Proceedings of the 15th International Symposium
Modern Trends in Livestock Production
October 29 –31, 2025, Belgrade, Serbia

DIETARY CAMELINA AND LINSEED CAKES MODULATE FATTY ACID CLASSES AND SENSORY TRAITS OF YELLOW MEALWORM LARVAE

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https://doi.org/10.5937/AnimTrend25187P

Abstract: This study evaluated the effects of including 5% and 10% camelina and linseed cake in the diet of *Tenebrio molitor* L., on the fatty acid (FA) composition and sensory traits of dried larvae. Five isonitrogen and isoenergy diets were tested: a control diet (CON), a diet with 5% camelina cake (CAM 5), 10% camelina cake (CAM 10), 5% linseed cake (LIN 5), and 10% linseed cake (LIN 10). Larvae were fed the diets from the 4th to the 9th week of age, then euthanized by freezing at –60 °C and freeze-dried for FA quantification. A portion of larvae was microwave-dried to conduct consumer sensory evaluation. Visual, olfactory and overall acceptance was assessed by 141 employers or students of the University of Padova. Results showed that incorporating camelina and linseed cakes at both 5% and 10% increased the amount of polyunsaturated fatty acids (PUFA) of larvae ($P = 0.0053$) and reduced their n-6/n-3 ratio ($P < 0.0001$). In terms of visual, olfactory, and overall consumer acceptance, larvae from the CON group received the lowest ratings, while those from the LIN 10 group were rated most favourably by consumers (3.90 *vs* 5.42, respectively; $P<0.0001$). In conclusion, this study demonstrated that dietary inclusion of camelina and linseed cakes enhanced the PUFA content and improved sensory acceptance of yellow mealworm larvae. Notably, the improvement in the n-6/n-3 ratio was proportional to the level of oilseed cake supplementation, with the lowest and most favourable ratio of 4.43 achieved in the LIN 10 group.

Key words: oilseed by-products, omega-3 fatty acids, polyunsaturated fatty acids, consumer preference, edible insects.

Introduction

Tenebrio molitor (TM) is one of the main insect species studied and farmed in Europe. This insect is particularly interesting due to its rich nutritional profile, ease of rearing, and lower environmental impact compared to conventional livestock, especially in terms of land and water use (van Huis et al., 2013; Grau et al., 2017; Gkinali et al., 2022). Particularly, TM larvae have been proposed as a valuable alternative to conventional feed ingredients such as soybean meal and fish meal, due to their comparable nutritional profile. TM larvae contain about 50% protein on dry matter (DM) with a good balance of essential amino acids, around 30% lipids rich in beneficial fatty acids, and considerable levels of minerals and vitamins (Nowak et al., 2016; Kröncke et al., 2019; Trukhanova et al., 2022). For these reasons, TM larvae have been included in the diet of different animal species to test how they affect performance and product quality. Inclusion levels up to 30% TM meal have been tested in aquaculture species, showing no negative effects on yield and even improving growth and feed conversion at moderate levels (Belforti et al., 2015; Sánchez-Muros et al., 2016; Gasco et al., 2016; Iaconisi et al., 2017; Piccolo et al., 2017; Mastoraki et al., 2020). In poultry diets, TM meal and oil have been used at levels of 5-10% and below 2.5% respectively, without adverse effects on feed intake, weight gain, or feed efficiency (Ramos-Elorduy et al., 2002; Biasato et al., 2018; Sogari et al., 2019; Hong et al., 2020; Dalmoro et al., 2023). Moreover, including 5% TM meal in laying hen diets has been shown to increase omega-3 levels in eggs and improve egg weight, freshness, and shell strength (Ait-Kaki et al., 2021; Rahmawati et al., 2022).

These findings suggest that TM could serve as a valuable alternative source of protein and lipids in animal feed. However, before this potential can be fully realized, industrial-scale production must be optimized, as several key factors influence productivity (Morales-Ramos et al., 2012; Deruytter et al., 2019; Eberle et al., 2022). One of these factors is the insect diet (substrate), which not only impacts growth performance, fecundity, and survival rate, but also determines the chemical composition of the larvae (Morales-Ramos et al., 2010; Oonincx et al., 2015; Van Broekhoven et al., 2015; Liu et al., 2020). For instance, van Broekhoven et al. (2015) found that including 40% yeast-derived protein in the diet of TM larvae altered their fatty acid profile by increasing the n-6/n-3 ratio, while also promoting faster growth, lower mortality, and greater weight gain. Similarly, Ruschioni et al. (2020) reported that adding 25% olive pomace to the diet increased the protein and amino acid content of the larvae, as well as larval and pupal weight. Rearing mealworms on brewer’s spent grain has also been shown to enhance omega-3 and omega-6 fatty acid levels and improve the feed conversion ratio (FCR) (Melis et al., 2019).

In the present study, the potential of supplementing the diet of TM with sources of n-3 FA was investigated. Specifically, camelina (*Camelina sativa* L. Crantz) cake and linseed (*Linum usitatissimum* L.) cake were each included in the diet at levels of 5% and 10%. Camelina is an oilseed crop of the *Brassicaceae* family that has attracted growing interest in recent years due to its favorable attributes, including adaptability to diverse environmental conditions, low input requirements, short growing season and a rich nutritional profile (Murphy et al., 2016). Camelina seeds contain a high oil content (30-49%) with substantial levels of omega-3 and omega-6 fatty acids, along with 24-31% protein and notable amounts of tocopherols, phytosterols, and phenolic compounds (Gesch et al., 2012; Berti et al., 2016; Boyle et al., 2018). Thanks to its nutritional profile, camelina could be used as an alternative to conventionally used soybean. In particular, camelina cake, a by-product of camelina seed pressing, represents an interesting substrate for rearing TM larvae. This by-product contains a high protein content (around 35%), a good amount of essential amino acids, and 10-22% oil with a high proportion of omega-3 fatty acids (Aziza et al., 2014). Similarly, linseed cake, a by-product of linseed oil production, is a valuable substrate for TM, as it provides a high protein content (approximately 36%) and 7-10% residual oil, along with good levels of lignans, antioxidants, and minerals (Poreda, 2017).

Giving these considerations, the present study aimed to evaluate the effect of including camelina and linseed cakes in the diet of TM larvae, assessing their impact on the FA profile (% total fatty acid methyl esters – FAME) on the consumer perception of dried larvae.

Materials and Methods

Experimental design and insects management

The experiment was conducted at the INEF (Insect Novel Ecologic Food) insect farm, (Piombino Dese, Treviso, Italy) and at the Department of Animal Medicine, Production and Health (MAPS) of the University of Padova (Legnaro, Padova, Italy). Four week old TM larvae were randomly assigned to five treatment groups receiving different diets: a control group fed a commercial diet (CON); a group fed a diet with 5% camelina cake inclusion (CAM 5); a group fed a diet with 10% camelina cake inclusion (CAM 10); a group fed a diet with 5% linseed cake inclusion (LIN 5); and a group fed a diet with 10% linseed cake inclusion (LIN 10). All diets were formulated to be isonitrogenous and isoenergy. Each treatment group consisted of 12 replicates.

TM larvae were housed in plastic crates measuring 60 × 60 × 14.5 cm and were provided feed *ad libitum*, along with fresh carrots as a source of water. Ingredients and FA classes of the experimental diets are presented in Table 1.

Larvae were raised for five weeks, and at 9 weeks of age, when they reached the selling size, they were separated from residual feed and frass, then euthanized by freezing at –60 °C for one hour using a shock freezer (Challenge 250 TC, Angelantoni, Perugia, Italy). Subsequently, they were transported to the LabCNX laboratory of the MAPS Department (University of Padova) for freeze-drying to perform FA analysis. A portion of larvae from each crate within each experimental group was microwave-dried using a Max Industrial Microwave 30B (Max Industrial Microwave, China) at 103 °C (12 kW) for 10 minutes. This method, routinely used by the INEF insect farm, was carried out to prepare samples for sensory analysis.

Table 1. Fatty acid classes (% FAMEs) of the experimental diets

	Experimental diets				
	CON	**CAM 5**	**CAM 10**	**LIN 5**	**LIN 10**
SFA	16.7	15.7	14.8	15.8	15.0
MUFA	25.9	28.3	30.9	26.1	26.2
PUFA	56.0	54.4	53.0	57.4	57.9
n-6	50.9	44.3	38.9	43.5	38.6
n-3	5.12	10.2	14.1	13.9	19.3
n-6/n-3	9.94	4.35	2.77	3.12	2.00

Chemical analyses: FA profile of diets and TM larvae

Lipid extraction to determine the FA profile of the diets and of TM larvae was performed by modified accelerated solvent extraction (M-ASE). For insect samples, extraction was carried out with a chloroform/methanol mixture (1:2 ratio) following the method of Lee et al. (1995), while hexane was used for the diets. The fat content of the samples was determined gravimetrically after vacuum evaporation under a nitrogen stream. Samples were then trans-methylated using a 4% methanolic solution of H_2SO_4 to produce fatty acid methyl esters (FAMEs). To achieve biphasic separation, 8 ml of distilled water and 4 ml of n-heptane were added, with these volumes adjusted proportionally due to the high lipid content of the samples. FAMEs were quantified by gas chromatography (Shimadzu GC17A). Results were expressed as a percentage of the total detected FAMEs, as described by Cullere et al. (2018).

Consumer sensory analysis

For the consumer sensory analysis, a total of 141 employees and students from the University of Padova participated on a voluntary basis. Since a preference test was chosen, participants did not require any prior experience with the specific food matrix, or the sensory method used. Instead, each participant received a detailed guide explaining the correct procedure for conducting the evaluation. Each participant then evaluated five TM dried larvae samples, representing the different treatments (labelled A, B, C, D, and E), for visual, olfactory, and overall acceptance. Each attribute was rated on a scale from 1 (extremely unacceptable) to 7 (extremely acceptable). The sensory evaluation did not include tasting the samples. TM larvae were subjected to microbiological analysis for the most common pathogens before the sensory test to ensure the safe of the product. However, consumers, were not allowed to touch the samples.

Statistical analysis

Data of FA profile of TM larvae were analysed with a one-way ANOVA following the GLM procedures of the SAS 9.1.3 statistical analysis software for Windows (SAS Institute, 2008), with treatment groups treated as fixed effect. Sensory analysis data were analysed using mixed model ANOVA including diets as fixed effect and effect of consumers as repeated random effect.

Results and Discussion

FA classes of TM larvae

The dietary inclusion of camelina and linseed cakes significantly affected the FA profile of TM larvae (Table 2). The control group showed the highest proportion of saturated fatty acids (SFA) compared to the other groups (27.5% *vs* 23.8% on average; P = 0.0001). Conversely, the control group had the lowest proportion of polyunsaturated fatty acids (PUFA), whereas all supplemented groups showed significantly higher values, except for CAM10 which had an intermediate value (23.6% *vs* 29.0% and 26.7% on average, respectively; P = 0.0053). For monounsaturated fatty acids (MUFA), the CAM10 group exhibited a significantly higher proportion compared to LIN5 group, while the remaining treatments exhibited intermediate values. Supplemented groups also showed an improved n-6/n-3 ratio. The inclusion of camelina and linseed cakes significantly increased the n-3 FA proportion (P < 0.0001), thus reducing the n-6/n-3 ratio, with the lowest value observed in the LIN10 group and the highest in the control group (4.43 *vs* 20.7, respectively; P < 0.0001).

These results suggest that feeding TM larvae with PUFA-rich substrates is proportionally reflected in the increased PUFA of the larvae. Notably, TM possesses delta-12 desaturase enzymes and endogenous FA synthesis capabilities. They can synthesize linoleic and α-linolenic acids *de novo*; however, like other terrestrial insects, they are deficient in producing 20:5 n-3 (EPA) and 22:6 n-3

(DHA). Furthermore, they are able to convert n-9 FA to n-6 FA (Brandstetter and Ruther, 2016). Therefore, the diet remains the main factor influencing the proportion of n-3 FA in this species.

Similar to the findings of the present study, Melis et al. (2019) observed that including brewer spent grain, rich in PUFA, in the larvae diet increased their n-3 and n-6 levels.

Consistently, Lawal et al. (2021) demonstrated that supplementing the diet of TM larvae linseed and chia seeds significantly increased the PUFA content and reduced the n-6/n-3 ratio. With an inclusion of 10% of linseed seeds, Lawal et al. (2021) reported a PUFA content of TM larvae of 21.3%, compared to 10.2% found in the present work. Moreover, they reported a n-6/n-3 ratio of 5.92, which was significantly lower than that of the control group, in line with the result of our study. However, it should be noted that in the current experiment, the inclusion of linseed was in the form of linseed cake. This potentially alters the diet's fat content and composition and, as a result, the FA profile of the larvae, explaining the differences in the values between the two studies.

In the present study, the dietary strategy not only increased PUFA levels but also reduced SFA content and the n-6/n-3 ratio, which is a favourable outcome for the potential use of mealworms as an alternative feed and food source. High n-6/n-3 ratios are generally associated with negative health effects, as they can promote inflammation, and increase the risk of cardiovascular diseases. For example, a ratio of 10 has been linked to adverse health outcomes (Simopoulos, 2008; Cao et al., 2024), while a favourable n-6/n-3 ratio is recommended to be around 3:1 to 1:1 (Kim et al., 2007).

Table 2. Effect of camelina and linseed cake dietary inclusion on fatty acid classes (% of Total FAME) of TM larvae

	Experimental groups					**RSD**	***P-value***
	CON	**CAM 5**	**CAM 10**	**LIN 5**	**LIN 10**		
SFA	27.5^{A}	23.7^{B}	23.3^{B}	23.9^{B}	24.2^{B}	1.97	< 0.0001
MUFA	46.6^{AB}	46.5^{AB}	47.5^{A}	44.8^{B}	46.2^{AB}	1.70	0.0165
PUFA	23.6^{B}	28.8^{A}	26.7^{AB}	29.8^{A}	28.4^{A}	4.03	0.0053
n-6	22.4	25.8	22.9	25.7	23.1	3.59	0.0729
n-3	1.22^{D}	3.07^{C}	3.85^{B}	4.18^{B}	5.28^{A}	0.55	< 0.0001
n-6/n-3	20.7^{A}	8.39^{B}	5.95^{BC}	6.16^{BC}	4.43^{C}	2.95	< 0.0001

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

The n-6/n-3 ratio is also a critical factor in animal diets, as it influences growth performance, animal health and quality of the end product. Reducing this ratio, particularly to levels around 4:1 or 2.5:1, through the inclusion of linseed oil or fish oil has been shown to improve the performance and immune response of broilers, enhancing their overall health and resulting in meat enriched with longchain n-3 PUFA (Ibrahim et al., 2018). Similarly, decreasing the n-6/n-3 ratio from 30:1 to 5:1 has been shown to reduce the feed conversion ratio (FCR) in broilers (Qi et al., 2010). Furthermore, in Heigai pigs, a lower dietary n-6/n-3 ratio reduced triglyceride and cholesterol levels, while increasing the omega-3 FA content in the *longissimus dorsi* muscle (Nong et al., 2020).

Sensory analysis of TM larvae

Results of consumers sensory analysis are presented in Figure 1. The inclusion of camelina and linseed cakes significantly influenced the sensory attributes of dried TM larvae. For visual, olfactory, and overall acceptance, the CON group received the lowest scores, while the LIN10 group achieved the highest scores (4.10 *vs* 5.05; 3.76 *vs* 5.65; 3.90 *vs* 5.42, respectively; $P < 0.0001$). Intermediate scores were observed for the other treatment groups.

These findings demonstrate that, even though the larvae were not tasted, consumers perceived clear differences in their appearance and olfactory attributes. One possible explanation for these results is the different lipid content observed in larvae. Larvae from the CON group showed a lower ether extract compared to the others, particularly the CAM10 group, which had the highest value. Intermediate ether extract levels were found in the remaining groups (11.5 *vs* 13.8 *vs* 12.2 g/100 g, on average, respectively). Lipids play a crucial role in flavour and off-flavour development and depending on the reactions or degradation processes involved, they can impart either pleasant or unpleasant notes to the product. For instance, when exposed to heat, especially at high temperatures, lipid degradation produces aldehydes that can participate in the Maillard reaction and Strecker degradation (Shahidi et al., 2014). The compounds generated by these combined processes contribute to the development of desirable aromas (Kerth et al., 2015; Shahidi et al., 2015). This may explain the higher olfactory acceptance of larvae with a higher lipid content compared to those from the CON group, where the lower lipid content may not have allowed these processes to occur, resulting in less pleasant odours.

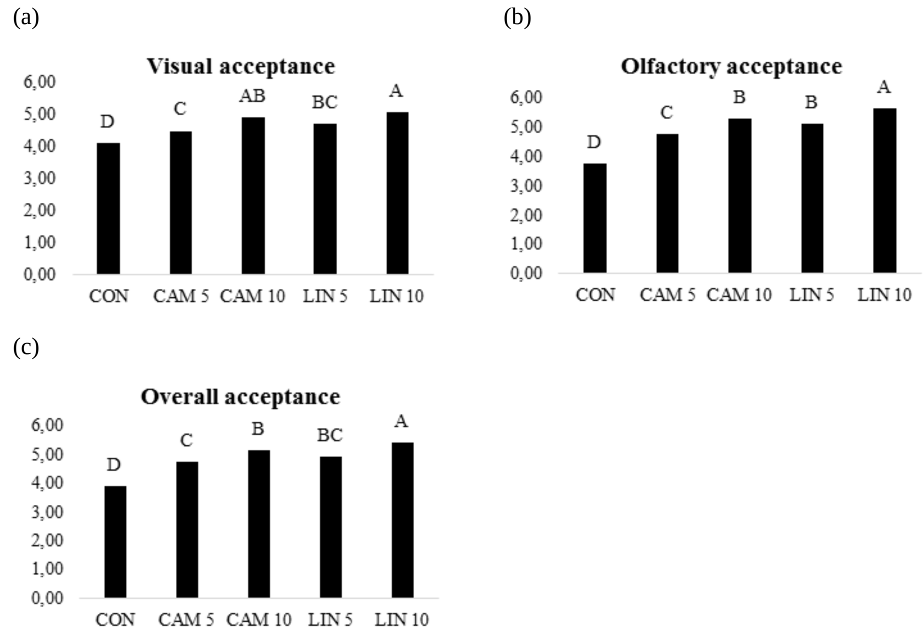

Figure 1. Effect of camelina and linseed cake dietary inclusion on visual **(a),** olfactory **(b)** and overall **(c)** acceptance of dried TM larvae

value from 1 to 7= 1: extremely unacceptable, 2: very unacceptable, 3: moderately unacceptable, 4: neither unacceptable or acceptable, 5: moderately acceptable, 6: very acceptable, 7: extremely acceptable; A-D Values within a row with different superscripts differ significantly at P< 0.0001;

Additionally, although TM larvae were dried using the same method, differences in the final product were possible. Particularly, larvae from LIN10 group showed a lower as compared to CAM5 group, while the other groups showed intermediate values (0.45 *vs* 0.69 and 0.61 on average, respectively; P< 0.0125).

Despite the product was dried, the residual moisture may have influenced the olfactory traits of larvae, resulting in a less appealing odour. Some microorganisms indeed, are able to survive also at low values of a_w, promoting reactions responsible for the formation of odorous compounds. This could have influenced the overall acceptance of the larvae of CAM5 and CON group.

Regarding visual acceptability, no differences in size were observed when comparing larvae across the groups. It is possible that the olfactory traits also influenced the visual and overall evaluation of the larvae.

Conclusion

In conclusion, the present study highlights the potential of incorporating camelina and linseed cakes in the diet of TM larvae, enhancing their FA profile. Specifically, the inclusion of 5% and 10% of camelina and linseed cakes significantly increased the PUFA content of larvae and decreased the n-6/n-3 ratio to more nutritionally desirable levels, particularly at the higher inclusion level of linseed cake. Additionally, dietary treatments significantly improved consumers acceptance of TM dried larvae especially with the 10% inclusion level of linseed cake. Overall, these findings demonstrate that oilseed cakes can be valuable, sustainable feed ingredients to improve the nutritional quality and consumer appeal of edible insects.

Ethics approval

Not applicable.

Acknowledgements

The authors are grateful to Elisabetta Garbin, Alberto Gottardo, and Sandro Tenti of the Laboratory “LabCNX” of the MAPS Department of the University of Padova (Italy) for the technical support and James Caon, the owner of the INEF farm where the trial took place. This research was fully supported by the University of Padova (Italy) funds (2023-prot. BIRD234733/23).

Conflict of interest

The authors declare no conflict of interest.

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