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# LINSEED TO INCREASE N-3 FATTY ACIDS IN *TENEBRIO MOLITOR* (COLEOPTERA TENEBRIONIDAE)

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Francardi V., Cito A., Fusi S., Botta M., Dreassi E. – Linseed to increase n-3 fatty acids in *Tenebrio molitor* (Coleoptera Tenebrionidae).

The yellow mealworm *Tenebrio molitor* is one of the most promising edible insect species for human consumption and its potential use as a supplement of unsaturated fatty acids (UFA) for human diet, has been recently confirmed.
The possibility to enhance polyunsaturated acids (PUFA), especially omega-3 (n-3) content, was evaluated in *T. molitor* mealworms. The purpose was to obtain an n-6/n-3 ratio more suitable for human consumption, which is useful for the secondary prevention of cardiovascular diseases. To this end, tests were carried out by adding linseed, as source of n-3 acid, to insect feeding diets. A decrease of saturated fatty acids (SFA) and an increase of PUFA contents, especially n-3 acid, was simultaneously observed in larvae fed on almost all the diets enriched with linseed. As a result, supplementation of feeding diets with linseed determined a favorable insect growth rate and a decrease of n-6/n-3 ratio to values more suitable for the prevention and treatment of cardiovascular diseases.

KEY WORDS: *Tenebrio molitor;* n-6/n-3 ratio; polyunsaturated fatty acids (PUFA); α-linolenic acid; linseed.

## INTRODUCTION

The potential use of the yellow mealworm *T. molitor* larvae as unsaturated fatty acids (UFA) supplement for human diet has been recently proposed (DREASSI *et al.*, 2017). The fat component of this coleopteran is indeed relatively low in saturated fatty acids (SFA) content but high in unsaturated fatty acids (UFA) content, mainly oleic acid (18:1n-9), among the monounsaturated fatty acids (MUFA); α-linoleic acid (18:2n-6) (n-6) and α-linolenic acid (18:3n-3) (n-3) among the polyunsaturated fatty acids (PUFA). LIVINGSTONE *et al*. (2012) reported that a high content of UFA in human diet might have beneficial effects on the cardiovascular system, partially replacing SFA intake.

PUFA, especially eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA), the long chain derivatives of α-linolenic acid, are the main fatty acids (FA) involved in the prevention of cardiovascular diseases (MANERBA *et al*., 2010). The mealworm *T. molitor* is able to synthesize *de novo* α-linoleic and α-linolenic acid; conversely, it does not contain EPA and DHA, as similarly observed in other terrestrial insect species (DADD, 1983; SÁNCHEZ-MUROS *et al.*, 2014).

Though a lower n-6/n-3 ratio (4:1) is reported as the most favourable value for the secondary cardiovascular prevention (SIMOPOULOS, 2002), the mealworm *T. molitor* is generally characterized by a n-6/n-3 ratio suitable for human consumption, ranging between 20:1 to 25:1 (DREASSI *et al*., 2017).

Diets are known to alter insect growth rate and nutritional quality and this suggests the possibility to increase the production and nutritional composition of insects to better suit consumer needs (ANDERSON, 2000; RICCIARDI & BAVIERA, 2016). In fact, several studies confirmed the possibility to increase PUFA content in insects through diet; for example, diets supplemented with fish offal increased n-3 fatty acid in the dipteran *Hermetia illucens* (L.) (Diptera: Stratiomyidae) (ST-HILAIRE *et al*., 2007).

Insect nutritional quality may change also according to the insect developmental stage, as observed in a recent study that reported a lower n-6/n-3 ratio in *T. molitor* larvae than in pupae raised on diets with a growing fat content (DREASSI *et al*., 2017).

The aim of the present study is to enhance PUFA content, especially n-3 FA, in *T. molitor* larvae to obtain n-6/n-3 ratios more similar to values recommended for human secondary prevention of cardiovascular diseases. For this purpose *T. molitor* larvae were reared on different diets suitable for *T. molitor* feeding (DREASSI *et al*. 2017), and supplemented with commercial linseed, known as "health seed" for its nutritive properties. Fat composition of larvae reared on the enriched diets was compared to those previously fed on the corresponding base diets.

The influence of the enriched diets on *T. molitor* larval growth was also investigated.

## MATERIALS AND METHODS

Four different base diets (D3-D6), previously selected for *T. molitor* larvae mass rearing (DREASSI *et al*., 2017), were supplemented with linseed ground to flour (10% w/w) and used as feeding substrates (hereafter indicated as D3+-D6+). FA compositions of the diets were determined on the basis of a single analysis. Carrot pieces were added to each diet twice a week to provide the tenebrionid with a moisture source.

*T. molitor* larvae were obtained from the commercial

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supplier "La Voliera" (Florence, Italy) but detailed information on the diet they had been previously provided with was not available. In the laboratory, larvae were divided in four groups and separately reared on the base diets (D3-D6) to complete their development until the adult stage. 250 young larvae (15-20 mm length) of the following generation were collected from each diet and reared on the corresponding diet enriched with linseed (D3+-D6+).

Fat content and fatty acids (FA) composition analysis was carried out from the insects reared on the enriched diets (D3+-D6+) in the same conditions previously reported for the base diets (D3-D6) (Dreassi *et al*., 2017). The influence of the enriched diets on *T. molitor* mealworms growth rate, based on the number of days elapsed from the beginning of the tests until or beyond 50% pupation (hereafter referred to as PT50) and cumulative mortality percentage (hereafter referred to as mortality) were evaluated, as previously described (Dreassi *et al*., 2017). Finally, fifty *T. molitor* newly formed pupae (free from chitin exuvia and excreta) were weighed and the obtained medium values used as an indirect index of the best feeding diet for the tenebrionid fitness, as performed for other coleopteran species (Hunt *et al*., 1992).

FA analysis of lipid fraction in all the diets and insects were performed with GC-FID and GC-MS, as previously reported (Dreassi *et al*., 2017).

Quantitative analyses (fat content and FA composition) were run at least in triplicate and data expressed as means ± standard deviations (SD). A T-test was performed to assess significant differences in fat content and FA composition between larvae reared on each base diet and those reared on the same diet enriched with linseed. Differences in growth parameters (PT50, cumulative mortality percentage and pupal medium weight) were also evaluated. All data were tested for normality and transformations (log and angular transformation respectively) were then applied before statistical analysis.

## RESULTS AND DISCUSSION

### Fat content and FA composition of the breeding diets

The diets enriched with linseed (D3+-D6+) showed an increase of 3-3.5% in total fat content based on dry matter compared to the base diets (D3-D6) (Table 1).

*Table 1* – Fat content and FA composition of the rearing diets used for *Tenebrio molitor* larvae.

| | Diets[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | D3[b] | D3+ | D4[b] | D4+ | D5[b] | D5+ | D6[b] | D6+ |
| Total fat content (% dry weight) | 6.23 | 9.81 | 7.34 | 10.81 | 7.92 | 11.33 | 9.34 | 12.61 |
| FA (mol%) | | | | | | | | |
| Capric acid 10:0 | 0.02 | 0.02 | 0.00 | 0.03 | 0.23 | 0.14 | 0.26 | 0.10 |
| Lauric acid 12:0 | 0.14 | 0.00 | 0.06 | 0.03 | 0.12 | 0.08 | 0.22 | 0.07 |
| Tridecylic acid 13:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Myristic acid 14:0 | 0.39 | 0.23 | 0.40 | 0.36 | 0.58 | 0.45 | 0.34 | 0.28 |
| 14:1n-5 acid | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14:2n-3 acid | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.12 | 0.00 |
| Myristoleic acid 14:1n-5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Pentadecylic acid 15:0 | 0.08 | 0.06 | 0.03 | 0.03 | 0.03 | 0.05 | 0.00 | 0.05 |
| Palmitic acid 16:0 | 16.18 | 5.78 | 19.55 | 15.36 | 19.40 | 16.04 | 19.31 | 13.19 |
| 16:1n-5 acid | 0.26 | 0.04 | 0.26 | 0.07 | 0.68 | 0.07 | 0.70 | 0.04 |
| Palmitoleic acid 16:1n-7 | 0.16 | 0.17 | 0.20 | 0.28 | 0.17 | 0.69 | 0.00 | 0.67 |
| 16:2n-4 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Margaric acid 17:0 | 0.06 | 0.09 | 0.03 | 0.09 | 0.04 | 0.09 | 0.12 | 0.07 |
| 17:1n-6 acid | 0.00 | 0.03 | 0.00 | 0.03 | 0.00 | 0.02 | 0.00 | 0.03 |
| Stearic acid 18:0 | 2.12 | 3.94 | 1.84 | 2.76 | 1.96 | 2.94 | 1.98 | 3.59 |
| Oleic acid 18:1n-9 | 31.55 | 24.62 | 35.84 | 30.61 | 35.72 | 28.07 | 34.68 | 24.14 |
| α-Linoleic acid 18:2n-6 | 46.07 | 33.62 | 39.64 | 32.25 | 38.93 | 30.50 | 40.28 | 24.61 |
| Arachidic acid 20:0 | 0.43 | 0.33 | 0.15 | 0.14 | 0.14 | 0.14 | 0.14 | 0.13 |
| α-Linolenic acid 18:3n-3 | 1.81 | 30.94 | 1.34 | 17.65 | 1.35 | 20.31 | 1.37 | 32.49 |
| Eicosenoic acid 20:1n-9 | 0.46 | 0.11 | 0.54 | 0.27 | 0.51 | 0.34 | 0.48 | 0.55 |
| 20:2n-6 acid | 0.05 | 0.03 | 0.05 | 0.04 | 0.04 | 0.07 | 0.00 | 0.00 |
| Behenic acid 22:0 | 0.21 | 0.00 | 0.07 | 0.00 | 0.07 | 0.00 | 0.00 | 0.00 |
| SFA | 19.63 | 10.45 | 22.13 | 18.81 | 22.59 | 19.94 | 22.37 | 17.48 |
| MUFA | 32.44 | 24.96 | 36.83 | 31.26 | 37.08 | 29.18 | 35.86 | 25.42 |
| PUFA | 47.93 | 64.59 | 41.04 | 49.93 | 40.33 | 50.88 | 41.78 | 57.10 |
| SFA/UFA | 0.24 | 0.12 | 0.28 | 0.23 | 0.29 | 0.25 | 0.29 | 0.21 |
| n-6/n-3 ratio | 25.51 | 1.09 | 29.54 | 1.83 | 28.79 | 1.51 | 26.91 | 0.76 |

[a] Diets composition (%w/w): D3 wheat flour (25), oat flour (25), corn flour (25), chickpea flour (25); D3+ linseed (10), wheat flour (22.5), oat flour (22.5), corn flour (22.5), chickpea flour (22.5); D4 oat flour (50), wheat flour (50); D4+ linseed (10), oat flour (45), wheat flour (45); D5 beer yeast (5), wheat flour (47.5), oat flour (47.5); D5+ linseed (10), beer yeast (5), wheat flour (42.5), oat flour (42.5); D6 beer yeast (0.5), wheat flour (33.17), oat flour (33.17), corn flour (33.17); D6+ linseed (10), beer yeast (0.5), wheat flour (29.8), oat flour (29.8), corn flour (29.8);
[b] data previously reported by Dreassi *et al.* (2017).

Though sharing the same most prevalent FA, the four enriched diets showed a lower SFA content, prevalently palmitic acid, while stearic acid slightly increased. Moreover, the MUFA content, mainly oleic acid, decreased compared with that recorded in base diets. In addition, a higher PUFA content was detected in all the diets enriched with linseed *vs* the corresponding base diets; α-linolenic acid content was 20-30 times higher in enriched *vs* base diets; conversely, α-linoleic content decreased after supplementation of each diet with linseed. As a result, the higher amount of α-linolenic acid determined a decrease of n-6/n-3 ratio in all the enriched diets (Table 1).

### Fat content and FA composition of the mealworms

The increased fat amount detected in the diets enriched with linseed was not observed in the mealworms fed on them. In fact, the mealworms fed on the enriched diets showed a total fat content similar to that of insects fed on the corresponding base diets (Table 2). The same most prevalent FA (myristic acid, palmitic acid, 16:1n-5 acid, palmitoleic acid, stearic acid, oleic acid, α-linoleic and α-linolenic acid) were found either in *T. molitor* larvae fed on the base diets or in those fed on the corresponding enriched diets. Conversely, a significantly decrease of SFA, prevalently palmitic acid, was detected in specimens fed on each enriched diet *vs* the corresponding base diet. A significantly decrease of MUFA, mainly oleic acid, was also detected in larvae fed on D3+, D4+ and D6+ diets, balanced by a significantly higher PUFA content that entailed a significant decrease of SFA/UFA ratio. Finally, the addition of the linseed (10% w/w) led to an increase of α-linolenic acid content in larvae and, consequently, to a significant decrease of the n-6/n-3 ratio in the mealworms fed on enriched diets (Table 2).

### *T. molitor* larvae growth and developmental rate

Growth rate data of *T. molitor* are reported in Table 3. The mealworms reared on D3+ diet reached or exceed 50% pupation (PT50) in significantly fewer days than those fed

*Table 2* – Fat content and FA composition of *Tenebrio molitor* larvae fed on four different diets and the same diets enriched with linseed (10% w/w).

| | Diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | D3[a] | D3+ | D4[a] | D4+ | D5[a] | D5+ | D6[a] | D6+ |
| Total fat content (% dry weight) | 16.76±1.32 | 15.88±0.69 | 15.48±1.86 | 16.01±1.45 | 15.61±2.43 | 16.84±1.55 | 15.58±0.87 | 14.88±1.24 |
| Fatty acid (mol%) | | | | | | | | |
| Capric acid 10:0 | 0.03±0.00 | 0.06±0.01 | 0.05±0.01 | 0.05±0.00 | 0.03±0.00 | 0.03±0.01 | 0.03±0.00 | 0.05±0.00* |
| Lauric acid 12:0 | 0.72±0.06 | 0.45±0.04* | 0.79±0.06 | 0.44±0.07* | 0.78±0.09 | 0.65±0.02 | 0.78±0.11 | 0.65±0.06 |
| Tridecylic acid 13:0 | 0.06±0.01 | 0.04±0.01* | 0.09±0.14 | 0.04±0.01 | 0.10±0.02 | 0.14±0.02 | 0.09±0.01 | 0.13±0.06* |
| Myristic acid 14:0 | 6.79±1.44 | 4.95±0.28 | 6.99±0.86 | 4.64±0.37 | 6.98±1.12 | 6.80±0.62 | 7.21±1.30 | 6.63±0.44 |
| 14:1n-5 acid | 0.40±0.10 | 0.30±0.01 | 0.43±0.07 | 0.27±0.00 | 0.50±0.14 | 0.38±0.01 | 0.45±0.11 | 0.33±0.03 |
| 14:2n-3 acid | 0.19±0.03 | 0.17±0.01 | 0.21±0.02 | 0.15±0.03 | 0.25±0.06 | 0.40±0.09 | 0.22±0.03 | 0.30±0.05 |
| Myristoleic acid 14:1n-5 | 0.01±0.00 | 0.01±0.01 | 0.01±0.00 | 0.01±0.00 | 0.01±0.00 | 0.02±0.00 | 0.01±0.00 | 0.04±0.00** |
| Pentadecylic acid 15:0 | 0.08±0.00 | 0.07±0.02 | 0.09±0.01 | 0.08±0.00 | 0.09±0.00 | 0.10±0.01 | 0.08±0.01 | 0.09±0.01 |
| Palmitic acid 16:0 | 20.79±0.79 | 17.11±1.33* | 19.46±0.37 | 16.63±0.72** | 20.06±1.14 | 15.71±0.40* | 20.52±0.55 | 15.95±0.52** |
| 16:1n-5 acid | 1.37±0.33 | 0.78±0.07 | 1.46±0.18 | 0.61±0.01* | 1.60±0.34 | 1.32±0.03 | 1.66±0.30 | 1.20±0.08 |
| Palmitoleic acid 16:1n-7 | 1.82±0.15 | 2.59±0.12* | 1.74±0.11 | 2.51±0.15** | 2.10±0.21 | 3.15±0.39* | 1.94±0.07 | 2.85±0.11** |
| Margaric acid 17:0 | 0.06±0.02 | 0.09±0.01 | 0.07±0.01 | 0.11±0.02* | 0.07±0.01 | 0.09±0.00* | 0.06±0.01 | 0.07±0.03 |
| 16:2n-4 acid | 0.26±0.02 | 0.18±0.03* | 0.27±0.01 | 0.17±0.01** | 0.32±0.08 | 0.15±0.03 | 0.24±0.02 | 0.24±0.02 |
| ni ($t_R$ 34.1min) | 0.05±0.01 | 0.39±0.28 | 0.07±0.02 | 0.31±0.14 | 0.06±0.02 | 0.12±0.12 | 0.04±0.01 | 0.25±0.05** |
| 17:1n-6 acid | 0.05±0.01 | 0.07±0.01 | 0.05±0.01 | 0.08±0.00* | 0.06±0.01 | 0.27±0.25 | 0.05±0.01 | 0.09±0.01* |
| Stearic acid 18:0 | 3.36±0.56 | 3.67±0.58 | 3.31±0.06 | 3.96±0.81 | 2.82±0.24 | 2.68±0.79 | 3.20±0.13 | 3.12±0.25 |
| Oleic acid 18:1n-9 | 44.80±0.46 | 42.78±1.11* | 44.53±1.28 | 40.89±0.97* | 43.60±2.07 | 43.81±1.13 | 46.21±0.57 | 42.91±1.47** |
| ni ($t_R$ 39.5min) | 0.17±0.01 | 0.19±0.00* | 0.18±0.00 | 0.22±0.00** | 0.18±0.01 | 0.26±0.05 | 0.18±0.00 | 0.24±0.02* |
| α-Linoleic acid 18:2n-6 | 18.51±1.51 | 22.32±1.69 | 19.71±0.87 | 23.16±1.70 | 19.84±2.47 | 19.29±1.09 | 16.63±1.41 | 20.65±0.16** |
| Arachidic acid 20:0 | 0.10±0.03 | 0.15±0.04 | 0.10±0.02 | 0.14±0.04 | 0.09±0.03 | 0.11±0.01 | 0.10±0.03 | 0.10±0.00 |
| α-Linolenic acid 18:3n-3 | 0.28±0.12 | 3.35±0.11** | 0.28±0.08 | 5.25±0.35** | 0.32±0.09 | 4.18±0.62** | 0.23±0.09 | 4.83±0.21** |
| Eicosenoic acid 20:1n-9 | 0.03±0.04 | 0.05±0.00 | 0.04±0.01 | 0.06±0.02 | 0.05±0.02 | 0.06±0.01 | 0.04±0.02 | 0.06±0.01 |
| 20:2n-6 acid | 0.02±0.01 | 0.12±0.05* | 0.02±0.01 | 0.11±0.01** | 0.02±0.01 | 0.18±0.02** | 0.02±0.01 | 0.11±0.01** |
| ni ($t_R$ 47.8 min) | 0.03±0.00 | 0.11±0.04 | 0.06±0.02 | 0.12±0.02* | 0.05±0.01 | 0.07±0.04 | 0.02±0.02 | 0.10±0.01* |
| SFA | 31.99±0.84 | 26.58±1.08** | 30.94±0.66 | 26.08±0.34** | 31.03±0.80 | 26.31±0.25** | 32.06±0.94 | 25.80±1.28** |
| MUFA | 48.49±0.84 | 46.77±1.06* | 48.26±1.03 | 44.61±1.17* | 47.92±1.85 | 49.18±1.31 | 50.37±0.76 | 47.71±1.24* |
| PUFA | 19.26±1.64 | 25.96±1.82* | 20.48±0.90 | 28.66±1.32** | 20.75±2.64 | 24.06±1.60 | 17.33±1.51 | 25.90±0.00** |
| SFA/UFA | 0.47±0.02 | 0.37±0.02** | 0.45±0.01 | 0.36±0.01** | 0.45±0.02 | 0.36±0.01** | 0.47±0.02 | 0.35±0.02** |
| n-6/n-3 ratio | 39.06±2.89 | 6.38±0.28** | 40.53±0.85 | 4.33±0.59** | 34.68±3.09 | 4.29±0.16** | 37.05±3.05 | 4.05±0.12* |

[a] data previously reported by Dreassi *et al.* (2017).
*** Values significantly different between mealworms fed on each base diet *vs* the corresponding enriched diet (* $p < 0.05$; ** $p < 0.01$)
ni = not identified

*Table 3* – *Tenebrio molitor* mean days (± SD) to reach PT50, cumulative mean percentage mortality and pupal medium weight (n= 50 pupae) fed on the different diets.

| | D3[a] | D3+ | D4[a] | D4+ | D5[a] | D5+ | D6[a] | D6+ |
|---|---|---|---|---|---|---|---|---|
| Days to reach PT50 (days) | 188.00±17.35* | 159.60±9.61* | 100.40±17.49 | 126.40±7.76** | 96.20±3.83 | 88.40±20.84 | 111.00±23.01 | 83.00±12.30 |
| Cumulative mortality (%) | 46.80±3.63 | 39.20±6.57 | 20.80±13.39 | 13.20±11.37 | 30.40±10.62 | 39.60±7.67 | 29.60±11.87 | 38.00±15.62 |
| Pupal weight (mg) | 109.18±16.62 | 115.84±20.07** | 132.68±15.84 | 120.80±17.70 | 144.38±16.61 | 156.42±25.38** | 138.96±22.05 | 164.46±15.74** |

[a] data previously reported in DREASSI *et al.* (2017).
*** Values significantly different between mealworms fed on each base diet *vs* the corresponding enriched diet (* $p < 0.05$;** $p < 0.01$)

on D3, while PT50 of *T molitor* reared on D4+ was recorded in significantly more days than that recorded in insects reared on D4. The shortest PT50s were observed for the mealworms fed on D5+ (88.40 days) and D6+ diets (83.00 days) even if results were not significantly different from those fed on the corresponding base diets. Moreover, no significant differences were highlighted between cumulative mortality percentages of the mealworms fed on the base diets (D4-D6) and those fed on the same diets enriched with linseed (D4+ -D6+).

Pupae fed on D3+, D5+ and D6+ diets were significantly heavier than those fed on the corresponding base diets.

The enrichment of the feeding diet of the mealworm *T. molitor* with linseed opens a concrete possibility of tenebrionid employment in preparation of human food supplements with interesting functional properties for human health: low SFA intake and an optimal n-6/n-3 ratio for cardiovascular prevention (SIMOPOULOS, 2002). Furthermore, the enrichment of the feeding diets with linseed produced at the same time a high quality of the fat fraction and a favourable insect growth rate.

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