First Optimization of Tomato Pomace in Diets for Tenebrio molitor (L.)(Coleoptera: Tenebrionidae)

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First Optimization of Tomato Pomace in Diets for *Tenebrio molitor* (L.)(Coleoptera: Tenebrionidae)

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Simple Summary: Rearing substrates based on agri-food by-products are ideal for converting bioactive-rich waste, and increasing the nutraceutical value of insects produced. This approach is limited by the need to provide nutritionally balanced diets for farmed insects. In this study, we evaluated the possible use of tomato pomace (TP), an agro-industrial waste from tomato processing, as a component in rearing substrate for Yellow Mealworm (*Tenebrio molitor*). We compared bran-based diets with increasing percentages of tomato pomace (0%, 27%, 41%, and 100%). As brewer's spent grain and yeast, protein sources are used in mixed diets to ensure equal protein content to diet control. Results showed no difference in larval performance between diets except for increased growth time in TP100%. Generally, the efficiency indices worsen proportionally with the increase in TP. Conversely, the presence of Lycopene and β-Carotene increased in the harvested larvae. The fatty acid composition is improved, with an increase in polyunsaturated fatty acids. Maximum qualitative increases were obtained with TP100%. Overall, the TP41% diet is the best balance between larval performance and qualitative improvement. Therefore, tomato pomace is suitable for the formulation of mealworm diets, even in high dosages when supplemented with protein.

Abstract: Tomato pomace (TP), an agricultural industrial waste product from the tomato processing industry, was valorized by being used as a rearing substrates for *Tenebrio molitor* (L.). This study evaluated bran-based diets with increasing tomato pomace (0%, 27%, 41% and 100%). Protein sources, as brewer's spent grain and yeast, are used in TP27 and TP41 diets, respectively, to ensure equal protein content to control diet (TP0). Results showed larval survival, larval and pupal weight no different between diets; however, growth time significantly increases in TP100 compared at all diets. The feed conversion rate progressively increases from 2.7 to 4.3, respectively from TP0 to TP100. Conversely, Lycopene and β-Carotene increases in the larvae. Fatty acid composition improves by increasing polyunsaturated fatty acids (mainly α-Linoleic acid). Although the best nutritional quality was obtained in T100, the TP41 is the optimal diet for balance between larval performance and qualitative improvement of larvae. Therefore, tomato pomace is suitable for the formulation of mealworm diets, even in high dosages when supplemented with sustainable protein sources.

Keywords: yellow mealworm, edible insects, by-products, rearing substrates, fatty acid, nutraceutical, antioxidant, lycopene, β-Carotene

1. Introduction

Use of by-products as growth substrate (from here below indicated as "diet") allows to reduce production costs in rearing edible insects and increase their role as "bioconverters" in the circular economy [1,2]. This approach is mainly focused on Black Soldier Fly (*Hermetia illucens* L.; Diptera: Stratiomizidae) but extended to Yellow Mealworm (*Tenebrio molitor* L.; Coleoptera: Tenebrionidae) with the choice of suitable by-products and the correct formulation of diets. The diet affects the growth performance of *T. molitor* larvae or mealworms (MLW) [3] and the productivity of their adults [4]. Diet also has an impact on the nutraceutical composition of collected larvae [3,5]. It has frequently been thought that the latter ability would enhance the protein content and amino acid composition [6]. Diet affects the fatty acid composition [3,7,8] and a diet high in linseed has been shown to increase

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polyunsaturated fatty acids (PUFA) [9]. Diets have also been shown to improve calcium content and produce a beneficial Ca:P ratio [5,10,11].

Recently, the increase of antioxidant substances through diets has focused the interest of some researchers [12–15].

This approach is limited not only by the need to provide balanced diets for the nutritional requirements of the farmed insect but also by the anti-nutritional substances present in some by-products. Typical is the presence of polyphenols, as in olive pomace [16].

World tomato production is estimated at 34-42 million tonnes per year by the Word Processing Tomato Council (www.wptc.to). Tomato pomace (TP) is a byproduct of the tomato processing industry, consisting of 5–10% of the fresh weight of tomatoes [17]. It consists of peels, seed and, residual pulp [18]. The nutritional composition is influenced by the proportions of the different components and by the transformation process. Generally, its composition is made up of fiber (53.0%), sugars (25.7%), protein (19.3%), and fat (5.9%), on a dry weight basis [19]. It is also a source of carotenoids, especially lycopene and β-carotene in the peel [20]. Inclusion in animal feed has been tested for poultry [21], quail [22],ruminants [23], dairy cows [24], and lamb [17].

Classified among the phytochemicals, carotenoids as well as polyphenols [25] show marked antioxidant [26] and anti-inflammatory activity [27].

Due to these properties, several studies have associated their use with multiple health benefits, in particular α- and β-carotene and β-cryptoxanthin as a valuable source of vitamin A [28], lutein and β-carotene as positive adjuvants in many eye-related diseases, including cataracts and age-related macular degeneration [29], and finally lycopene and b-carotene as skin protectors from UV rays [30], adjuvans in the prevention of cancer [31] and heart health [32].

Use of this by-product to feed insects has been little investigated, although recently tomato was tested by *H. illucens* [33] and tomato pomace was included as a supplement (10% w/w) in diets for *T. molitor* [13]. A greater supplement would alter the initial nutritional composition of the diet; therefore, high-dose tomato pomace requires the formulation of specific isoproteic diets.

This work aims to evaluate diets assembled with increasing doses of tomato pomace, evaluating its influence on the growth performance and nutraceutical quality of the larvae to optimise the diet.

2. Materials and Methods

2.1. Yellow Mealworms Colony

Mealworms used in this work were reared at the insectarium of CIHEAM-Bari (in the Apulia region). *T. molitor* was fed a bran-based diet and yeast (ratio 95:5); the wet supplement was distributed twice a week with pieces of pumpkin (*Cucurbita moschata*, cv. Butternut). Rearing and subsequent experiments are conducted in a climatic room (28.0±1° C, 70±5% RH and 0L:24 D photoperiod).

2.2. Substrate composition/preparation

Four different by-products were used to formulate the tested diets. Local farms supplied tomato pomace as a by-product of the production of tomato sauce. The pomace consisted mainly of peels and seeds, while the presence of pulp was scarce. Bran was purchased from the mill (Molino "Cimminelli", Montegiordano, CS, Italy) and derived from durum wheat milling. Brewer's spent grain was supplied from small local brewers (Brewery "Jazz Beer", Bernalda, MT, Italy). Zootechnical yeast, as a protein supplement, was purchased from Zabele Srl (Padova, PD, Italy). By-products were preliminarily dried at 60°C for 24 h in Food Dehydrator (COSORI, mod. CP267-FD-RXS, Anaheim, CA 92806, USA). Subsequently, matrices were sieved using a 2 mm manual sieve and the coarse part ground, ensuring homogeneity to avoid the influence of particle size [34]. The nutrient composition was determined on a representative sample using AOAC methods [35] (Table 1).

Table 1. Nutrient composition of by-products preliminarily conditioned (%DM).

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By-product	Dry Matter	Crude protein	Crude Fat	Crude fiber	Ash	Carbohydrate
Bran	91.2	16.7	6.5	36.1	4.2	30.2
Tomato pomace	92.1	9.5	3.2	67.1	3.9	8.9
Brewer's spent grain	93.4	24.7	4.8	42	2.6	24
Yeast	93.0	47.6	2.4	6.8	8.0	13.8

Diets were formulated with bran and increasing doses of tomato pomace. Mixtures also with brewer's spent grain (TP27) or yeast (TP41) produced isoproteic diets, similar to the control diet (TP0). Finally, a diet with only tomato pomace (TP100) was tested. Table 2 reports the macro-nutrient and energy values (the latter calculated by conversion factors in Regulation (EU) 1169/2011, Annex XIV). All diets were assembled in the form of "cookies", partly similar to previous procedures.[6,36], to avoid self-selection in mixed diets [37] and to facilitate the separation of the frass.

Table 2. Diets composition and nutritional values* (% DM).

Diet	Bran (%)	Tomato pomace (%)	Brewer's spent grain (%)	Yeast (%)	Protein value (%)	Carbohydrate (%)	P:C	Crude fiber (%)	Fat (%)	Energy (kcal (100 g)
TP0	100	-	-	-	16.7	30.2	1:1.8	36.1	6.5	318.3
TP27	50	27	23	-	16.6	23.0	1:1.4	45.8	5.21	296.9
TP41	50	41	-	9	16.5	20.0	1:1.2	46.2	4.78	281.4
TP100	-	100	-	-	9.5	8.9	1:0.9	67.1	3.2	236.6

* calculated values

2.3. Experimental set-up

At the beginning of the experiment, six week-old larvae (28±1mg) were distributed in groups of 20 larvae [38] in plastic cups (bottom diameter 6 cm). Larvae were fed *ad libitum* with their respective diets and, wet supplements provided twice weekly. Complete randomization was applied to the experimental design, with 10 replicates/treatment and 20 larvae/replicate.

For each replicate, the final larval weight, the residual substrate, and the frass were weighed with an analytical scale (Mettler-Toledo, mod. B2002-S; precision ±0.1 mg).The collected larvae were starved for 48 h, blanched at 100°C for 5 min, and dried at 60 °C for 24 h in Food dehydrator in accordance with Melgar-Lalanne et al. [39]. Dried larvae, substrate, and frass were stored at -18 °C and powdered before chemical analysis. The latter was performed on three replicas per diet.

2.4. Mealworm growth perfomance

At the formation of the first pupa in the replica, were measured Larval survival (Equation (1)), Growth time (Equation (2)), Larval weight (Equation (3)), Pupal weight (Equation (4)), Feed Conversion Rate or FCR (Equation (5)) where FC represents the feed ingested excluding the wet supplement, Specific Growth Ratio or SGR (Equation (6)) where lFW and lIW represent final and initial fresh larval weight. The Efficiency of Conversion of Ingested feed or ECI (Equation (7)) and Efficiency of Conversion of Digested feed or ECD (Equation (8)) [40] were calculated considering the weight gained (WG), the feed consumed (FC) and Frass as dry weight.

$$\text{Larval survival (\%)} = \text{n. initial larvae/n. final larvae and pupae} \times 100 \quad (1)$$

$$\text{Growth time (d)} = \text{n. days between start of experiment and emergence of first pupa} \quad (2)$$

$$\text{Larval weight (mg)} = \text{weight larvae collected/n. larvae collected} \quad (3)$$

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$$\text{Pupal weight (mg)} = \text{weight pupae collected/n. pupae collected} \quad (4)$$

$$\text{FCR} = \text{FC/WG} \quad (5)$$

$$\text{SGR (\% day}^{-1}\text{)} = 100 \times (\ln FW - \ln IW)/\text{days} \quad (6)$$

$$\text{ECI (\%)} = [WG/(FC] \times 100 \quad (7)$$

$$\text{ECD (\%)} = [WG/(FC\text{-}Frass)] \times 100 \quad (8)$$

2.5. Carotenoids analysis

Carotenoids were extracted from feed, mealworms and feces. According to Leni et al. [33], extraction was conducted with some modifications. Four hundred g of homogenized sample was mixed with 10 ml of hexane/ethanol/acetone (50:25:25) extraction mixture containing 0.1% ascorbic acid. After stirring at 200 rpm for 1 h on ice and under subdued light (Universal Table Shaker 709), the samples were centrifuged at 2,800 rpm at 4 °C for 20 min (Laborfuge 400R –Heraeus Instruments), and the supernatant was separated from the pellet. Separation and quantification of licopene and *β*-carotene were conducted according to Anthon and Barret [41], with some modifications. Distilled water (1.5 ml per 10 ml of extract) was added to the extract to cause phase separation. After stirring for 1 min under subdued light, the samples were centrifuged at 2,800 rpm at 4 °C for 10 min, and the upper hexane phase was recovered and used for spectrophotometric carotenoids quantification (Multiscan Go Spectrophotometer). Samples were read at 503 and 444 nm. The Concentration of Lycopene and β-Carotene was calculated using the following Equations (9) and (10):

$$C_{\text{lycopene}}\ (\text{mg/kg}) = (6.95 \times Abs.503 - 1.59 \times Abs.444) \times 0.55 \times 537 \times V/W \quad (9)$$

$$C_{\beta\text{-carotene}}\ (\text{mg/kg}) = (9.38 \times Abs.444 - 6.70 \times Abs.503) \times 0.55 \times 537 V/W \quad (10)$$

where:

- 0.55 = The final hexane layer volume ratio to the volume of mixed solvents added for hexane:acetone:ethanol (2:1:1)
- W (mg) = The weight of sample analyzed
- V (ml) = The volume of mixed solvents added
- 537 = The molecular weights of lycopene and β-carotene(g/mole).

2.6. Lipid analysisin mealworms

The defatting process of mealworm powder (MLWP) was performed according to Gkingali et al. [42] with some modifications. In brief, a three-step extraction procedure using n-hexane removed the fat from the MLWP. The sample was first mixed with n-hexane at a ratio of 1:5 w/v. The mixture was then shaken for 1 hour at 150 rpm (25 °C) using a rotary shaker (Universal Table Shaker 709). Following a centrifugation of the resultant slurry at 8,500g for 10 min at 10 °C, the organic phase in the supernatant was decanted. The supernatant is separated from the sediment and stored separately. The process was twice repeated, adding more hexane to the sediment each time. Supernatants were collected in a pre-weighed round-bottom flask, and the n-hexane was evaporated using a rotary evaporator (Steroglass Rotary Evaporator InstrumentsKentron-Strike 202). The final sediment was left to stand at room temperature to eliminate any remaining solvent. The resultant defatted larvae powder (DLP) and lipid extract were kept in a freezer at -18 C° until used. The oil extraction yield (or Crude Fat %) was calculated according to Equation (11) [43]:

$$\text{Oil extraction yield (\%)} = [\text{mass of extracted fat(g)/solids of the initial sample (g)}] \times 100 \quad (11)$$

Lipids extracted from MLWP were directly trans esterified by producing fatty acid methyl esters (FAME) by applying the technique described by Tasselli et al [44]. The separation of FAMEs was carried out using an Agilent GC7890A gas chromatograph out fitted with a split-splitless injector and a flame ionisation detector (FID) at the settings specified by Di Fidio et al. [45]. The retention times of the fatty acids were compared to FAME standards (Sigma-Aldrich), and their percentage was estimated using the combined area of the present peaks.

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The fatty acid profile data were processed by deriving indices. Indices of atherogenicity (AI) and thrombogenicity (IT) were calculated using Equations (12) and (13) previously described by Ulbricht and Southgate [46]:

$$IA = \frac{C12{:}0 + (4xC14{:}0) + C16{:}0}{PUFA(\sum n-6 + \sum n-3) + C18{:}1 + \sum MUFA} \quad (12)$$

$$IT = \frac{C14{:}0 + C16{:}0 + C18{:}0}{(0{,}5xC18{:}1) + (0{,}5x\sum MUFA) + (0{,}5xn-6) + (3xn-3) + \frac{n-3}{n-6}} \quad (13)$$

Theoxidizability (COX) value was calculated using the following Equation (14) previously described to Fatemi et al. [47]

$$COX = \frac{(1xC18{:}1) + (10{,}3xC18{:}2) + (21{,}6xC18{:}3)}{100} \quad (14)$$

The Hypocholesterolemic/Hypercholesterolemic (HH) ratio was calculated as reported by Santos-Silva et al. [48] using the following Equation (15):

$$HH = \frac{(C18{:}1, cis-9) + \sum PUFA}{C14{:}0 + C16{:}0} \quad (15)$$

The Unsaturated index (UI) was calculated was calculated using the following Equation (16) previously described to Chen et al.[49]:

$$UI = [(1 \times \%monoenoics) + (2 \times \%dienoics) + (3 \times \%\ trienoics) + (4 \times \%\ tetraenoics) + (5 \times \%\ pentaenoics) + (6 \times\% \ hexaenoics)] \quad (16)$$

2.7. Protein analysis

An alkaline protein extraction was performed on the DLP samples using the protocol made by Zhao et al. with minor modifications [50]. One g of sample was treated with 15 ml of 0.250 M NaOH at 40°C under agitation for 1 h in a thermostatic orbital shaker (ThermoScientific Forma, model 420) and centrifuged at 8,500 rpm for 30 min at 4°C for 3 times. The extraction procedure was repeated three more times in total. The supernatant and gel layer from all extractions were pooled and used for the quantification. The determination of crude protein content was performed according to Kotsou et al., with modifications [14]. Teen μL of the appropriately diluted supernatants pooled was transferred to the wells of a 96-well plate, and 200 μL of diluted Bradford reagent was added and then shaken for 30 seconds in a plate reader. The plate was incubated for 10 minutes at room temperature in the dark. The absorbance was measured at 595 nm with a Multiscan Go Spectrophotometer. A standard calibration curve was prepared using bovine serum albumin.

2.8. Statistical analysis

Larval performance data were initially submitted for normality and homogeneity of variance tests. A one-way ANOVA was applied at FCR, SRG, and ECI values, followed by Tukey-Kramer HDS test post-hoc to identify the differences between the diets. Alternatively, the non-parametric Kruskal–Wallis test and pairwise multiple comparisons with Bonferroni correction were applied to the other measured parameters.Significance was assumed at $p< 0.05$.All data were statistically processed by SPSS software version 26.0 (IBM Corporation, Armonk, NY, USA). The data of qualitative and quantitative analysis are shown as the mean values ± standard deviation (SD), from three replicas.

3. Results

3.1. Larval performances

Larval survival was close to 100% in all diets tested. No statistically significant differences were found between diets ($H = 1.05$; df = 3; p=0.788), including the tomato pomace diet (TP100) (Table 3).

Table 3. Results of larval performances between diets.

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Diet	Survival (%)	Growth time (d)	Larval Weight (mg)	Pupal Weight (mg)
TP0	99.5±1.6	32.0±5.5 a	100.0±14.1	117.5±14.0 ab
TP27	100.0±0.0	38.5±3.9 a	104.0±9.7	114.0±13.5 ab
TP41	99.5±1.6	37.7±5.0 a	109.0±11.0	127.0±20.2 b
TP100	99.5±1.6	63.4±18.5 b	91.0±17.9	101.0±9.9 a

Mean ± standard deviation values with the same letter, within columns, are not significantly different (Kruskal–Wallis test and Pairwise multiple comparisons with Bonferroni correction), at $\alpha = 0.05$.

Values of larval growth time showed significant differences between diets ($H = 21.23$; df = 3; $p<0.000$). The presence of tomato pomace increased the growth time by 5-6 days in the TP27 and TP41 diets, but these longer periods were not significantly different compared to the 32 days of the control diet (TP0). In contrast, the TP100 diet recorded growth times double (+33.4 days)that of the control and significantly longer than the other diets.

At harvest, the mean larval weight was not significantly different between diets ($H = 6.41$; df = 3; $p=0.093$).The lowest weight (91.0 mg) was achieved by the TP100 diet, and the highest weight (109.0 mg) by the larvae of the TP41 diet. The analysis of the weights of the first pupa showed significant differences between diets ($H = 12.61$; df = 3; $p<0.006$), although significant differences were found only between the TP41 (127.0 mg) and TP100 (101.0 mg) diets (Table 3).

3.2. Efficiency indicators

Generally, the utilization efficiency of the tested diets decreased with increasing doses of tomato pomace in the diets. The Feed Conversion Rate was significantly different between diets ($F = 73.2$; df = 3, 36; $p< 0.001$) with minimum values (FCR= 2.7) in the control, without tomato pomace, and progressively increasing until reaching the maximum in T100 (FRC=4.3) (Table 4).

The Specific Growth Ratio significantly differed between diets ($F = 34.1$; df = 3, 36; $p <0.001$). The highest values were obtained with the control diet (4.9%), while slightly lower values were recorded with the two diets mixed with tomato pomace (TP27 and TP41). The TP100 diet achieved significantly lower values (2.5%).

The Efficiency of Conversion of Ingested feed significantly differed between diets ($F = 85.8$; df = 3.36; $p < 0.001$). The ECI values decreased significantly as the amount of tomato pomace increased in the tested diets (Table 4).

The Efficiency of Conversion of Digested feed was significantly different between diets ($H = 27.3$; df = 3; $p <0.001$). There was no significant difference between the TP0 and TP41 diets, while the TP27 diet showed significantly higher ECD values (42.8%). Finally, significantly higher values than all other diets were obtained in the TP100 diet, with an ECD of 65.9% (Table 4).

Table 4. Efficiency indicators of diets tested.

Diet	FCR	SRG (% day^{-1})	ECI (%)	ECD (%)
TP0	2.7±0.2 a	4.9±0.7 a	15.4±1.1 a	34. 6±3.3 a
TP27	3.2±0.1 b	4.1±0.4 b	13.1±0.5 b	42.8±4.5 b
TP41	3.8±0.3 c	4.3±0.4 b	10.8±0.7 c	30.0±6.3 a
TP100	4.3±0.4 d	2.5±0.7 c	9.8±1.0 d	65.9±12.7 c

Mean±standard deviation values with the same letter, within coloums, are not significantly different (Tukey-Kramer HDS test for FCR, SRG and ECI; Pairwise multiple comparisons with Bonferroni correctionfor ECD), at $\alpha = 0.05$.

3.3. Licopene and β-carotene Quantification

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The values of carotenoids, lycopene and β-carotene, present in feed (diets), stored in mealworms, and excreted with frass, have been collected in Table 5. In feed, the addition of tomato pomace shows a proportional increase in the amount of lycopene, and β-carotene compared to the control: from 2.66 ug/g TP0 to 179.75 ug/g TP100 for lycopene; from 0.30 ug/g TP0 to 241.5 ug/g TP100 for β-carotene. Furthermore, an inversion of the lycopene/β-carotene (L/C) ratio between feeds is also evident. While the TP0 diet shows an L/C value >1 (8.8), all the supplemented feeds (TP21, TP47, and TP100) show a value < 1 (~ 0.5).Values referring to the larvae highlighted a general tendency towards the accumulation of both analyzed carotenoids. In the TP47 and TP100 diets, compared to TP0, there are incremental signals of both lycopene (0.61 ug/g and 1.19 ug/g against 0.08 ug/g) and β-carotene (2.56 ug/g and 7.28 ug/g versus 1.43 ug/g). However, the larvae are richer in β-carotene than in lycopene. In larvae, the increase of both carotenoids is evident only at TP27 (maximum at TP100), unlike the progressive increase observed in diets. The lycopene content in frass varies from 0.70 ug/g in the TP0 to 39.67ug/g in the TP100, while the B-carotene content varies from 12.09 ug/g in the TP0 to 147.46 ug/g in the TP100; so as seen for the larvae, the frass is richer in β-carotene than in lycopene.

Table 5. Licopene and β-Carotene Quantification[1].

Diet	Feed		Mealworm		Frass	
	Lycopene (ug/g)	β-Carotene (ug/g)	Lycopene (ug/g)	β-Carotene (ug/g)	Lycopene (ug/g)	β-Carotene (ug/g)
TP0	2.66±0.24	0.30±0.07	0.08±0.06	1.43±0.99	0.70±0.04	12.09±0.26
TP27	22.68±0.79	45.30±1.95	0.08±0.01	1.11±0.16	12.44±0.35	50.99±0.57
TP41	52.43±1.71	95.09±0.70	0.61±0.33	2.56±0.76	24.10±0.32	76.32±1.24
TP100	179.75±2.74	241.47±2.53	1.19±0.27	7.28±0.06	39.67±1.56	147.46±4.61

[1] Values are reported on dry mass and are expressed as means ± standard deviation of three independent analyses. (n = 3).

3.4. Larval nutritional value

Figure 1 shows the fat extracted (% w/w of MLW powder) from larvae fed different diets enriched with tomato pomace (from 27 to 100%). In our results, the control larvae (TP0) contained 30.76% crude fat. Adding tomato pomace to the feed in percentages of 27% (TP27) does not generate any difference compared to the control diet. However, when tomato pomace was present at 41% (TP41) and 100% (TP100), there was a decrease in the fat content of the larvae. Specifically, TP41 larvae contained 19.6% less fat than control larvae, while TP100 larvae contained as much as 67.9% less fat. Total fatty acid content (TFA) analysis also shows a decreasing trend in values compared to TP0 (81%). In particular, TP41larvae contained about 77% TFA, while TP100 contained 53%, following the trend of crude fat.

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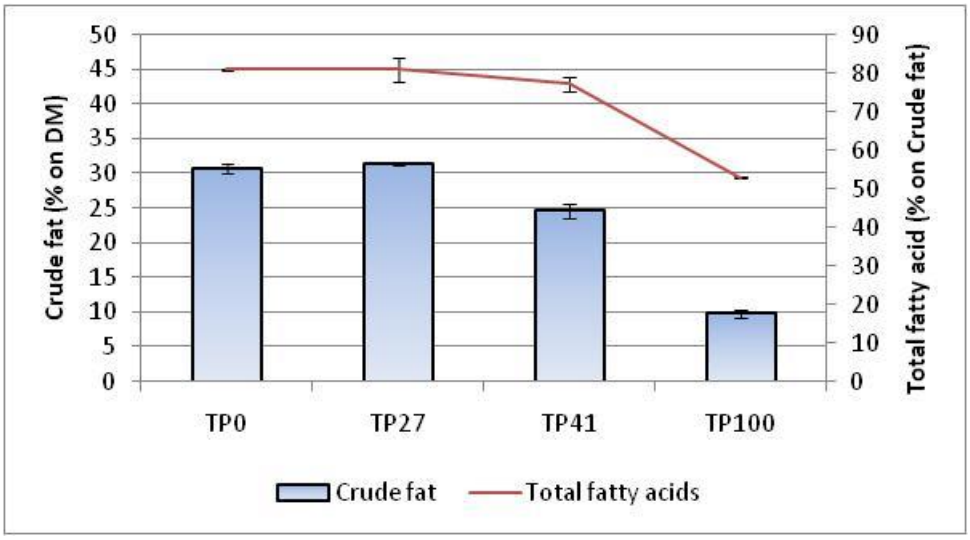

Figure 1. Fat and fatty acid content in mealworms fed on different diets.Values are are expressed as means ± standard deviation of three independent analyses. (n = 3).

The fatty acid composition of mealworms shows a total of 7 fatty acids detected and measured in all treatments (Table 6). In TP0 larvae, the main unsatured fatty acids(UFAs) found were oleic acid (OA) (50.2%), followed by linoleic acid (LA) (25%), while of the satured fatty acids (SFAs), the most abundant was palmitic acid (PA) (15%).While introducing a diet supplementation of tomato pomace produced no significant change in the amount of SFAs compared to the control, it produced a significant qualitative and quantitative change in UFAs. In fact, in diets TP27, TP41, and TP100, there is a decreasing trend in the percentage of oleic acid (OA), which falls from 50% (TP0) to 26%, and a simultaneous significant increase in PUFAs. The content of linoleic acid increases from 25% (TP0) to 40%, while that of linolenic acid varies greatly from 0.42% to 2.68%.The PUFA:SFA ratio, calculated to assess our samples' cardiovascular health benefits, is higher in all 3 case studies (TP27, TP41, and TP100) than the control TP0 larvae (1.18). Their PUFA:SFA ratio shows an increasing trend, with values between 1.35 (TP27) and 2.30 (TP100). The influence of diet on the n-6/n-3 ratio highlights that all larvae fed with tomato pomace supplementation significantly reduced their n-6/n-3 ratio. The greatest 70% reduction was obtained with the TP100 diet, followed by 46% of the TP41, compared to the TP0, which had an n-6/n-3 ratio of 61.5.

Table 6. Fatty acid profile of the lipid extract of MLW powder fed different diets (% TFA)[1.]

Fatty Acid (%)		Diets			
Common Name	**Lipid number**	**TP0**	**TP27**	**TP41**	**TP100**
Caprilic acid	C8:0	0	0	0	0
Capric acid	C10:0	0	0	0	0
Lauric acid	C12:0	0	0	0	0
Myristic acid	C14:0	3.68±0.04	3.81±0.03	3.42±0.12	2.66±0.06
Palmitic acid	C16:0	15.60±0.04	14.8±0.09	15.28±1.13	13.80±0.09
Palmitoleic acid	C16:1	1.56±0.27	4.19±0.03	1.44±0.29	1.00±0.01
Stearic acid	C18:0	2.89±0.20	3.16±0.08	3.38±0.17	5.59±0.05
Oleic acid	C18:1	50.20±0.20	44.7±0.28	42.87±0.229	26.20±1.46
α-Linoleic acid	C18:2n-6	25.70±0.31	28.9±0.32	32.61±1.24	48.10±1.27
α-Linolenic acid	C18:3n3	0.42±0.04	0.51±0.09	0.99±0.11	2.68±0.08

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Arachidic acid	C20:0	0	0	0	0
Behenic acid	C22:0	0	0	0	0
Erucic acid	C22:1	0	0	0	0
Lignoceric acid	C24:0	0	0	0	0
Σ SFA		22.14±0.07	21.76±0.19	18.71±1.25	22.02±0.19
Σ MUFA		51.75±0.19	48.84±0.32	44.32±0.52	27.22±1.46
Σ PUFA		26.10±0.19	29.40±0.40	33.60±1.35	50.8±1.4
Σ UFA		77,9±0,08	78,2±0,27	77,9±0,39	78±0,20
PUFA : SFA ratio		1.18	1.35	1.79	2.30
MUFA:PUFA ratio		1.98	1.66	1.32	0.53
ω6 : ω3 ratio		61.47	56.13	32.99	17.95

[1] Values are reported on % Total Fatty Acid (TFA) and are expressed as means ± standard deviation of three independent analyses. (n = 3).

3.4.1. Lipid quality indices

Diet had a direct influence on both COX and UI. As shown in Table 7, in all cases of tomato-fed larvae (TP27, TP41, and TP100), there was a substantial increase in the COX value compared to the TP0 value, equal to 3.2. The TP41 and TP100 diets, in particular, increased by +25% and +80% respectively. The UI data also follow an increasing trend; in fact, compared to the control values TP41 and TP100, they show an increase of +7.7% and +25% respectively. Among the lipid quality indices related to the incidence of coronary heart disease, IA, IT, and HH do not appear to be significantly influenced by diet, except for the TP100 diet. Larvae fed with 100% tomato pomace showed IA and IT decreased, respectively by -20% and -12% compared to the control, and HH increased by +18% compared to the control.

Table 7. Lipid quality indices of TFA obtained from MLW fed different diets[1].

Index	Diets			
	TP0	**TP27**	**TP41**	**TP100**
COX	3.2±0.43	3.53±0.18	4.01±0.3	5.79±0.14
IT	0.55±0.05	0.54±0.03	0.53±0.05	0.48±0.01
IA	0.39±0.06	0.38±0.01	0.37±0.02	0.31±0.02
HH	3.96±0.21	3.98±0.55	4.09±0.31	4.68±0.24
UI	104.4±0.78	108.1±0.92	112.5±0.15	131.4±0.91

[1] Values are expressed as means ± standard deviation of three independent analyses. (n = 3).

Indices abbreviations: COX (Calculated oxidizability value); IT (Indices of thrombogenicity); IA (Indice of atherogenicity); HH (Hypocholesterolemic/Hypercholesterolemic ratio); UI (Unsaturation index)

3.4.2. Crude proteine

Larvae fed on control, TP27, and TP41 diets showed similar crude protein content (Figure 2). Their values vary from 47.3% to 49.2%, expressed on the DM of larvae defatted. In contrast, crude protein in larvae on the TP100 diet decreased at 42%.

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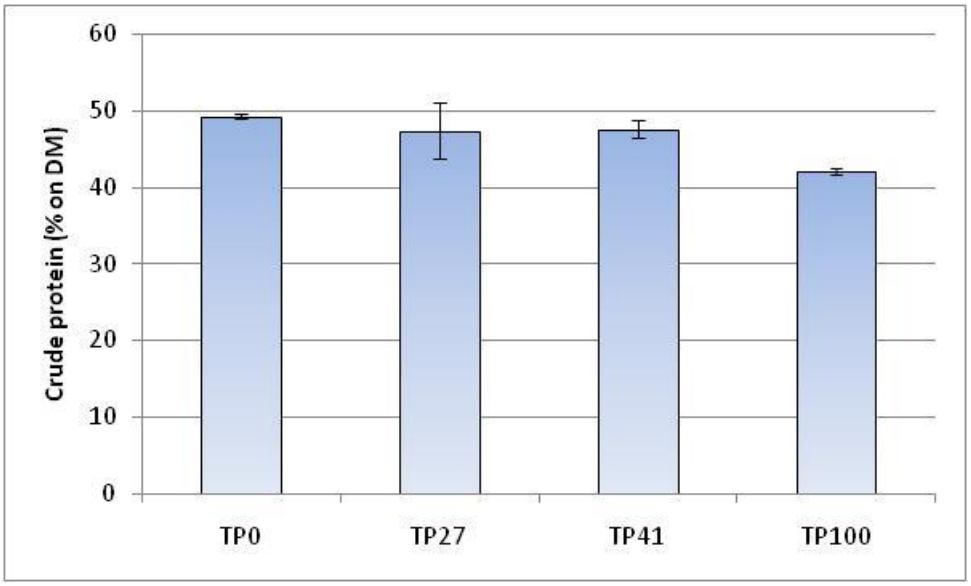

Figure 2. Crude protein quantification in defatted larvae fed on different diets. Values are reported as means ± standard deviation of three independent analyses. (n = 3).

4. Discussion

The use of by-products of vegetable origin in the feeding of edible insects must consider the presence of toxic secondary metabolites or with a negative influence on their development. The major secondary metabolite of tomato fruit is the glycoalkaloid tomatine, known for its repellent and antifeeding effects [51,52]. This abounds in unripe fruit and degrades with ripening [53], so it is not a limiting factor in the use of tomato pomace. The ripe fruit contains polyphenolic compounds such as gallic acid, chlorogenic acid, caffeic acid, rutin, kaempfero-3-O-glucoside, naringin, quercetin, and naringenin [54]. Some of these compounds have shown a negative and dose-dependent influence on the feeding of *Lepinotarsa decemlineata* (Say) [55]. Others, such as chlorogenic acid, have a repellent and antifeeding effect [56] or, like quercitin, they can be phagostimulant or phagodeterrent according to the doses [57]. As far as we know, it is not known whether the industrial tomato transformation process degrades the secondary metabolites, but the absence of mortality in the TP100 diet suggests their absence or presence at non-lethal doses. This is contrary to the hypothesis of Ruschioni et al. [16] in the presence of high mortality in larvae fed on olive pomace.

The nutritional composition is another important element in the use of by-products. The tomato pomace used in this study is particularly poor in macro-nutrients, probably because it derives from a very efficient industrial process [19]. Its content in protein (P 9.5%), carbohydrate (C 8.9%), and lipids (L 3.2%) is unfavorable in carbohydrates when compared to optimal compositions such as P 20-25%, C 65-75%,and L 3- 12% [36] or P 19.9-22.8%, C 67.3-71.5% and L 8.6-10% [6].

TP's protein to carbohydrate (P:C) ratio is 1:0.9 and was similar to the 1:1 ratio, considered the best among those evaluated by Rho and Lee [50]. However, these authors tested adults on synthetic diets and reported the "tendency to prioritise the regulation of carbohydrate intake over that of protein intake" [58]. More specifically, the ratio would be 1:1.6 for males and 1:1.3 for females [59]. This would indicate the need to supplement TP with carbohydrates. However, the TP100 diet results indicate that protein and carbohydrate deficiency had a more prevalent effect on their P:C ratio. TP100 larvae had lower protein content than larvae from TP0, TP27, and TP41 isoproteic diets, in contrast to the expected maximum protein accumulation with a ratio between 1:1 and 2:1 [58].

The TP100 diet has the lowest energy value (236.6 kcal/100 g) and the TP0 diet has the highest (318.3 kcal/100 g), but they all have lower energy values than the poorest diet (353 kcal/100 g) reported by other authors [16]. The low energy value of TP is determined by the high fiber content (67.1%), considered unfavorable if more than 5-10% in diets for *T. molitor* [60].

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A comparison of the diets tested showed limited differences in larval performance. Major and minor larval and pupal weights were obtained in the TP41 and TP100 diets, respectively; however, only the pupal weight in the TP41 diet was significantly higher. The significant increase in larval growth time in the TP100 diet (twice as much as compared to the control) is a potential limitation in using pure TP due to the consequent increase in breeding costs. The increase in larval growth times can be mainly attributed to the low protein content, as there are no differences between isoproteic diets. This hypothesis is in agreement with the reduction of larval growth times observed in diets richer in proteins [61].

Results on the use of diets have highlighted the significant increase in the FCR value as the dose of TP increases. This result is expected in TP100 as a remedy to compensate for the low concentration of nutrients [58]. Furthermore, the increase in FCR values in isoproteic diets suggests a positive correlation to the fiber content and a negative correlation to the energy value of the diet. Our FCR values (from 2.7 to 4.3 for TP0 and TP100, respectively) are slightly higher than some control diets, such as wheat bran and yeast (FCR=2.3) [38], chicken feed (FCR=1.57), and wheat bran (FCR=2.08) [3]. It is important to point out that the FCR value (3.8) recorded in the TP41 diet is similar to 3.44 of the commercial diet for *T. molitor* used by van Broekhoven et al [62], however, with similar protein values (16.5 and 17.1%, respectively).

The tested diets have SRG values in line with the seed clearing process by-products values (2.7 to 7.2% day^{-1}) [38], but lower than the mixed diets (8.2 to 11.9% day^{-1}) [6]. The particularly low value (2.5% day^{-1}) of the TP100 diet is probably influenced by the long larval growth time.

The ECI values significantly decreased from 15.4% to 9.8% with the increase of the TP in the diets; they are lower than commercial diet (18.96%) [62] and chicken feed (almost 22%) [3]. However, we find our results better comparable to Kroncke and Benning [6] and Morales- Ramos et al. [63] for the similar mode of diet administration. Administration through "cockies" greatly reduces self-selection in mixed diets, thus reducing the possibility of self-reducing the negative impact of unbalanced diets or fibres.In this case, our ECI values fall within the range 5.5-18.4% described by Kroncke and Benning [6] and higher than the range 7-10% [63], where the two best diets have ECI values similar to the TP100 diet (with 9.8%).

The ECD values (30.0 to 65.9%) were higher compared to the values (17 to 20%) found in larval density tests [64]. The limited knowledge on ECD and the high value of the TP100 diet suggest more studies, hypothesizing better conversion of the digested diet if it is poor in nutrients.

The positive influence of tomato pomace on larval quality is evidenced by carotenoids and fatty acid composition. The degree of accumulation of carotenoids observed in the larvae is very low if correlated with that contained in the substrate and in the faeces. This reduced efficiency of larval accumulation against an evident enrichment in frass carotenoids is in agreement with other data present in the literature on mealworms fed with former foodstuffs [65] and *H. illucens* fed with agri-food by-products (ground and coarse tomato) [33]. The use of commercial β-carotene supplements administered to insects shows larval accumulation values comparable to our results [66], as well as MLW fed with leaves of *Moringa oleifera* (Lam.) [15] or with carrot pomace [67].

The lycopene content in the larvae appears to be much lower than the β-carotene content accumulated in the substrate and faeces. This data is in agreement with the reduction of the mass balance also found for other non-provitamin A carotenoids such as zaexanthin and lutein in *T.molitor* [65] and in *H. illucens* [33] probably due to bioconversion phenomena by the 'insect (β-cyclase and carotene-9',10'-monooxygenase) [68,69] or by the gut microbial community [70].

Of great interest is the observation of the effect of diet on the quantity and quality of lipids and FAs. In the MLW, lipids are second only to proteins in quantity [71]. TP0 larvae show a CF value (~30.8%) that is very comparable with other previously published data in which there is great variability in its concentration (from 22% to 42%) [14,43,72]. Insects and, in particular, MLW are equipped with a sophisticated enzymatic kit (Elongase and Desaturase) that allows them to synthesise *de novo* fatty acids and, in particular, PUFAs [9,73,74].

Furthermore, they can modulate the degree of lipid accumulation and change their profile in FA depending on the developmental stage, sex, growth environment, and especially the type of feed used [75–80].

Larvae fed with 100% TP showed a reduction in the percentage of CF compared to the control. This decrease agrees with many studies showing how caloric restriction, total carbohydrate intake in the diet, and, in particular, the addition of fatty acid and carotenoid supplements affect fat synthesis and accumulation in insect larvae and other animals [15,59,62,81,82]. Low levels of intramuscular fat are found in lamb-fed diets enriched in lycopene [83] and in pork fed diets, rich in linoleic acid [84], and supplemented with 15% TP [85].

The FAs composition of TP0 larvae also confirms it as one of the most abundant sources of OA, PA, and especially linoleic acid (LA) compared to other animal sources that are rich in fatty acids and especially omega-6 such as chicken fat and egg yolk [86]. LA and linolenic acid (ALA) are PUFAs defined as 'essential' for the human body, which is unable to synthesise them [87] and are therefore essential for human health and, in particular, for the prevention of cardiovascular disease, one of the leading causes of death worldwide [9].

The increase in the amount of ω-3 in feeds due to TP led to a general increase in PUFAs, especially LA and ALA, and to a decrease in both OA and ω-6/ω-3 ratios in especially TP41 and TP100 diets (-45%, -70%). Diets high in the ω-3/ω-6 ratio cause an increase in PUFA and ω-3/ω-6 ratios in larvae [88], and can modulate the activity of both [89] Δ-12 desaturase, which converts ω-9 oleic acids into ω-6 linoleic acids [90], and Elongase (TmElo1 and TmElo2) involved in the synthesis of PUFAs [74].

Our results are also in agreement with other data where MLW are fed feed supplemented with linseed, grape seeds, and winery waste sludge and show a reduction in MUFA content [9,12]. In contrast, diets with distillery by-products (grape pomace, exhausted grape marcs, grape skin pulp [12], or sunflower [91]) produced a significant increase in MUFAs and, in particular, OA, while the inclusion of olive pomace in the feed composition did not affect the FA composition of body lipids [16]. Our results are also in agreement with the improvement in the quality and quantity of PUFAs previously obtained with the addition of fish oil [92]. All this emphasizes how physiological mechanisms of MLW adaptation play a key role in the quality of the lipid profile of larvae on par with diets [62,65,88,93].

Incorporating TP into feeds also increased the wholesomeness of mealworms for human and animal consumption, as indicated by the lipid indices obtained. The increase in COX emphasizes the positive influence of diets rich in PUFAs on the stability and shelf life of by-products obtainable from MLW [14,94,95], while the increase in UI, comparable to some macroalgae *(Hypnea esperi, Gracilaria fergusonii, Codium vermilara*) [49], shows the strong impact of diet on increasing the percentage of high-quality PUFAs useful for reducing the risk of heart disease [96], preventing and managing type 2 diabetes, insulin resistance [97], osteoarthritis [98] and neurological disorders [99].

The absence of adverse effects on the IA, IT, and HH indices, however, makes these larvae comparable to other diets applied to MLW [15,89] and other valid novel foods such as brown seaweed, whose consumption produces the best results for human health as it has a positive effect against cardiovascular diseases [100].

Therefore, the use of an optimal TP-based diet can support the production of mealworms with higher nutraceutical value. Whole larvae and mealworm oil can be commercial products with specific health characteristics since the presence of carotenoids and the best chemical composition are crucial for the future use of this insect as feed and food [95,101].

5. Conclusions

Tomato pomace is a by-product rich in fiber, nutritionally poor and unbalanced. Its use in high doses in mealworm diets must include other sources of carbohydrates and proteins. In this study, the optimal diet was assembled with wheat bran (50%), tomato pomace (41%), and yeast (9%).This diet had no negative impact on larval performance and increased the content of carotenoids and polyunsaturated fatty acids. Further studies should point to the replacement of yeast with a cheaper protein source. The use of pure tomato pomace further increases the content of lycopene and β-carotene in the larvae and doubles the PUFA values. However, such a poor diet doubles the larval

growth time and reduces the protein and fat content of the larvae. The latter use could only find application in the economic valorization of the greater nutraceutical value of the larvae.

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