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Proceeding Paper

Improvement of *Tenebrio molitor* Larvae Farming and Fatty Acids Composition by Supplementation with Vegetable Waste †

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Abstract: *T. molitor* larvae were fed with wheat bran (W) and supplemented (1:1) with cucumber (C+W) or tomato (T+W) agricultural wastes, from conventional or ecological farming, for 6 weeks. Weekly and fortnight measurements of larvae weight/tray and length were taken, respectively. At the end of the study, the fatty acid composition of the larvae was analyzed by GC-MS. At day 43, the weight of larvae supplemented with wastes almost doubled that reached by the control larvae (647–720 vs. 370 g/tray), which were 15% smaller. Supplementation decreased the larval fat content and increased the polyunsaturated fatty acids between 22–37%, being linoleic acid the most abundant.

Keywords: *Tenebrio molitor*; insect farming; fatty acids; agricultural waste; diet supplementation; larval production; growing parameters

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1. Introduction

Concerning about food quality and environmental conservation gave rise to ecological farming in order to protect agriculture from industrialization. Different studies have demonstrated that ecological farming favours the biodiversity, reduces the use of pesticides, energy, and fertilizers. Some advantages compared to the conventional farming are the higher nutritional value of fruits and vegetables and the increased content in bioactive compounds, whose synthesis in the plant is triggered by abiotic or biotic stress. However, vegetables can also suffer nutritional deficiencies, vulnerability to pesticides, diseases and a lower self-live period. Some strategies are carried out to cover such disadvantages and obtain ecological products that meet consumer standards, but they are not always efficient enough, which lead to generate huge amount of agricultural wastes. Nowadays, the revalorization of wastes either from conventional or ecological farming is critical to reduce the carbon footprint and obtain economic profitability.

Circular economy has become one of the most important matter worldwide, whose objective is to create a sustainable food system and obtain food products considering their quality and safety while protecting the environment [1]. Recently, insect-based bioconversion has been suggested as a potential strategy for revalorizing agri-food wastes and contribute to build a circular economy system [2,3]. This novel approach is based on the fact that insect rearing can convert tons of food waste into valuable products for animal and human nutrition, as well as generating frass with suitable characteristics to be used as organic biofertilizer in ecological cultivation [4,5]. Additionally, this type of farming presents several environmental advantages such as low emissions of ammonia and greenhouse gases. *Tenebrio molitor* is one of the most used insects in bioconversion. They can easily adapt their larval development time, weight, and nutritional composition, depending on the provided diet. Generally, they are fed with grains, but supplementing or partially replacing their diet with vegetable wastes could be an alternative to save money and avoid the use of resources intended for human consumption while reducing environmental impact. In addition, vegetable wastes are rich in water content and an excellent source of bioactive compounds, which exert biological activity when consumed. Most common bioactive compounds in vegetables include phenolic compounds, terpenoids, vitamins, and sulphur compounds, which may bring benefits to insect growing and may provide health-promoting properties when insect-based products are consumed.

The present study aims to evaluate the effect of supplementing the diet of *T. molitor* larvae with vegetable wastes (cucumber and tomato, from conventional or ecological farming) on their growth performance and their fatty acid composition.

2. Material and Methods

2.1. Insect Farming

Insect farming was carried out at the Insectalia S.L. company facilities. *T. molitor* larvae were fed with wheat bran as a control diet (W) or supplemented (1:1) with vegetable wastes: cucumber (C+W) or tomato (T+W) proceeding from conventional or ecological farming. Water was added to the control diet to compensate the wastes moisture. Five trays containing 100 g of larvae were used for each studied diet condition and they were grown in a controlled-chamber room in 12 h light-dark cycles (27 °C and 50% humidity). Larvae were fed once per week for 6 weeks. The first 3 weeks, 150 g of wheat bran and 150 g of corresponding waste were added to each tray. On week 4th the quantity was increased to 200 g per tray and weeks 5 and 6, 400 g per tray. After that, they were starved for 48 h, collected and frozen at −20 °C.

2.2. Growth Performance Measurement

In order to measure the growth performance of larvae, each tray was sieved to separate frass from larvae. Larval weight measurements were taken weekly on a precision balance for each tray. Thereafter, the quantities of wheat bran plus water or vegetable wastes corresponding to each week were added. Length was measured fortnight by using a vernier calliper and was taken as the average of 5 larvae per tray.

2.3. Fatty Acid Profile

Fatty acid methyl esters (FAMEs) were obtained following the AOAC official method 996.06 and analysed in an Agilent 7890B gas chromatograph coupled to a 7200 quadruple-time-of-flight mass spectrometer with electron impact ionization. FAMEs were separated in a HP-88 capillary column (30 m, ø 0.25 mm and a film thickness of 0.2 µm), using Helium as carrier gas at a flow rate of 1 mL/min. Sample (1 µL) was injected with a split ratio of 1:20 (*v/v*). The oven temperature was increased from 80 °C to 145 °C at a speed of 8 °C/min, this temperature was maintained for 26 min, then it was programmed at 200 °C at a speed of 2 °C/min, maintaining this temperature for 1 more minute and finally up to 220 °C at a rate of 8 °C/min. Injector and transfer line temperatures were maintained at 250 and 240 °C, respectively. The instrumental conditions of the detector were the following: ionization source temperature, 230 °C; ionization energy, 70 eV; mass range, 50–500 m/z; solvent delay, 2 min. Extraction and derivatization were done in triplicate. Tentatively identified FAMEs were confirmed by comparison with Supelco’s mix of 37 FAMEs.

2.4. Statistical Analysis

Statistical analyses were carried out using the Statgraphics Centurion XVI.I software (Statgraphics Technologies Inc., VA, USA). Results were reported as the mean ± standard deviation. Results were subjected to an analysis of variance (ANOVA) followed by Tukey post hoc test in order to establish statistical differences among mean values. The statistical significance level was set up at $p < 0.05$.

3. Results and Discussion

3.1. Effect of Diet on Growing Parameters of T. molitor Larvae

The weight of *T. molitor* larvae increased over time, being more pronounced from day 15th to day 43rd. Diet influenced the larval weight and significant differences were observed among the supplemented and control groups from day 22 onwards (Figure 1). However, no differences were reported between the weight of larvae fed with cucumber or tomato wastes, regardless the type of farming (conventional or ecological). Larvae fed with just wheat bran showed significant lower weight than those supplemented with vegetable wastes. At day 43, larvae fed with cucumber or tomato wastes (conventional or ecological crops) reached 0.647–0.720 kg/tray, being almost twice the weight of larvae fed with wheat bran (0.370 kg/tray). Therefore, supplementation favours the growing of larvae since their weight increased 7 times from the beginning of the study. On the other hand, the weight of larvae fed with wheat bran increased by less than 4 times.

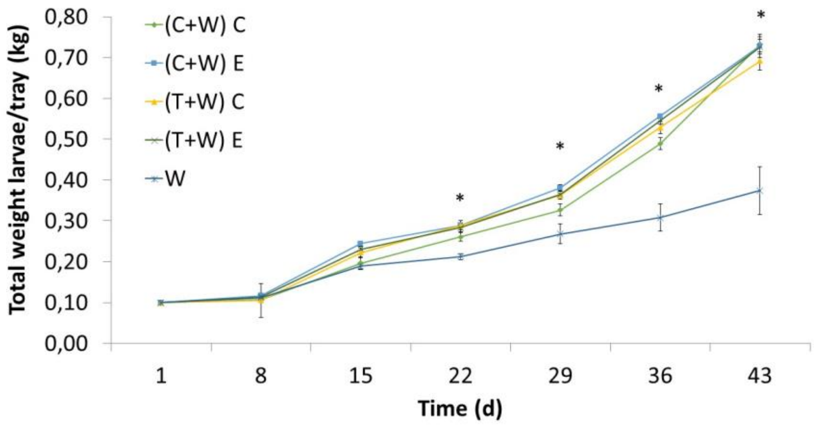

Figure 1. Larval weight of *T. molitor* determined weekly while feeding for 43 days with different diets [(C+W) C: Cucumber waste + wheat bran, conventional crop; (C+W) E: Cucumber waste + wheat bran, ecological crop; (T+W) C: Tomato waste + wheat bran, conventional crop; (T+W) E: Tomato waste + wheat bran, ecological crop; W: Wheat bran]. Asterisks (*) indicate statistical difference ($p < 0.05$) between the larvae weight fed with W (control diet) and that of those supplemented with vegetable wastes at the same rearing time. Values are expressed as mean ± standard deviation (n = 5).

The length of larvae increased over time, being more sharped from day 15th. Diet also affected the larval length and differences were easily detectable among the supplemented and control diets after day 36 (Figure 2). However, the length of larvae supplemented with agricultural wastes was similar regardless the kind of waste (cucumber or tomato) and the type of farming (conventional or ecological). At the end of the study, larvae fed with vegetable wastes had higher size (20 mm) than those fed with the control diet (17 mm).

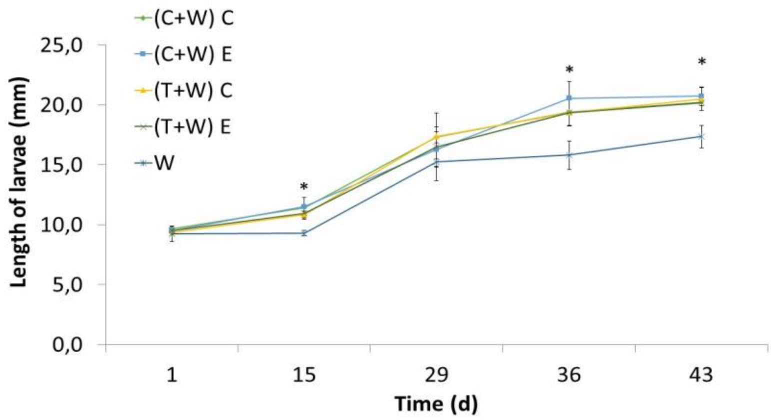

Figure 2. Larval length of *T. molitor* determined every fortnight while feeding for 43 days with different diets [(C+W) C: Cucumber waste + wheat bran, conventional crop; (C+W) E: Cucumber waste + wheat bran, ecological crop; (T+W) C: Tomato waste + wheat bran, conventional crop; (T+W) E: Tomato waste + wheat bran, ecological crop; W: Wheat bran]. Asterisks (*) indicate statistical difference ($p < 0.05$) between the larval length fed with wheat bran (control diet) and that of those supplemented with vegetable wastes at the same rearing time. Values are expressed as mean ± standard deviation (n = 5).

Some authors have found that nitrogen content and carbohydrates/protein ratio in diet influence the development time and growth of *T. molitor* and other insects. Nitrogen is required for protein biosynthesis, therefore, a lower nitrogen content is related to reduced growth [6]. Zhang et al., [7] suggested that supplementing diet with vitamins or proteins could reduce mortality and favour the growth. Supplementing the traditional wheat bran diet with vegetable waste increases the available nitrogen and provides extra minerals (K, Cu, S, Cl), vitamins (mainly B5 and B6 in the case of cucumber waste, and C and E in the case of tomato waste) (data not shown) and other bioactive compounds like carotenoids and phenolic compounds, which may accelerate the growth of the larvae. Additionally, the presence of moisture in diet has huge relevance for the performance of *T. molitor* [8]. Probably, the water contained in the agricultural wastes was preferable or better assimilated by larvae than that added to the wheat bran control diet, which could be evaporated before the water retained in the vegetables. No differences among conventional and ecological crops were found in the studied growing parameters; therefore, both wastes could be revalorized by insect bioconversion when used as larval growth promoters.

3.2. Effect of Diet on Fatty Acid Composition of T. molitor

Main fatty acids found in *T. molitor* larvae are compiled in Table 1. The content from highest to lowest was linoleic acid > elaidic acid > myristic acid = palmitic acid > stearic acid. Obtained results demonstrated that the diet influenced the fatty acid profile and the polyunsaturated/saturated ratio in *T. molitor* as reported by other authors [8,9]. Regardless the type of farming, cucumber and tomato wastes supplementation led to increase the content in linoleic acid (between 21.2–37.6% more than in the larvae fed with the control diet), reaching the highest content (41.01%) when the diet was supplemented with tomato waste from ecological farming. Besides, those larvae whose diet was supplemented with vegetable wastes presented lower content in myristic acid, reaching the lowest one (11.98%) in (T+W) E larvae. Such modifications led to an enhancement in the polyunsaturated/saturated fatty acid ratio in all supplemented larvae compared to the control larvae. The total fat contents of larvae were also influenced by the provided diet (data not shown), showing a significant 12% reduction compared to the control larvae when cucumber either from conventional or ecological farming were used as dietary supplements.

Diet influences the lipid accumulation and fatty acid composition. The lipid profile and content has been described to be more affected by the contents of non-fibrous carbohydrates, starch and protein of the feeding substrates [9,10] than by their fatty acid contents [11]. Acetyl-CoA carboxylase and fatty acid synthase are involved in de novo biosynthesis of fatty acids from carbohydrates [11]. The biosynthetic pathway to produce linoleic acid from myristic acid involves a series of enzymatic reactions including chain elongation and desaturation, in which several additional enzymes are implicated. Probably, larvae adapted their metabolism to the provided diet in order to be more effective to preserve or obtain energy. As far as we know, there are no available studies that compare the effects of using conventional or ecological vegetable wastes as insect dietary supplements on the nutritional composition of larvae, although it can be hypothesized that their differences in micronutrients and bioactive compounds could also affect to the obtained results.

Therefore, cucumber and tomato wastes (either ecological or conventional) could be used as a supplement to improve the percentage of linoleic acid and the polyunsaturated/saturated fatty acid ratio in larvae. Both modifications in the fatty acid profile of foods have been related to lower risk of suffering certain cardiovascular diseases [11].

Table 1. Effect of different larvae diet [(C+W) C: Cucumber waste + wheat bran, conventional crop; (C+W) E: Cucumber waste + wheat bran, ecological crop; (T+W) C: Tomato waste + wheat bran, conventional crop; (T+W) E: Tomato waste + wheat bran, ecological crop; W: Wheat bran] on flour fatty acids composition at the end of the study (day 43).

Larvae	Fatty Acids (%) *							
	Myristic Acid C14:0	Palmitic Acid C16:0	Stearic Acid C18:0	Elaidic Acid C18:1n9t	Linoleic Acid C18:2	Saturated Fatty Acids	Monounsaturated Fatty Acids	Polyunsaturated Fatty Acids
(C+W) C	13.08 ± 0.98 bc	13.25 ± 0.12 bc	5.18 ± 0.56 ab	22.20 ± 0.60 a	39.73 ± 0.18 b	33.42 ± 0.78 c	25.00 ± 0.44 b	41.71 ± 0.30 b
(C+W) E	14.63 ± 0.14 b	14.70 ± 0.28 a	4.68 ± 0.3 bc	22.11 ± 0.05 a	36.13 ± 0.78 d	37.05 ± 0.61 b	25.15 ± 0.08 b	38.00 ± 0.69 d
(T+W) C	14.78 ± 1.40 b	13.70 ± 0.40 b	4.49 ± 0.41 bc	21.41 ± 1.56 a	38.09 ± 0.29 c	35.76 ± 1.34 b	24.57 ± 1.11 b	39.84 ± 0.18 c
(T+W) E	11.98 ± 0.89 c	12.34 ± 0.09 d	5.59 ± 0.54 a	23.18 ± 0.60 a	41.01 ± 0.23 a	31.72 ± 0.47 c	25.63 ± 0.5 b	42.76 ± 0.05 a
W	21.20 ± 2.10 a	12.64 ± 0.77 cd	4.22 ± 0.41 c	23.34 ± 2.10 a	29.81 ± 0.38 e	41.68 ± 1.81 a	27.76 ± 1.37 a	31.14 ± 0.35 e

* Fatty acids were quantified in a relative way, expressing the % concentration of each individual compound with respect to the total content of FAMEs identified in the samples. Different letters indicate significant ($p < 0.05$) differences among fatty acids content in flours.

4. Conclusions

Obtained results demonstrated that supplementation with vegetable wastes can significantly reduce the *T. molitor* larvae rearing time, between 3–4 weeks, which brings benefits in terms of savings in commodities and energy as well as the reduction of production time, which entails competitive improvements for insect breeders. Furthermore, the polyunsaturated fatty acids content of obtained larvae was improved when diet was supplemented with vegetable wastes, which provide an added value to the feed that is developed from them.

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