised by gut microorganisms, a process affecting PUFAs, but also stearic acid (C18:0) and palmitic acid (C16:0), which can be metabolised to C17:0 and C15:0, respectively (Hoc *et al.*, 2020; Renna *et al.*, 2024). Then, FFAs should be absorbed and incorporated into DAGs. This process helps explain the presence of uncommon fatty acids, such as conjugated linoleic acid (C18:2n6 c10t12), in larvae. The influence of TAG profiles and isomerisation on fatty acid absorption in BSF remains understudied and warrants further research.

High lactose inclusion had an adverse effect in combination with peanut oil during larval growth, resulting in lower yield rates, primarily due to reduced survival. A similar effect was observed by Carpentier *et al.* (2024), supporting the potential toxicity of lactose to BSF larvae. Excess dietary sugar can be deleterious to animals due to the intracellular formation of advanced glycation end products (AGEs) (Garrido *et al.*, 2015). In healthy animals, AGE production is limited by the utilisation of dietary sugars for fatty acid synthesis and, ultimately, TAG formation. Saturation of this pathway can lead to metabolic disorders such as type 2 diabetes. In *D. melanogaster*, Garrido *et al.* (2015) demonstrated

that TAG synthesis constitutes a metabolic strategy to mitigate the toxicity of high dietary sugar levels. They also showed that it delays development by a few days, consistent with observations in this study. This developmental delay may result from the need for detoxification through the expression of acetyl-CoA carboxylase (ACC) and fatty acid synthases (FASNCG3523 and FASNCG3524). Investigating the expression of the glyoxalase system in BSF larvae could provide insights into the differential toxic effects of lactose and maltose in this model. Furthermore, defining the dietary sugar threshold above which BSF larvae are forced to continue lipid storage synthesis could be a critical parameter for rearing optimisation. While high carbohydrate levels may produce larger larvae, they can also increase mortality and reduce production yield by prolonging batch duration.

At the end of the experiment, frass from larvae fed 8% lactose contained unexpectedly high lipid levels. Together with the lower RR values observed for these treatments, this indicates reduced overall substrate utilisation and suggests that LR was lower with high lactose inclusion. However, the increase in residual lipids alone does not fully account for the higher residual dry matter observed in these treatments. This suggests that the utilisation of other dietary nutrients may also have been affected by high lactose inclusion, contributing to the lower RR values. As a reminder, for assimilation, TAG must first be emulsified and then hydrolysed by lipases into DAG, MAG and FFA (Delamotte and Montagne, 2024). Regarding emulsification, TAGs are nonpolar molecules, so lipases act at the lipid-aqueous interface in the gut. Since insects do not produce bile salts, various strategies have evolved to enhance the accessibility of dietary fats to lipases (Turunen, 1979; Verger *et al.*, 1973). In *Musca domestica* L. 1758, it has been proposed that dietary TAG must first be emulsified by lysophosphatides (produced from digested phosphatides in the anterior midgut) to allow digestion in the middle and posterior midgut (Barroso *et al.*, 2021). Dietary lactose can interfere with emulsification, thereby limiting lipase activity.

Regarding hydrolysis, lactose may have inhibited the expression of midgut TAG lipases. In *D. melanogaster*, digestive lipase expression can be suppressed under starvation conditions or in response to high dietary glucose. Specifically, glucose stimulates the secretion of the transforming growth factor β (TGF- β) ligand from the fat body, which then represses the expression of digestive enzymes commonly upregulated during starvation (Chng *et al.*, 2014). Interestingly, high concen-

trations of lactose and maltose may differentially affect lipase expression. Maltose digestion produces two glucose molecules, whereas lactose produces one glucose and one galactose molecule. Consequently, lactose is expected to cause a smaller increase in glucose levels than maltose, potentially making it a weaker repressor. The effect of hemolymph galactose concentration on TGF- β signaling should be investigated. Another well-known regulator of TAG lipase is the nuclear receptor DHR96v (Sieber and Thummel, 2009). Metabolites of lactose or maltose could act as antagonists or agonists, respectively, altering DHR96 activity and, in turn, lipase function. Even if lipases were present, they could be inactivated by non-optimal pH or the absence of essential cations, such as Ca^{2+} , which are crucial for TAG digestion (Verger *et al.*, 1973). Finally, lactose could have impaired the absorption of FA and MAG. These nutrients can be absorbed passively through the peritrophic membrane in the insect midgut or actively via binding proteins (Barroso *et al.*, 2021).

Despite a reduced dietary LR in larvae fed lactose, overall lipid content remained comparable to that of maltose-fed larvae. However, their fatty acid profiles differed markedly: maltose promoted the accumulation of SFA, whereas lactose favoured the accumulation of UFA. In maltose-fed larvae, SFA are mainly characterised by a higher lauric acid level. Lauric acid is recognised as a typical fatty acid of reserve lipids synthesised *de novo* from carbohydrates in the black soldier fly (Carpentier *et al.*, 2024; Hoc *et al.*, 2020; Suryati *et al.*, 2023; Zhu *et al.*, 2019). Initially, the lower lauric acid content in larvae fed lactose suggested a higher accumulation of dietary fatty acids. In other words, the monoacylglycerol pathway appeared to be favoured, facilitating the incorporation of dietary fatty acids through the acylation steps of the dihydroxyacetone phosphate (DHAP) and glycerol-3-phosphate (G3P) pathways. In *D. melanogaster*, FASN mutants (lacking the ability to synthesise fatty acids from dietary sugars) can be rescued by dietary lipids, leading to a more unsaturated fatty acid profile (Garrido *et al.*, 2015). Interestingly, high lactose levels appeared to have the opposite effect. The elevated lipid content in frass indicated that fatty acid synthase (FASN) was likely activated. Moreover, the high levels of unsaturated fatty acids, particularly oleic acid (C18:1n9), suggest that delta-9 desaturase activity was also induced. Oleic acid is not an essential fatty acid and its *de novo* synthesis by BSF is well documented (Hoc *et al.*, 2020). Elevated linoleic acid levels are surprising, as it is considered an essential fatty acid for BSF and other Dipteran species studied. Unlike *Periplaneta americana* (L. 1758), Dipter-

ans are thought to have lost the ability to produce delta-12 desaturase and therefore cannot convert oleic acid into linoleic acid (Guillou *et al.*, 2010; Malcicka *et al.*, 2018). In this study, the dietary net lipid removal from the substrate was estimated. Although the assimilated fraction cannot be precisely determined, lactose-fed larvae led to the removal of about 3 g of dietary lipid, which – given the dietary fatty acid profile – is more than enough to account for the linoleic acid present in larval lipids and to exclude lactose-induced *de novo* linoleic acid synthesis.

As saprophagous feeders, BSF larvae develop in a substrate harbouring a microbial community. This community degrades the substrate by utilising nutrients and can affect larval development, with effects that are either adverse or beneficial (reviewed in). Microbial digestion of soluble sugars produces various metabolic compounds (Caplice and Fitzgerald, 1999). Among these compounds, short-chain fatty acids (SCFAs) have been recognised to exert systemic effects in *D. melanogaster*. For instance, acetate influences the immune deficiency pathway, lipid metabolism and lipid droplet size (Grenier and Leulier, 2020; Kamareddine *et al.*, 2018). SCFAs act as ligands for specific G protein-coupled receptors, FFA2 and FFA3, located on enteroendocrine cells, which modulate carbohydrate and lipid metabolism (Bolognini *et al.*, 2016; Kamareddine *et al.*, 2018). The nature of dietary carbohydrates influences the SCFA profile produced by anaerobic gut bacteria and these receptors recognise SCFAs differently depending on their carbon chain length (Bjursell *et al.*, 2011; Campbell *et al.*, 1997). Consequently, lactose and maltose may promote distinct microbial communities and, therefore, different SCFA profiles. These profiles could have influenced energy homeostasis in BSF larvae (Grenier and Leulier, 2020; Miyamoto *et al.*, 2016). While fat metabolism is well documented in *D. melanogaster*, it remains understudied in BSF, despite the high industrial potential of BSF oil. In their study, Zhu *et al.* (2019) identified pathways of fat accumulation in BSF larvae and linked them to gene expression and temporal variation. Although the authors attributed this temporal variation solely to larval developmental stage, it is plausible that temporal substrate degradation and the release of microbial metabolites may also partly drive variation in gene expression or help explain discrepancies between observed fatty acid profiles and gene expression.

In conclusion, this study demonstrates that increasing soluble carbohydrate levels in the diet raises lipid content in BSF larvae, regardless of carbohydrate type. However, only lactose induces a more unsaturated fatty

acid profile, as previously reported by Carpentier *et al.* (2024). High lactose levels maintain stable lauric acid content and do not significantly alter the overall fatty acid profile compared with maltose. Nevertheless, high lactose inclusion reduced dietary LR and was associated with lower substrate conversion efficiency. These findings suggest that high dietary lactose alters nutrient utilisation and lipid metabolism in BSF larvae. The reduced LR observed under lactose-rich conditions, together with the changes in fatty acid composition, may indicate a greater contribution of *de novo* fatty acid synthesis to lipid storage. The observed increase in oleic acid further suggests possible stimulation of delta-9 desaturase activity, although the underlying mechanisms remain unclear. Investigating whether lactose is metabolised directly by the larvae or by substrate-associated microorganisms and characterising the resulting metabolites, could provide a foundation for understanding these mechanisms.

Interest in organic waste management using BSF larvae is increasing due to the growing diversity of tested by-products. Moreover, the plasticity of stored TAG represents a significant opportunity for this sector to compete with oil producers across industries and to reduce dependence on imported materials, such as coconut oil, in non-tropical countries. Although insights from *D. melanogaster* have guided some mechanistic hypotheses, it is essential to note that BSF larvae may exhibit distinct lipid metabolism and carbohydrate digestion and direct extrapolations should be made with caution. In this context, it is evident that, compared with *D. melanogaster*, the characteristics of BSF lipid metabolism remain incompletely understood. Specific investigations into the effects of nutrients on BSF life-history traits and composition are therefore needed. The potential benefits are numerous: creating synergies, avoiding deleterious effects from by-product mixtures and anticipating BSF oil composition, which is crucial for the downstream applications of insect-derived oil.

Acknowledgements

The authors would like to thank Mrs E. Bera, Mr A. Anglicus, Mr D. Eggermont, Mrs S. Vancraenenbroeck and Mr N. Deville for their valuable assistance with the experimental work.

Conflicts of interest

The authors declare no conflict of interest

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TABLE A1 Proximate composition of the experimental diets containing maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO) and peanut oil (PNO)

Modality	Dry matter content $\pm$ SD (%)	Carbohydrate	Crude protein	Crude fat $\pm$ SD (%) ¹
Control	36.77 $\pm$ 0.91	69.59	13.00	3.50 $\pm$ 0.16 a
PNO-CEL	36.89 $\pm$ 0.51	64.19	13.00	8.82 $\pm$ 0.18 b
RSO-CEL	36.87 $\pm$ 0.58	64.19	13.00	8.70 $\pm$ 0.43 b
PNO-LAC4	37.17 $\pm$ 0.66	64.19	13.00	8.79 $\pm$ 0.43 b
PNO-MAL4	37.04 $\pm$ 0.22	64.19	13.00	8.77 $\pm$ 0.29 b
RSO-LAC4	37.15 $\pm$ 1.17	64.19	13.00	8.61 $\pm$ 0.60 b
RSO-MAL4	36.23 $\pm$ 0.47	64.19	13.00	9.01 $\pm$ 0.02 b
PNO-LAC8	37.45 $\pm$ 0.13	64.19	13.00	8.58 $\pm$ 0.25 b
PNO-MAL8	37.50 $\pm$ 0.40	64.19	13.00	8.43 $\pm$ 0.20 b
RSO-LAC8	37.37 $\pm$ 0.46	64.19	13.00	8.62 $\pm$ 0.39 b
RSO-MAL8	37.05 $\pm$ 0.53	64.19	13.00	8.36 $\pm$ 0.93 b
Statistical analysis	LM: $F_{10,22} = 1.032, p = 0.450$	–	–	LM: $F_{10,22} = 41.098, p < 0.001$

Soluble sugars and oils were replaced by cellulose in Control substrate. Carbohydrate, crude protein and crude fat are expressed as a mean percentage of the dry matter content ($n = 3$). Dry matter and crude lipids were determined experimentally; crude protein and carbohydrates were estimated based on the composition of the chicken feed and ingredient proportions.

TABLE A2 Fatty acid profile of the isolipidic experimental diets containing rapeseed oil (RSO) and peanut oil (PNO)

Fatty acid	RSO diet	PNO diet
CI2:0	0.49 $\pm$ 0.07	0.33 $\pm$ 0.09
CI4:0	0.31 $\pm$ 0.07	0.18 $\pm$ 0.04
CI6:0	9.88 $\pm$ 0.04	10.94 $\pm$ 0.74
CI6:1	0.23 $\pm$ 0.03	0.11 $\pm$ 0.01
CI8:0	1.77 $\pm$ 0.07	2.17 $\pm$ 0.02
CI8:1n9 cis + trans	39.41 $\pm$ 0.83	48.46 $\pm$ 3.27
UND1	1.91 $\pm$ 0.09	0.80 $\pm$ 0.08
CI8:2n6c	38.53 $\pm$ 0.72	31.87 $\pm$ 2.52
CI8:3n3	5.44 $\pm$ 0.16	1.36 $\pm$ 0.20
C20:0	0.49 $\pm$ 0.12	0.75 $\pm$ 0.11
C20:1n9	0.69 $\pm$ 0.14	0.88 $\pm$ 0.13
C22:0	0.29 $\pm$ 0.05	1.16 $\pm$ 0.12
C22:1n9	0.20 $\pm$ 0.07	0.16 $\pm$ 0.04
C24:0	0.24 $\pm$ 0.09	0.74 $\pm$ 0.06
Σ SFA	13.48 $\pm$ 0.48	16.28 $\pm$ 0.85
Σ MUFA	40.54 $\pm$ 0.95	49.60 $\pm$ 3.86
Σ PUFA	43.98 $\pm$ 0.67	31.84 $\pm$ 2.62

The results of the fatty acid profile are expressed as a mean percentage of the total fatty acid ($n = 5$).

TABLE A3 Initial individual weight of *Hermetia illucens* larvae harvested 5 days post-hatch for feeding experiment

Soluble carbohydrate inclusion rate	Condition	Mean weight of larvae $\pm$ SD (mg) at day 0
0%	Control	6.9 $\pm$ 0.4
	PNO-CEL	6.9 $\pm$ 0.6
	RSO-CEL	6.9 $\pm$ 0.6
	PNO-LAC4	6.9 $\pm$ 0.5
	PNO-MAL4	6.8 $\pm$ 0.4
4%	RSO-LAC4	7.1 $\pm$ 0.5
	RSO-MAL4	7.0 $\pm$ 0.4
	PNO-LAC8	6.9 $\pm$ 0.4
	PNO-MAL8	7.1 $\pm$ 0.4
	RSO-LAC8	7.0 $\pm$ 0.3
8%	RSO-MAL8	7.0 $\pm$ 0.5
	Statistical analysis	LM: $F_{10,32} = 0.122,$ $p = 0.993$

TABLE A4 Instrumental details of GC-MS method used for fatty acid identification

<u>GC-MS parameters</u>		
Carrier gas	Helium	
Flow control mode	Linear velocity	
Linear velocity	41.4 cm/s	
Column flow	1.3 ml/min	
Oven Temperature program	1 min at 50 °C; from 50 to 150 °C at 30 °C/min; from 150 to 260 °C at 4 °C/min	
Injection mode	Split mode	
Split ratio	25	
Injection volume	0.5 µL	
Injector Temperature	250 °C	
<u>Instrumentation</u>		
Autosampler	AOC-20i Plus Autoinjector	Shimadzu
GC	Nexis GC-2030	Shimadzu
Detector	MS	Shimadzu
Column	SupelcoWAX 30 m × 0.25 mm × 0.25 µm	Supelco
<u>MS Parameters</u>		
Ion source temperature	220 °C	
Interface temperature	260 °C	
Acquisition mode	Scan m/z from 45 to 550	
<u>Software</u>		
Data acquisition and processing	GCMS Solution 4.53 SP1	Shimadzu
Library	FAMEs Fatty Acid Methyl Esters: Mass Spectral Database	Wiley Science Solutions

TABLE A5 Mean frass quantity, residual lipid in frass and larval lipid mass at the end of the experiment

Modality	Frass ± SD (g)	Residual lipid in frass (mg/collected larvae)	Larval lipid mass (mg/collected larvae)
Control	23.09 ± 0.31 bc	1.57 ± 0.54 e	12.35 ± 0.51 e
PNO-CEL	21.50 ± 0.16 c	4.89 ± 1.28 cde	20.64 ± 1.21 d
RSO-CEL	21.47 ± 0.88 c	3.35 ± 0.87 de	18.31 ± 1.99 d
PNO-LAC4	25.82 ± 0.83 ab	10.08 ± 0.76 b	27.74 ± 1.58 bc
PNO-MAL4	24.80 ± 1.48 ab	9.51 ± 1.27 b	28.43 ± 1.28 bc
RSO-LAC4	26.18 ± 0.80 a	9.33 ± 2.86 b	27.22 ± 2.75 c
RSO-MAL4	25.05 ± 0.72 ab	9.29 ± 1.34 bc	29.51 ± 2.20 bc
PNO-LAC8	29.99 ± 2.40 a	19.50 ± 2.49 a	29.66 ± 0.56 bc
PNO-MAL8	20.58 ± 0.80 c	7.78 ± 0.71 bc	35.33 ± 0.72 a
RSO-LAC8	26.11 ± 1.38 a	18.79 ± 2.97 a	31.91 ± 3.40 ab
RSO-MAL8	20.51 ± 1.05 c	7.47 ± 1.09 bcd	36.13 ± 1.28 a
Statistical analysis	LM: $F_{10,32} = 17.776, p < 0.001$	LM: $F_{10,32} = 43.361, p < 0.001$	LM: $F_{10,32} = 64.248, p < 0.001$

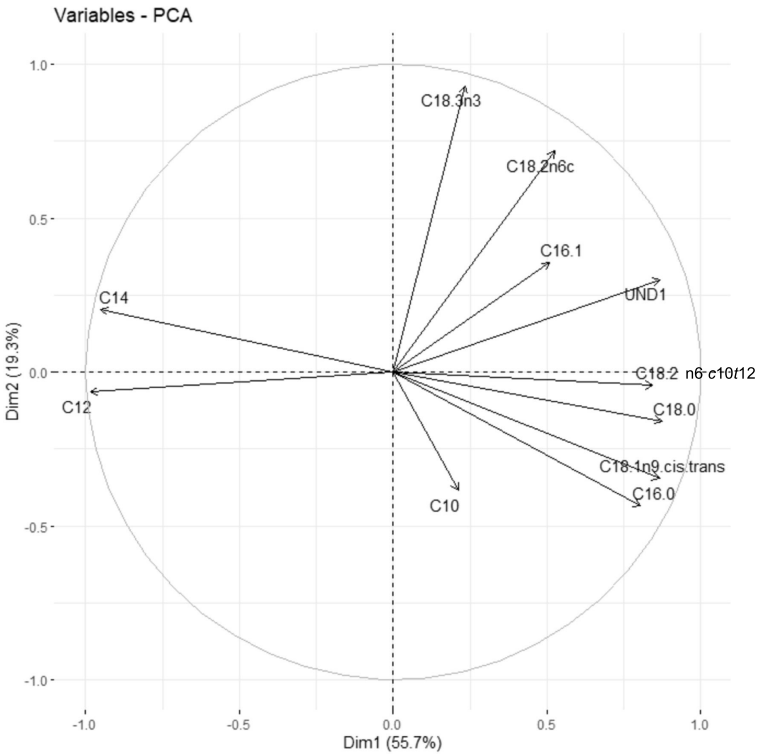


FIGURE A1 PCA circle correlation for fatty acid profiles of *Hermetia illucens* larvae fed illucens fed maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO-CEL) and peanut oil (PNO-CEL).

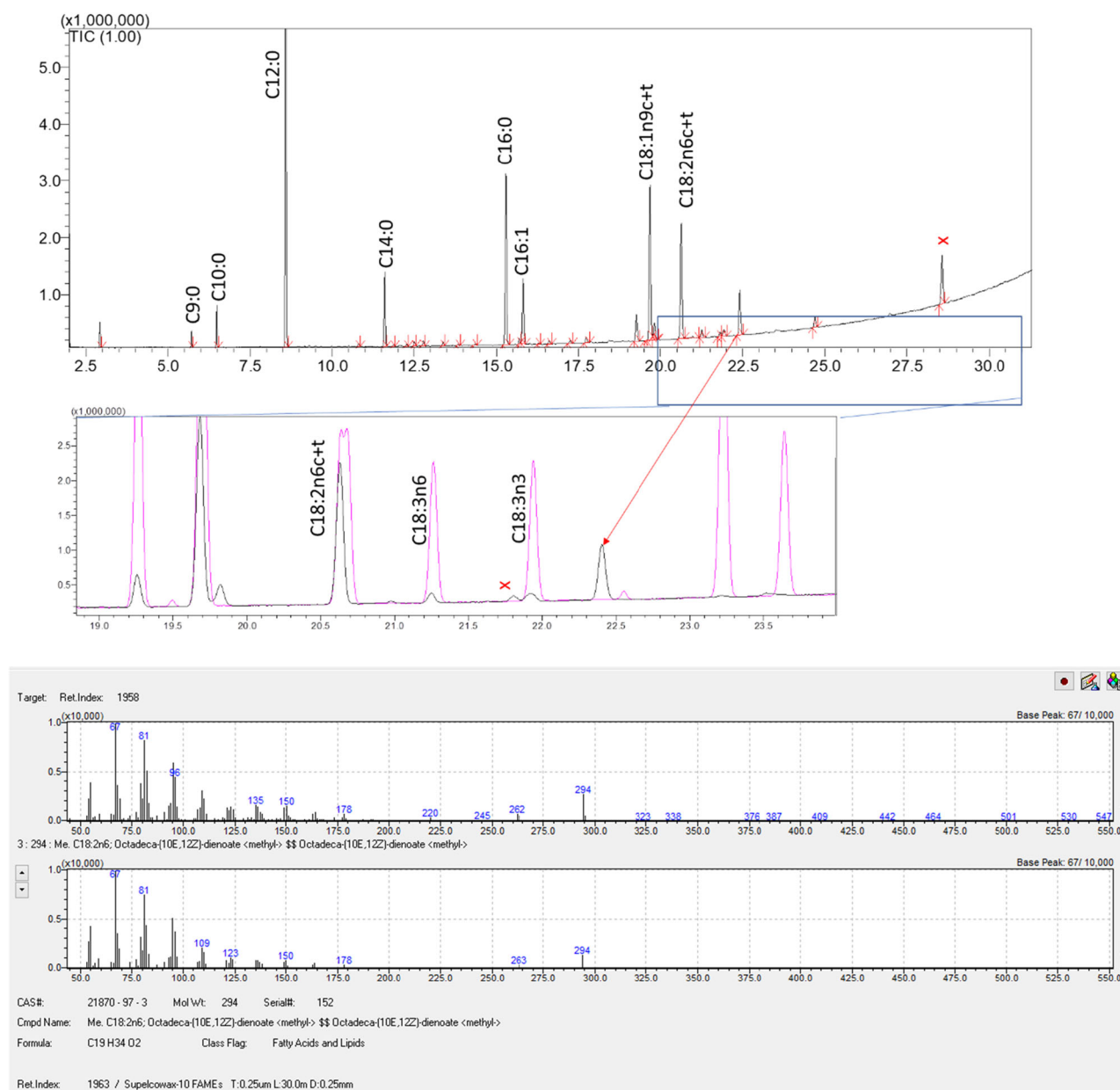


FIGURE A2 GC-MS identification of unidentified FAME for fatty acid profiles of *Hermetia illucens* larvae fed *illucens* fed maltose (MAL) and lactose (LAC) at 4% (4) and 8% (8) inclusion rate in combination with rapeseed oil (RSO-CEL) and peanut oil (PNO-CEL). Putative identification was done based on retention index (RI = 1958, RI literature = 1963) and MS similarity (93%).