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Enrichment in specific fatty acids profile of *Tenebrio molitor* and *Hermetia illucens* larvae through feeding

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ABSTRACT

Tenebrio molitor (mealworms) and *Hermetia illucens* (Black soldier fly) larvae were analysed for the effect of feed composition on the fatty acid profiles. The larvae were raised on various feeds to which the basal diet had been supplemented to various levels with seed meals (flax seed, chia seed, hemp seed, and rapeseed). Fatty acid classes and composition of both insect larvae were similar in *T. molitor* and *H. illucens* larvae fat; however, the actual percentage composition differed; saturated (28.61% and 86.75%), monounsaturated (MUFA) (52.89% and 7.94%), and polyunsaturated (PUFA) fatty acids (18.49% and 5.31%). The supplementation of the basal diet resulted in larvae fat with increased omega-3 fatty acids levels, and subsequently a lower omega-6 to omega-3 ratio (*T. molitor*; 4.28:1 in the diet with 10% chia seed, *H. illucens*; 3.52:1 in the diet with 20% hemp seed) than those of the basal diets (50:1 and 9.91:1 in *T. molitor* and *H. illucens* respectively). In most of the larvae samples, the ratio achieved was closer to that recommended for a healthy diet.

Introduction

Edible insects can be a major element of sustainable food in the future due to the increasing global population and the accompanying demand for food containing enough nutrients such as proteins (Van Huis et al., 2013). *T. molitor* and *H. illucens* are two of the most promising insects with potential as a beneficial and alternative food source. The potential of using *T. molitor* larvae as a source of unsaturated fatty acid (UFA) to supplement the human diet has been proposed (Dreassi et al., 2017). *T. molitor* larvae have a relatively low amount of saturated fatty acid (SFA), and subsequently higher UFA. The UFA present includes oleic (18:1ω9), linoleic (18:2n-6; ω6) and α-linolenic (18:3; ω3) acids and are usually present in different percentages depending on the diet (Ghosh et al., 2017; Ravzanaadii et al., 2012).

On the other hand, *H. illucens* has a high amount of SFA and lower UFA content, but has a lower ω6:ω3 ratio of 0.9:1 (Guil-Guerrero et al., 2018) compared to *T. molitor* having around a ω6:ω3 ratio of $\geq$ 20 (Cito et al., 2017). Human diet containing a high UFA may have beneficial effects on the cardiovascular system by replacing saturated fats intake, partly (Livingstone et al., 2012). The long-chain derivatives of α-linolenic acid (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) are the PUFA that are involved in preventing cardiovascular diseases (Manerba et al., 2010). Similarly observed in other terrestrial insect species, *T. molitor* can synthesize linoleic and α-linolenic *de novo;* however, EPA and DHA are absent in this insect. The ω6:ω3 ratio in *T. molitor* (i.e. 20:1 to 25:1) is a range suitable for human consumption (Dreassi et al., 2017). However a lower ratio of 4:1 is said to be more favourable in preventing secondary cardiovascular diseases (Simopoulos, 2002). The ω6:ω3 ratio is also a commonly used index for determining healthy fat. Diet with a higher ratio is linked to the development of disorders like cancer and coronary heart disease (Bophimai and Siri, 2010; Milićević et al., 2014).

Studies have shown that the growth rate and nutritional components of insects are influenced by their diet (Dreassi et al., 2017; Francardi et al., 2017; Liland et al., 2017; St-Hilaire et al., 2007), and this makes it possible to alter the nutritional composition of insects via diet (Anderson, 2000; Ricciardi and Baviera, 2017). Feeding trial studies have been reported on *H. illucens* larvae raised on commercial laying hen feed supplemented with a fish meal (Barroso et al. 2017), or on seaweed-enriched media (Liland et al., 2017), or fish offal (St-Hilaire et al., 2007).

To successfully utilize the lipid components of *T. molitor* and *H. illucens* larvae as an alternative food, there is a need for more research on the diet needed to improve their beneficial FA composition ratio to levels that are most suitable for meeting nutritional needs. Aside the studies by Cito et al. (2017) and Dreassi et al. (2017), little is known on the roles of seed meal supplemented diets on the FA profile of edible insects. Therefore, this study aimed to identify the impact on the fatty acid composition of *T. molitor* and *H. illucens* larvae fed with a broader

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range of seed meals known to be rich in healthy FA (i.e., flax seed, chia seed, hemp seed, and rapeseed). The goal was to improve the FA profiles of *T. molitor* and *H. illucens* larvae diet modification, thereby further enhancing the food/feed value of these insect larvae.

Materials and methods

Collection of samples, and feed preparation

Young *T. molitor* larvae were obtained from and reared at the Insect Room of the Zoology Department facility of the University of Otago, New Zealand. *H. illucens* larvae were provided and reared by Prescient Nutrition; Palmerston North, New Zealand. The basal diets of both larvae were supplemented with whole-seed meals. The basal diets were whole wheat meal and wheat bran for *T. molitor* and *H. illucens* larvae respectively. The same percentage and type of seed meals were used to supplement the diet of both insect larvae. The basal diet compositions were 100% whole-wheat meal (W100) and wheat bran (B100) for *T. molitor* and *H. illucens* respectively. The supplemented diets used were composed of 80% wheat meal/bran and 20% flax seed (for FLAX20); 90% wheat meal/bran and 10% flax seed (FLAX10); 80% wheat meal/bran and 20% rapeseed (RAPE20); 80% wheat meal/bran and 20% chia seed (CHIA20); 90% wheat meal/bran and 10% chia seed (CHIA10) and 80% wheat meal/bran and 20% hemp seed (HEMP20).

Experimental design of feeding trials

For each feeding diet, about 70 *T. molitor* larvae (15 mm -20 mm) were manually sorted from available lot. Feeds (150 g) were weighed into plastic containers, with holes in the lids for aeration. The larvae were placed in the containers, and fresh carrot slices were used as a source of moisture and replaced twice weekly. The feeds were changed twice throughout the trial. The feeding was stopped after three weeks (21 days) when the larvae reached lengths of between 25 mm and 30 mm. Feeding of *T. molitor* larvae was done as three biological replicates (i.e., three containers of insects per feed, in a climate-controlled insect room at 25 ˚C with 70% humidity level. The *H. illucens* were reared and supplied by Prescient Nutrition, Palmerston North, New Zealand. Seven egg clutches collected from an established *H. illucens* colony (raised on wheat bran) were placed in different trays containing wheat bran and placed in a growing unit maintained at 25 ˚C and 70% humidity. After one week (which is the time it took for the eggs into develop to small tiny larvae), the diet mixtures prepared were used to feed the larvae except for the control trial (B100). The feeding mixtures were added to the trays as required over a period of 4 weeks. The diets were constituted using water at a ratio of 2:1 diet to water. About 200 larvae from each feeding tray were harvested at the sixth instar, before reaching the prepupae stage. The growth was halted by immediate freezing and storage at -20 ˚C. Feeding of *H. illucens* was performed as one biological replicate per feed.

Fat extraction and methylation of fatty acids

Prior to fat extraction, the frozen larvae samples were dried. *T. molitor* larvae were freeze-dried (Labconco Freezone, USA) and *H. illucens* larvae were vacuum oven-dried at a low temperature of 55 ˚C. The dried samples were ground to a powder with a mortar and pestle (particles passing ~ 300 μm sieve). Total fat extraction from ground larvae samples (triplicate; $n = 3$) was achieved using Soxhlet method (AOAC, 2003) with hexane as the extraction solvent (RCI, Labscan Ltd, Bangkok, Thailand). The fat samples were stored at -20 ˚C until use. The FA methylation was performed on each triplicate sample ($n = 3$), using boron trifluoride as the derivatizing agent. Fat (20 mg) was weighed into a screw-top glass test tube and diethyl ether (10 ml; Ajax Finechem, Auckland, New Zealand) was added. An aliquot of 2 ml (containing 4 mg of fat) of this was placed into a new test tube, to which 0.5 M methanolic KOH (2 ml) was added. The mixture was refluxed for 20 min at 80 ˚C and allowed to cool to room temperature. Diethyl ether (2 ml) and distilled water (5 ml) was added to the cooled solution, and the top layer was discarded. The bottom layer was acidified with few drops (4–6) of HCl (Ajax Finechem, Auckland, New Zealand), and diethyl ether (2 ml) was added. The organic layer (top layer) was transferred into a new test tube, and boron trifluoride (100 µl) (CSA, USA) was added. It was heated for 20 min at 80 ˚C and saturated NaCl (5 ml; Merck KGaA, Darmstadt, Germany) was added to the cooled solution and vortexed at high speed for a minute. FA methyl esters (FAMEs) were transferred into a 2 ml sample vial and stored at -4 ˚C until analysis by Gas Chromatography.

Analysis of fatty acids

A Gas Chromatography equipped with a flame ionization detector (Agilent 6890N, CA, USA) equipped with an auto-sampler (Agilent 7683, China) was used to analyse the FAMEs. The column oven temperature program was, 40 ˚C for 3 min, 5 ˚C/min to 225 ˚C/min, then 10 ˚C/min to 245 ˚C and finally held for 2 min. Injector detector ports were set at 240 ˚C and with 10:1 inlet split ratio and the ionization detector port was at 250 ˚C. The carrier gas used was hydrogen, and its flow was maintained at a constant 2.2 mL throughout. Identification of peaks was achieved by matching retention times with known FAME standards (FAMQ-005) (NuCheck Prep, Elysian, Minnesota and Sigma, St Louis, Missouri). The percentage composition of each identified compound in the mixture was obtained from the percent peak area data obtained from the GC data processing software.

Statistical analysis

The fat content of the larvae samples is reported as g/100g of sample. Individual fatty acids detected from FA composition analysis are presented as a percentage of the total FA and results were presented as mean ± standard deviation of triplicate experiments. All other experiments (feeding trials and analysis) were performed in three independent replicates. Analysis of variance (using IBM SPSS® software, version 25) was performed on mean values of individual FA detected in the larvae fat; and significant differences were declared at $p \leq 0.05$.

Results

Fat content T. molitor *and* H. illucens *larvae fat*

The fat contents of both larvae investigated are presented in Table 1. The total fat in *T. molitor* was higher than *H. illucens*. *T. molitor* had fat content between 32.26 g/100 g in HEMP20 to 39.61 g/100 g in W100, whereas fat of *H. illucens* was between 18.19 g/100 g in B100 and 28.85 g/100 g in RAPE20. Except in HEMP20 (32.26 g/100 g), the total fat content in the rest of the diets, including the basal diet, were not significantly different in *T. molitor* (see Table 1). The fat content of *T. molitor* larvae was within the vicinity value of 32.7% reported in similar study on *T. molitor* (Ravzanaadii et al., 2012). However, there are some orthopterans with lower fat content, including *Acheta domesticus* (15.31%) and *Chorthippus parallelus* (10.21%) (Paul et al., 2017).

In *H. illucens* the fat obtained from B100 (18.19%) is significantly lower than all the other samples (21.75%–28.85%), except CHIA10 (18.62%). These values are higher than *H. illucens* larvae fed laying hen feed (LHF) (8.5% total fat) and LHF + 40% fish meal (10.2% total fat), but comparable with the 21% total fat obtained when the larvae were fed LHF + 40% fish meal two days before harvest (Barroso et al. 2017). Another study reported a 15.7% fat content, when fed LHF (Guil-Guerrero et al., 2018). The fat contents of some of the samples (such as RAPE20 (28.85%) and FLAX20 (27.67%)) are higher than the 21.42% fat reported in *H. illucens* fed 100% cow manure (St-Hilaire et al., 2007).

Table 1
Fat content of larvae samples reared on various feeds ((g/100g, Mean±SD, $n = 3$).

Diets	Basal diet	FLAX20	FLAX10	RAPE20	CHIA20	CHIA10	HEMP20
T. molitor	39.61±0.39^{a}	38.03±0.62^{a}	39.18±0.90^{a}	38.47±0.7^{a}	38.52±1.78^{a}	37.14±0.73^{a}	32.26±1.08^{b}
H. illucens	18.19±0.27^{d}	27.67±0.88^{a}	25.03±0.22^{b}	28.85±0.41^{a}	21.75±0.42^{c}	18.62±0.44^{d}	23.86±0.07^{b}

Diet composition (%w/w): Basal diet was W100: wheat meal (100) for *T. molitor* larvae; and B100: wheat bran diet (100) for *H. illucens* larvae; FLAX20: wheat meal (80), flaxseed (20); FLAX10: wheat meal (90), flaxseed (10); RAPE20: wheat meal (80), rapeseed (20); CHIA20: wheat meal (80), chiaseed (20); CHIA10: wheat meal (90), chiaseed (10); HEMP20: wheat meal (80), hempseed (20),

^{abcd} Values with different superscript letters within a row are significantly different

Table 2
Percentage of fatty acid composition in *T. molitor* raised on various diet (% of total FA).

Fatty acid	Common name	W100	FLAX20	FLAX10	RAPE20	CHIA20	CHIA10	HEMP20
C12:0	Lauric acid	0.57±0.02^{ab}	0.50±0.06^{ab}	0.47±0.04^{ab}	0.52±0.06^{ab}	0.59±0.09^{a}	0.51±0.06^{ab}	0.54±0.09^{ab}
C14:0	Myristic acid	5.01±0.08^{a}	4.68±0.20^{a}	4.76±0.31^{a}	5.03±0.53^{a}	4.90±0.05^{a}	4.76±0.25^{a}	4.70±0.91^{a}
C15:0	Pentadecanoic acid*	0.17±0.05^{a}	0.16±0.02^{a}	0.11±0.01^{a}	0.18±0.03^{a}	0.16±0.03^{a}	0.11±0.02^{a}	0.13±0.01^{a}
C16:0	Palmitic acid	19.05±0.02^{ab}	17.68±0.06^{e}	19.18±0.18^{a}	18.37±0.19^{c}	18.54±0.25^{bc}	18.21±0.10^{cd}	17.78±0.30^{de}
C17:0	Margaric acid	0.10±0.02^{a}	0.10±0.01^{a}	0.10±0.01^{a}	0.10±0.01^{a}	0.12±0.03^{a}	0.12±0.03^{a}	0.14±0.04^{a}
C18:0	Stearic acid	3.60±0.08^{a}	3.70±0.07^{a}	3.69±0.16^{a}	3.64±0.23^{a}	3.61±0.07^{a}	3.93±0.09^{a}	3.62±0.30^{a}
C20:0	Arachidic acid	0.11±0.01^{a}	0.11±0.02^{a}	0.09±0.00^{a}	0.16±0.03^{a}	0.14±0.04^{a}	0.13±.0.02^{a}	0.05±0.04^{a}
ΣSFA		**28.61±0.06^{a}**	**26.93±0.27^{b}**	**28.39±0.35^{a}**	**28.00±0.53^{ab}**	**28.07±0.16^{ab}**	**27.77±0.18^{ab}**	**26.95±1.00^{b}**
C16:1	Palmitoleic acid	1.74±0.01^{b}	1.53±0.04^{bc}	1.66±0.07^{b}	1.49±0.09^{bc}	1.56±0.02^{bc}	1.65±0.04^{b}	1.33±0.16^{c}
C18:1tω9	Oleic acid (trans)	0.08±0.01^{ab}	ND	0.09±0.04^{a}	0.08±0.01^{ab}	0.09±0.02^{a}	0.08±0.03^{ab}	0.12±0.06^{a}
C18:1cω9	Oleic acid (cis)	49.86±0.08^{a}	45.99±0.34^{bc}	48.59±0.75^{ab}	50.57±0.81^{a}	45.53±0.29^{c}	46.68±0.12^{bc}	42.32±2.06^{d}
C22:1ω9	Erucic acid	1.21±0.05^{a}	1.18±0.26^{a}	ND	1.09±0.18^{a}	1.13±0.27^{a}	0.84±0.29^{a}	ND
ΣMUFA		**52.89±0.09^{ab}**	**48.70±0.52^{c}**	**50.34±0.71^{bc}**	**53.24±0.91^{a}**	**48.31±0.03^{c}**	**49.25±0.39^{c}**	**43.77±1.96^{d}**
C18:2ω6	Linoleic acid	18.13±0.12^{b}	17.97±0.10^{b}	18.19±0.24^{b}	18.14±0.35^{b}	16.87±0.06^{c}	18.63±0.11^{b}	26.67±0.69^{a}
C18:3ω3	α-Linolenic acid	0.36±0.02^{e}	6.40±0.15^{a}	3.08±0.13^{c}	0.62±0.03^{e}	6.75±0.10^{a}	4.36±0.10^{b}	2.61±0.27^{d}
ΣPUFA		**18.49±0.12^{e}**	**24.37±0.25^{b}**	**21.27±0.36^{d}**	**18.76±0.38^{e}**	**23.62±0.15^{bc}**	**22.99±0.21^{c}**	**29.28±0.96^{a}**
ω6:ω3		50±2.85^{a}	2.81±0.05^{ef}	5.92±0.16^{de}	29.31±1.15^{b}	2.50±0.03^{f}	4.28±0.07^{ef}	10.27±0.81^{c}
SFA:UFA		0.40±0.00^{a}	0.37±0.01^{b}	0.40±0.01^{a}	0.39±0.01^{ab}	0.39±0.00^{ab}	0.38±0.00^{ab}	0.37±0.02^{b}

Mean ±SD, n = 3, ND: Not detected,

* : Systematic nameDiet composition (%w/w): see footnote on Table 1,

^{abcdef} Values with different superscript letters within a row are significantly different

The variations in the fat content of insects are usually due to type of the species, extraction method, diet, and rearing conditions (Yi et al., 2013; Cito et al., 2017). Therefore, the varying fat contents observed in this study can be attributed to the fat content of the diets on which they were raised. All seed meals used are rich sources of fat, with rapeseed (47.7%), hemp seed (46.67%), and flax seed (42.16%) having higher fat contents than chia seed (33.33%) (Orsavova et al., 2015; USDA, 2019). Adding the seed meals to the diet is expected to increase the overall fat content of the feed mixtures. Upon supplementing the basal feed with the seed meals, the fat content values obtained in *H. illucens* larvae reflected these differences. CHIA10 (18.62%) and CHIA20 (21.75%) had the lowest fat values, while for the rest, the lowest was 23.86% in HEMP20 and a higher value of 28.85% in RAPE20. On the other hand, the percentage fat in *T. molitor* was generally high, and the values (37.14% in CHIA10 - 39.18% in FLAX10) showed no significant difference, except in HEMP20 (32.26%). For *T. molitor*, the W100 (39.61%) also had a high fat content, not significantly different from the other supplemented samples, however, the reverse was the case in B100 for *H. illucens* (18.19%). The percentage fat value obtained in *T. molitor* larvae was not significantly affected by the supplementation, however, this was not the case with its fatty acid composition.

Fatty acid composition of T. molitor *larvae*

The results presented in Table 2 show that the FA composition of the seed meals influenced that of the *T. molitor* larvae composition. As shown, rapeseed containing a high oleic acid value (63.3%) (Orsavova et al., 2015) resulted in an increased oleic acid value in the *T. molitor* larvae raised on RAPE20. The oleic acid in RAPE20 (50.57%) was the highest, although not significantly different from W100 (49.86%). However, the resulting FA enrichment, occurring due to the supplementation, was more visible in samples like HEMP20, in which the high linoleic acid (LA) in hemp seed (59.4%) resulted in an increased LA value in HEMP20 (26.27%), compared with the 18.13% in W100 larvae. These contributed to a subsequent increase in the PUFA content in the samples, i.e. 18.49% in W100 and 29.28% in HEMP20. Similar increments in LA value were observed in the other larvae samples.

Although a wide variation exists between the percentages SFA, MUFA, and PUFA content of fat in *T. molitor* larvae fed the basal diet (W100) and those fed the seed meals (Table 2). For instance, in W100, the UFA (52.89% MUFA, 18.49% PUFA) were higher than SFA (28.61%). The most prevalent SFAs were palmitic acid (C16:0), myristic acid (C14:0), and stearic acid (C18:0). Oleic acid (C18:1cω9) and α-linoleic acid (C18:2ω6) were the most abundant UFA.

On supplementing the feeds, it was observed that SFA present in larvae fed W100 (28.61%) was similar to those fed RAPE20 (28.00%), and CHIA20 (28.07%). Cito et al. (2017) reported a similar SFA value (28:78%) in *T. molitor* larvae fed wheat flour, oat flour, and yeast. A lower SFA value (22.26%) was reported when larvae were reared on wheat bran only (Ravzanaadii et al., 2012). The PUFA contents also varied (see Table 2).

Higher values of 29.28%, 24.37%, and 23.62% were recorded in larvae fed HEMP20, FLAX20, and CHIA20, respectively. Hemp, flax, and chia seeds contain 36.67%, 29.14% and 23.67% PUFA contents, respectively USDA (2019). This shows that supplementing the diet with the feed reflected on the UFA composition of larvae fats. These PUFA values are attributed to the increased amount of linoleic acid (LA) in most of the larvae fat.

From Table 2, it is also observed that supplementation caused an increase in the proportion of ω3 FA in the larvae. This resulted in a subsequent reduction in the ω6:ω3, when compared with a ratio of 50:1 (in W100). For example, the addition of 10% flax seed and chia seed greatly reduced the ω6:ω3 ratio to 5.92:1 (FLAX10) and 4.28:1 (CHIA10). Increasing the proportion of these seeds in the meal reduced the ω6:ω3. FA

Table 3
Percentage of fatty acid composition in *H. illucens* fat raised on various diet (% of total FA).

Fatty acid	Common name	B100	FLAX20	FLAX10	RAPE20	CHIA20	CHIA10	HEMP20
C10:0	Capric acid	5.46±0.01^{a}	2.09±0.03^{de}	2.62±0.11^{c}	2.38±0.08^{cd}	1.42±0.15^{f}	3.98±0.33^{b}	1.67±0.1^{ef}
C12:0	Lauric acid	62.05±0.88^{a}	55.42±0.46^{ab}	44.97±4.21^{c}	52.97±2.27^{b}	36.53±2.64^{d}	52.54±2.66^{b}	33.38±1.99^{d}
C14:0	Myristic acid	7.53±0.03^{c}	8.89±0.1^{a}	7.22±0.11^{cd}	8.33±0.07^{b}	7.06±0.07^{de}	6.69±0.35^{e}	5.71±0.18^{f}
C15:0	Pentadecanoic acid*	0.13±0.02^{d}	0.24±0.01^{bc}	0.25±0.04^{b}	0.32±0.03^{a}	0.25±0.02^{b}	0.18±0.02^{d}	0.19±0.01c^{d}
C16:0	Palmitic acid	9.41±0.3a^{b}	9.42±0.1^{ab}	11.53±1.06^{a}	10.78±0.63^{ab}	11.94±0.7^{a}	5.84±5.05^{b}	10.52±0.43^{ab}
C17:0	Margaric acid	0.14±0.02^{c}	0.17±0.01^{bc}	0.27±0.05^{a}	0.21±0.03^{abc}	0.22±0.03^{ab}	0.19±0.02^{bc}	0.21±0.03^{abc}
C18:0	Stearic acid	1.49±0.09^{c}	1.21±0.01^{c}	2.13±0.37^{ab}	1.23±0.13^{c}	2.26±0.22^{ab}	1.71±0.13^{bc}	2.59±0.22^{a}
C20:0	Arachidic acid	0.54±0.01^{e}	2.39±0.05^{b}	1.79±0.14^{c}	1.92±0.1^{c}	2.38±0.13^{b}	0.88±0.05^{d}	4.85±0.16^{a}
ΣSFA		**86.75±0.54^{a}**	**79.82±0.23^{b}**	**70.79±2.66^{c}**	**78.14±1.50^{b}**	**62.07±1.65^{d}**	**72.01±1.53^{c}**	**59.13±1.43^{d}**
C15:1	Pentadecenoic acid*	0.41±0.01^{c}	0.37±0.01^{c}	0.65±0.05^{a}	0.51±0.03^{b}	0.43±0.02^{c}	0.52±0.03^{b}	0.41±0.02^{c}
C16:1	Palmitoleic acid	2.13±0.03^{bc}	2.31±0.03^{b}	2.29±0.09^{b}	2.72±0.07^{a}	1.97±0.07^{cd}	1.87±0.12^{d}	1.59±0.01^{e}
C18:1tω9	Oleic acid (trans)	0.16±0.02^{ab}	0.24±0.03^{a}	0.23±0.07^{ab}	0.17±0.03^{ab}	0.23±0.1^{ab}	0.10±0.00^{b}	0.22±0.02^{ab}
C18:1cω9	Oleic acid (cis)	4.52±0.19^{d}	5.75±0.07^{cd}	7.75±0.98^{b}	10.83±0.93^{a}	6.05±0.4^{cd}	4.59±0.23^{d}	7.22±0.53^{bc}
C20:1	Gondoic acid	0.14±0.02^{c}	0.22±0.01^{bc}	0.25±0.04^{b}	0.24±0.06^{b}	0.20±0.01b^{c}	0.13±0.02^{c}	0.42±0.05^{a}
C22:1ω9	Erucic acid	0.58±0.11^{bc}	0.07±0.02^{c}	1.28±0.50^{a}	0.09±0.07^{c}	0.06±0.02^{c}	0.66±0.06^{b}	0.07±0.04^{c}
ΣMUFA		**7.94±0.36^{b}**	**8.96±0.08^{b}**	**12.45±1.66^{a}**	**14.57±1.14^{a}**	**8.95±0.57^{b}**	**7.85±0.35^{b}**	**9.94±0.64^{b}**
C18:2tω6	Linoleic acid (trans)	ND	0.36±0.01^{a}	0.08±0.03^{c}	ND	0.26±0.01^{b}	0.08±0.02^{c}	0.10±0.02^{c}
C18:2cω6	Linoleic acid (cis)	4.82±0.15^{d}	4.96±0.07^{d}	7.12±0.59^{c}	6.39±0.36^{c}	9.67±0.47^{b}	7.71±0.44^{c}	24.00±0.81^{a}
18:3ω3	α-Linolenic acid	0.49±0.02^{e}	5.79±0.09^{d}	9.56±0.44^{c}	0.67±0.01^{e}	18.94±0.61^{a}	12.33±0.73^{b}	6.84±0.02^{d}
C20:3ω3	Eicosatrienoic acid *	ND	0.06±0.01^{b}	ND	0.11±0.04^{a}	0.05±0.00^{b}	ND	ND
C20:5ω3	Eicosapentaenoic acid*	ND	0.05±0.01^{bc}	ND	0.11±0.01^{a}	0.06±0.02^{b}	0.02±0.03^{bcd}	0.01±0.02^{cd}
ΣPUFA		**5.31±0.17^{e}**	**11.21±0.17^{d}**	**16.76±.01^{c}**	**7.29±0.36^{e}**	**28.98±.09^{a}**	**20.15±1.20^{b}**	**30.94±0.80^{a}**
ω6: ω3		9.91±0.17^{a}	0.90±0.00^{d}	0.75±.03^{d}	7.14±0.55^{b}	0.52±0.01^{d}	0.63±0.00^{d}	3.52±0.12^{c}
SFA/UFA		6.56±0.30^{a}	3.96±0.06^{b}	2.44±0.31^{c}	3.59±0.33^{b}	1.64±0.12^{d}	2.58±0.19^{c}	1.45±0.08^{d}

Mean ±S.D, $n = 3$, ND; Not detected,
* : Systematic name, Diet composition (%w/w): see footnote on Table 1,
^{abcdef} Values with different superscript letters within a row are significantly different

ratio in larvae fats to values below 4:1, as evidenced in FLAX20 (ratio of 2.81:1) and CHIA20 (ratio of 2.5:1) feed results. The FA composition of the seed meals used reflected in the ratios of essential fatty acid in *T. molitor* larvae fat. Flax seed and chia seed, for example, are both considered to be rich sources of the essential omega-3 FA (Orsavova et al., 2015). In the study by Francardi et al. (2017), the addition of 10% linseed (flax seed) to the diet of *T. molitor* resulted in ω6:ω3 of 4.05:1, compared to a value of 21.78:1 for *T. molitor* raised on wheat flour, oat flour and yeast (Cito et al., 2017). The significantly low ω6:ω3 ratios observed in the current study (i.e., below 6:1) are attributed to the relatively high omega-3 FA levels present in the seeds meals used in insect feeds.

Fatty acid composition of H. illucens *larvae*

The FA composition of the diet positively affected the FA of some of larvae raised on them. Supplementing the basal feed with 20% rapeseed increased the oleic acid value of *H. illucens* larvae from 4.52% (B100) to 10.83% (RAPE20). The oleic acid in rapeseed (63.3%) being significantly higher than wheat bran (13.3–15%) (Jung et al., 2010; Orsavova et al., 2015). Similarly, the substitution of the basal feed with 20% hemp seed meal, resulted in an increased LA content in the *H. illucens* larvae. An increment from 4.85% in B100 to 24% in HEMP20 was observed. Both hemp seed and wheat bran have higher and comparable LA content (59.4% in hemp seed, about 52.2–60% in wheat bran) (Jung et al., 2010; Orsavova et al., 2015). However, the resulting FA composition in this study shows that *H. illucens* larvae fat had a higher LA value when 20% hemp seed (24%) is used to supplement the wheat bran feed than when raised on only wheat bran (4.85%) (Table 3). A similar value of LA (26%) was recorded by Barroso et al. (2017) when *H. illucens* were raised on laying hen feed. The increased LA value subsequently resulted in a higher PUFA in HEMP20 (30.94%) larvae compared with B100 (5.31%). The increased PUFA in *H. illucens* could be due to the increased oleic and LA contents of seed meals used in the insect diets.

The percentages of SFA, MUFA and PUFA varies among the larvae fed the various diets (Table 3). The most abundant FA in *H. illucens* diets were lauric acid, myristic and palmitic acid (for the SFA), oleic acid (for the MUFA), and α-linoleic acid and α-linolenic acid (for PUFA). The high SFA content found in *H. illucens* larvae fed wheat bran only (B100; 86.75%), in this study is similar to finding in another study where a high SFA (81.9%) was reported for *H. illucens* larvae raised on commercial laying-hen feed (Guil-Guerrero et al., 2018). Supplementing the basal wheat bran diet in the current study resulted in lowering of the SFA content of larvae fat to between 59.13% (in HEMP20) and 79.82% (in CHIA20).

In the basal diet; B100, the SFA and ω6:ω3 compositions were significantly different from the rest of the diet ($p \leq 0.05$). In addition, it contained higher percentages of capric and lauric acid (5.46% and 62.05% respectively) compared to other diets. However, the pentadecyclic acid and margaric acid detected were of slightly lower values. High SFA in the larvae ranged from 59.13% in HEMP20 to 79.82% in CHIA20, and was higher in B100 (86.75%). The lauric acid present of *H. illucens* was very high; 62.5% in B100. High contents of this fatty acid were also detected in the other samples: FLAX20 (55.42%), FLAX10 (44.97%), RAPE20 (52.97%), CHIA20 (36.53%), CHIA10 (52.54%), and HEMP20 (33.38%). These high percentages of lauric acid are also similar in *H. illucens* larvae fed fish offal and cow manure (42.57% lauric acid) (St-Hilaire et al., 2007) and laying hen feed (57.2% lauric acid) (Guil-Guerrero et al., 2018).

Supplementing the basal diet increases the percentage of α-linolenic acid (ALA), an essential FA present in most of the samples thereby reducing the ω6:ω3 ratio. Among the PUFA, ALA and linoleic acid (LA) are the major components and are present in varying amounts. A higher amount of ALA was found to be 18.94% and 12.33% in CHIA20 and CHIA10 respectively. These values were significantly higher than those in the basal diet B100 (0.49%), and in RAPE20 (0.67%). With a ω6:ω3 ratio of 3.52:1, CHIA20 has a ratio that is closer to the 4:1 associated with the prevention of secondary cardiovascular diseases Simopoulos (2002). The ω3 FA found in *H. illucens* is mainly ALA, while in some others, eicosatrienoic acid and eicosapentaenoic were detected in small amount.

Discussions

The study of the dietary requirement, metabolism, transport, storage, and utilization of lipids in insects has been a hot topic in the

past decades (Canavoso et al., 2001). Fatty acids play several important roles such as serving as 'powerhouse' of energy reserve, as precursors for the synthesis of pheromones, ensuring the fluidity of cell membranes and playing important roles in insect metamorphosis (Lockey, 1988; Stanley, 2006). Most insects require dietary sources of lipophilic compounds such as sterols, carotenoids, PUFAs, fat-soluble vitamins, and prostaglandins, but these requirements differ significantly between species (Canavoso, Jouni et al. 2001). Lipophorins are responsible for the transport of lipids in insects, and these lipids are stored as in the fat bodies, in a form that is predominantly triacylglycerides (~ 90%) Arrese and Soulages (2010). It is also widely known that insects show significant plasticity in lipid accumulation, and changes in FA profile is largely driven by feed/substrate raised on, growth climate, stage of development or instar, secondary isotopic effects, etc. (Bjørge et al., 2018; Hoc et al., 2020; van Dooremalen & Ellers, 2010).

In this study, the fatty acid compositions of two promising insect larvae species for consumption were analysed to determine a suitable feed substrate in an attempt to improve their fatty acid composition for a healthier diet. The categories of larvae FA (in particular, the predominance of palmitic, oleic, and linoleic acids) in this study are similar to those reported in previous studies (Cito et al. 2017, Francardi et al. 2017). For *T. molitor* larvae, the shortest chain fatty acids detected were lauric acid (C12:0) (see Table 2). In their study, Cito et al. (2017) reported the presence of capric acid (C10:0, 0.02%) in *T. molitor* larvae reared on laying-hen feed. Moreover, those larvae fed 100% wheat flour bread had a SFA composition (29.60%) similar to those reported in the current study (i.e., W100; 28.61%). The SFA in the present study ranged between 26.93 and 28.79% and was similar to (e.g. 28.78% reported by Cito et al. (2017)) or higher than (e.g. 22.26% reported by Ravzanaadii et al. (2012)) those reported in other studies. Some insect order (Lepidoptera) with similar SFA composition as *T. molitor* are Monarch butterfly larvae (*Danaus plexippus;* 30.0%) and Malachite larvae (*Siproetastelenes;* 27.6%) (Guil-Guerrero et al., 2018). The SFA composition of HEMP20 (26.95%) and FLAX20 (26.93%) were similar to cotton oil (25.5%), higher than wheat germ oil (18.8%) and soybean oil (14.29%), and lower than coconut oil (82.48%), cocoa butter (59.7%) and palm kernel oil (81.5%). Fats from some animal sources are also more saturated; butter (60%), tallow (49.8%), and lard (39.2%) (USDA, 2019).

Some insects (such as *T. molitor* and *H. illucens*) contain delta-12 desaturase enzymes and have endogenous FA synthesis capacities. They are therefore able to convert ω-9 oleic acids to ω-6 linoleic acids (Brandstetter & Ruther, 2016). Consequently, feeding of *T. molitor* larvae with diets that are high in ω-3:ω-6 ratios causes an increase in PUFA proportions and in ω-3/ω-6 ratios in the larvae (Fasel et al., 2017). Importantly, changes in the proportion of ω-3 fatty acids (i.e., α-Linolenic acid) are largely driven by diet (Cookman et al., 1984; Dreassi et al., 2017).

Linoleic acid present in *T. molitor* (16.87% in CHIA20, and 26.67% in HEMP20) is one of the two essential PUFAs (the other being α-Linolenic acid) that are associated with the ability to reduce the risk of heart attacks and reduce inflammations. Additionally, the reduction in the ω6:ω3 ratio (see Table 2) achieved in *T. molitor* larvae in the study has an important health implication; as the appropriate levels of these dietary essential fatty acids are necessary for regulating and improving serum lipids and postprandial triacylglycerol concentrations (Mori et al., 2000). Several studies have also confirmed its effects on the cardiovascular system by reducing and preventing the risk of disease (Kris-Etherton et al., 2002). The optimum ω6:ω3 ratio also plays an important role in preventing and managing type 2 diabetes, insulin resistance, and neurological disorders (Calon and Cole, 2007; Delarue et al., 2004).

The categories of FA, including a high SFA levels detected in *H. illucens* studied here were similar to those reported in previous studies (Barroso et al., 2017; Caligiani et al., 2018; Hadj Saadoun et al., 2020; Lim et al., 2019; St-Hilaire et al., 2007). Except in the control diet (86.75% SFA), the range of SFA (70.79%- 79.82%) recorded in the other samples are comparable to *H. illucens* fed alfalfa meal (73.0% SFA) and a diet with 60% cows' milk whey and mixtures of wheat bran, alfalfa meal and cornmeal (74.5% SFA) (Hadj Saadoun et al., 2020). The use of cows' milk whey as a moisture source, instead of water provided other nutrients such as lactose and mineral salts, thereby increasing larval weight and growth time (Hadj Saadoun et al., 2020). *H. illucens* fed B100 (62.05%) has similarly high lauric acid content to the larvae fed waste coconut endosperm and mixed-bacteria powder (63.10%) (C.-Y. Wong et al., 2019). The lauric acid values obtained for most of larvae studied (52.97%–44.97%) were higher than *H. illucens* larvae fed soybean curd residue and *L. buchneri* (39.60%) (Somroo et al., 2019). These values are however lower than when waste coconut endosperm and *S. cerevisiae* was used as a feed (C. Y. Wong et al., 2020).

Studies have shown that even if rearing substrates are rich in polyunsaturated fatty acids, the fatty acid profiles of the insect larvae may be rich in saturated fatty acids. This phenomenon has been observed in *H. illucens* (Giannetto et al., 2020; Meneguz et al., 2018). A similar observation was made in the current study when *H. illucens* were fed the various seed meals: the total SFA content in larvae fed various seed diets (between 59.13% and 79.82%) and basal diet (86.75%) were significantly high (see Table 3). It is known that 12 to 18 carbon fatty acids can be biosynthesized *de novo* by several insect species, including *H. illucens* (Hoc et al., 2020). The interesting thing is that these fatty acids can be synthesized from the carbohydrates in the diet, and this process is regulated by genes coding for acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). ACC is responsible for the synthesis of malonyl-CoA from acetyl-CoA, and FAS catalyzes the coupling of two-carbon units to an acetyl-CoA to make the fatty acid molecule (Giannetto et al., 2020). It is also known that *H. illucens* can bioaccumulate dietary unsaturated fatty acids (especially oleic acid, linoleic acid, and α-linoleic acid) and metabolise them into lauric acids (Hoc et al., 2020). The phenomenon mentioned above may account for the relatively high levels of lauric acids observed in the current study. It is observed that the larvae raised on the basal diet B100 (which is low in PUFA, and high in carbohydrates) contained the highest proportion of lauric acid (62.05%). In contrast, larvae fed with PUFA-rich seed meals were relatively low in lauric acid (i.e., between 33.38% in HEMP20 and 55.42% in FLAX20). This too is a reflection of the fact that PUFA in seed meal or substrates can bioaccumulate to some extent in insect materials.

This study also confirmed a large quantity of SFA in *H. illucens* fat (62.07% to 86.75%). *H. illucens* larvae raised on an equal amount of cow manure and fish offal reported a high range of SFA (41.95% to 61.91%) with lauric acid (42.57%), palmitic acid (11.14%), and oleic acids (12.28%) in larvae fat (St-Hilaire et al., 2007). Similarly, a high SFA (81.9%) containing lauric acid (57.2%), palmitic acid (9.3%) and oleic acids (6.5%) were reported in *H. illucens* raised on laying-hen feed (Guil-Guerrero et al., 2018). Liland et al. (2017) reported that *H. illucens* larvae reared on 100% brown algae contained lauric acid (40.2%), palmitic acid (14.8%), oleic acid (8.9%), and SFA (67.9%). The SFA content in the larvae raised on various levels of brown algae diet were between 50.9% and 68.3%. The oleic acid content of RAPE20 (10.83%) in this current study is comparable to the 10.6% found in *H. illucens* fed 50% brown algae in the work of Liland et al. (2017). The high lauric acid in *H. illucens* is beneficial due to its antiviral and antimicrobial functions and a healthy role played in fat and cholesterol accumulation and storage (Sun et al., 2002). Monolaurin, together with lauric acid, its precursor, has significant antimicrobial activity against some microorganisms Dayrit (2015). In addition, it has also been shown to increase both total cholesterol and high-density cholesterol (good cholesterol) levels (German and Dillard, 2004). In their study, Giannetto et al. (2020) inferred that *H. illucens* larvae and prepupae can biosynthesise ω-3 and ω-6 FA, when they observed significantly high levels of these FA in the fatty acid profile of the insect materials. However, a mechanistic study by Hoc et al. (2020) has shown that *H. illucens* may bioaccumulate these fatty acids (up to 15%), and about two-thirds of these fatty acids are metabolized into the saturated acids lauric acid or myristic acid.

Conclusion

The study investigated the roles of seed meal supplemented diets on the lipid composition and fatty acid profiles of *T. molitor* and *H. illucens* larvae. It was confirmed that feeding insect larvae with different substrates can affect the composition of the larvae fat. Moreover, there were higher amounts of PUFA and MUFA in the fat of the larvae fed on seed meals compared to those fed on basal diets. This shows that the FA composition profiles of *T. molitor* and *H. illucens* larvae meal can be modified by manipulating their diet composition, and "healthy" FA components, especially the omega-3 PUFA, can be improved via feeding trials.

T. molitor larvae fed with a diet containing 10% chia seed and flax seed showed a "healthier" profile with regards to the ω6:ω3 ratio than those fed with the corresponding base diet. However, in the case of *H. illucens*, supplementing the basal diet with 20% hemp seed and rapeseed meal improved this "healthy" fatty acids ratio. The previous reports on *H. illucens* have been based on using the larvae as a bio-converter of organic waste and agricultural by-products. Resulting in its utilization in the production of bio-diesel and animal-grade feedstuff. However, this study suggests that rearing the larvae on cleaner foodstuff as done in the present study, can make its valuable components, such as the lipids, useful for edible purposes. This study suggests that supplementing *T. molitor* diet with healthy seed meals creates the possibility of producing edible larvae products with improved dietary properties, namely optimal ω6:ω3 ratio and low SFA. *H. illucens*, however, may be useful for food and feed purposes.

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Conflict of Interest

Authors declare no conflicts of interest.

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