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Agricultural by-products from Greece as feed for yellow mealworm larvae: circular economy at a local level

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RESEARCH ARTICLE

Abstract

Organic side-streams and agricultural wastes represent a big pool of untapped and underrated resources which could be efficiently exploited for insect rearing. We evaluated the suitability of eleven agricultural by-products, stemming from the production of cotton, sugar beet, sunflower, barley, oats, peas and vetch, for the development of larvae of the yellow mealworm, *Tenebrio molitor*. In a first bioassay, by-products were screened singly to evaluate their potential to support the development of middle sized (4th-6th instar) larvae. In a second bioassay, by-products were supplemented with carrot, and their potential to support complete larval development (first instar until first pupation) was evaluated. Lastly, a third bioassay was conducted in which the more promising by-products were used as components of isonitrogenous diets for middle sized larvae, at two nitrogen levels (2.7 and 3.2%). Our results show the suitability of several agricultural by-products from Greece for *T. molitor* larvae. The best results though were obtained with the oat and the barley by-product (class II), tested either singly or mixed in diets. These by-products can be utilised singly or in composed diets and can greatly decrease the feed costs of mealworm production. Moreover, the results obtained from the isonitrogenous diets bioassay, show that besides nitrogen content, other factors need to be considered when formulating optimal diets. Our study aims to implement circular economy practices in insect farming at a local level.

Keywords: edible insects, feed conversion efficiency, larval development, nutritional value

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1. Introduction

Insect production is a rapidly growing agricultural activity that has attracted a lot of scientific and commercial interest during the last decade (Van Huis, 2020) and is expected to further expand in the near future (Meticulous Market Research, 2019). However, one of the main barriers identified for the further development of the insect sector is the high production cost and subsequently the high price of the insect meal in the market (Arru *et al.*, 2019; Cadinu *et al.*, 2020). Feed costs contribute substantially to the total costs of insect production (Roffeis *et al.*, 2018).

The inclusion of organic side-streams and wastes with low or zero economic value can mitigate the cost of insect feed (Gasco *et al.*, 2020; Varelas, 2019). Agricultural farming and agro-industrial systems produce a huge amount of waste and by-products, which constitute a largely untapped pool of valuable resources suitable as insect substrates (FAO, 2013). Valorisation of such resources was studied for several species including the black soldier fly (*Hermetia illucens*; Bava *et al.*, 2019; Bosch *et al.*, 2019), the long-horned grasshopper (*Ruspolia differens*; Sorjonen *et al.*, 2020), the lesser mealworm (*Alphitobius diaperinus*; Gianotten *et al.*, 2020; Van Broekhoven *et al.*, 2015), the superworm (*Zophobas morio*, Van Broekhoven *et al.*, 2015), and the yellow mealworm (*Tenebrio molitor*, Oonincx *et al.*, 2015; Van Broekhoven *et al.*, 2015).

The latter species *T. molitor* L. (Coleoptera: Tenebrionidae) is one of the most commonly produced insect species for food and feed (Rumbos *et al.*, 2020). A diet composed of bran supplemented with a protein source (e.g. yeast, soy protein, casein, albumin, etc) is commonly used in both lab cultures and commercial production (Ribeiro *et al.*, 2018). However, several studies have evaluated the potential of organic waste and by-products as feed for *T. molitor* (Harsányi *et al.*, 2020; Kim *et al.*, 2017; Li *et al.*, 2020; Mancini *et al.*, 2019; Oonincx *et al.*, 2015; Ramos-Elorduy *et al.*, 2002; Ruschioni *et al.*, 2020; Shu *et al.*, 2018; Stull *et al.*, 2019; Van Broekhoven *et al.*, 2015). Several organic wastes have been evaluated as *T. molitor* larvae feeding substrates, such as vegetable and garden waste, and cattle and horse manure (Harsányi *et al.*, 2020). Also, by-products from food manufacturing (beet molasses, potato steam peelings, spent grains and beer yeast, bread and cookies remains and maize distillers' dried grains) (Oonincx *et al.*, 2015; Van Broekhoven *et al.*, 2015) and by-products from the olive oil industry have been evaluated with variable results (Ruschioni *et al.*, 2020).

The aforementioned studies suggest potential to valorise locally available by-products. Therefore, the present study evaluates the suitability of Greek by-products as feed for *T. molitor* larvae.

2. Materials and methods

Insects

Larvae of *T. molitor* were obtained from the colonies maintained at the Laboratory of Agricultural Zoology in the University of Thessaly, Greece. These were reared in plastic boxes (48 cm length × 28 cm width × 10 cm height) with a rectangular screened opening (19×27 cm) on the top to allow box aeration. Stock colonies were kept at constant conditions, i.e. 26 °C, 55±5% relative humidity (RH) and continuous darkness. *T. molitor* larvae were reared on a diet comprised of wheat bran (90%) and dry instant yeast (10%), supplemented with fresh potato slices twice a week. Adults were left to oviposit for 1 week in white wheat flour. Then, they were removed, and newly emerged larvae were left to feed on the flour until experimentation.

By-products

Eleven by-products, stemming from the production of cotton, sugar beet, sunflower, barley, oats, peas and vetch, were selected based on their high local availability (Table 1). Specifically the following by-products were tested: sugar beet pulp meal (in pellets), cotton cake, cotton seed meal, sunflower meal (in pellets), as well as by-products of the seed cleaning process of barley [remains in the sieves (class I); beard hairs, husks and other non-seed materials (class II)], oat (beard hairs, husks and small seeds), pea [bean pods and other non-seed materials, few small and broken seeds (class I); small and broken seeds (class II)] and vetch [bean pods and other non-seed materials, small and broken seeds (class I); small and broken seeds (class II)]. These were ground (Thermomix TM31-1C, Vorwerk Elektrowerke GmbH & Co. K, Wuppertal, Germany), hand-sieved through a 2-mm sieve and stored at 20 °C until further use. Prior to experimentation, they were placed under experimental conditions (26±1 °C, 55±5% RH) for 7 d, to equilibrate with the relative humidity level.

Order of experiments and bioassay details

The by-products were evaluated as feed for *T. molitor* larvae via three bioassays. In the first bioassay, by-products were screened singly to evaluate their potential to support the development of middle sized (4^{th}-6^{th} instar) larvae. In the second bioassay, by-products were supplemented with carrot, and their potential to support complete larval development (first instar until first pupation) was evaluated, including feed conversion efficiency, and feed costs per ton of produced larvae. Lastly, a third bioassay was conducted in which the more promising by-products were used as components of isonitrogenous diets, at two nitrogen (N) levels. These were evaluated for middle sized (4^{th}-6^{th} instar) larvae until first pupation for the same parameters as the second bioassay.

Bioassay I: Single by-products without a moisture source

Larvae were sieved at 600 and 500 µm. This yielded 4^{th} to 6^{th} instar larvae in the latter sieve, as determined by head capsule size (Morales-Ramos *et al.*, 2014). These larvae were divided in groups of twenty individuals and their group weight was recorded. Each group was placed in a plastic cylindrical vial (7.5 cm in diameter, 8.8 cm in height), together with 20 g of wheat bran (control) or one of the by-products (Table 1). There were six replicates per dietary treatment. Larvae were left undisturbed for 4 weeks, after which they were separated from their feed, and survival and group weight were recorded. Then, larvae were provided with new feed (20 g) to grow for another 4 weeks, after which they were processed as described above. This procedure was repeated one more time, resulting in three periods of 4 weeks.

Bioassay II: Single by-products with a moisture source

The wheat bran and the eleven by-products were further ground (Thermomix TM31-1C, Vorwerk Elektrowerke GmbH & Co. K) and hand-sieved through a 500-µm sieve. One gram of these feeds, together with a carrot slice (0.6±0.1 g) and twenty newly hatched larvae (<2-d old), were placed

Table 1. Proximate composition (% DM), energy content (kJ/g DM) and cost (€/ton) of a control diet and eleven by-products based on duplicate analysis.

By-product	Dry matter	Nitrogen	Lipids	Ash	Energy	Price	Supplier
	%	% DM	% DM	% DM	kJ/g	€/ton	
Wheat bran (control)	86.7	2.7	2.2	4.6	16.7	170	Mallias N., Bros O.E.
Sugar beet pulp meal	93.7	4.4	1.8	7.5	17.3	210	Mallias N., Bros O.E.
Cotton cake	91.0	4.2	6.8	4.5	18.5	240	Mallias N., Bros O.E.
Cotton seed meal	92.0	4.1	14.0	4.2	20.0	350	Local farmer
Sunflower meal	91.7	4.4	1.6	7.0	17.1	220	Mallias, N., Bros O.E.
Barley by-product (class I)	90.3	1.3	0.6	13.5	14.5	100	Fyto-Animal Services
Barley by-product (class II)	88.6	1.9	0.6	3.3	16.2	140	Fyto-Animal Services
Oat by-product	91.2	3.1	3.4	9.3	16.9	120	Fyto-Animal Services
Pea by-product (class I)	91.0	1.7	0.0	23.1	10.9	100	Fyto-Animal Services
Pea by-product (class II)	91.0	4.1	1.2	4.6	16.3	220	Fyto-Animal Services
Vetch by-product (class I)	89.5	3.5	3.0	11.	15.1	100	Fyto-Animal Services
Vetch by-product (class II)	91.9	4.0	1.0	11.4	14.7	270	Fyto-Animal Services
Yeast (control ingredient)	97.1	8.0	1.7	6.7	19.9	8,000	Angel Yeast Co., Ltd

in the aforementioned vials. There were six replicates per dietary treatment. Carrot was replaced three times per week to function as a moisture source and increase growth, and feed conversion efficiency (Liu *et al.*, 2020; Oonincx *et al.*, 2015). The vials were visually inspected three times per week for feed depletion. If the feed was depleted, more feed was added and the weight was recorded. The larvae were left undisturbed for 4 weeks, after which larval survival and group weight were determined bi-weekly until the first pupation. Then, larvae were separated as a group from their feed, fasted for 24 h, and placed at -20 °C until further analysis. Development time, feed utilisation parameters, growth rate and feed costs per ton of weight gain were quantified.

Bioassay III: Isonitrogenous diets composed of by-products

Suitable by-products, as shown via the first two bioassays, were compounded into two sets of isonitrogenous diets at 2.7 and 3.2% N. Inclusion levels and proximate composition are shown in Table 2 and 3, respectively. For the lower N level, wheat bran served as a control (A1), and in the higher N level wheat bran was mixed with instant yeast (9:1), to serve as a control (B1). The experimental procedure of Bioassay II was followed except for the use of fourth to sixth instar larvae.

Chemical analysis

Proximate composition and energy content of by-products and yellow mealworms were determined based on methods described in AOAC (1995). Dry matter was determined via oven-drying at 105 °C until a constant weight was reached. Nitrogen content was determined by Kjeldahl analyses (behr Labor-Technik GmbH, Düsseldorf, Germany, K12-block standard digestion system, Programmable infrared digestion device, S4 distillation unit). Crude fat was determined by exhaustive Soxhlet extraction using petroleum ether (40-60 °C, BP) using a Soxtherm Multistat/SX PC (Sox-416 Macro, Gerhard, Königswinter, Germany). Ash fraction was determined by incineration in porcelain crucibles at 600 °C for 5 h in a muffle furnace (Nabertherm L9/12/ C6, Lilienthal, Germany). Gross energy content was determined adiabatically using an IKA oxygen bomb calorimeter (C5000; IKA Werke GmbH, Staufen, Germany).

Calculations and statistical analysis

Survival rate was calculated by dividing the number of animals at the end of an experiment by the starting number of animals. Development time was calculated as the time between the start of an experiment until the first pupation within a vial. When calculating feed intake and conversion all provided feed was assumed to have been ingested, whereas carrot weight was excluded from the calculations.

Feed utilisation parameters were calculated based on Waldbauer (1968) via the following equations:

$$\text{Feed conversion ration (FCR)} = \frac{\text{Feed ingested}}{\text{Live weight gained}} \qquad (1)$$

$$\text{Efficiency of Conversion of Ingested food (ECI)} = \frac{\text{Weight gained}}{\text{Feed ingested}} \text{ x } 100\% \qquad (2)$$

Table 2. Inclusion percentages of by-products in two sets of isonitrogenous diets (A=2.7% N; B=3.2% N).

	Diet									
By-product	**A1 (control A)**	**A2**	**A3**	**A4**	**A5**	**B1 (control B)**	**B2**	**B3**	**B4**	**B5**
Wheat bran	100					90				
Barley by-product (class I)			41.0	49.0				15.0	30.0	
Barley by-product (class II)		55.0			44.5		31.5			10.0
Oat by-product			23.0		27.5			42.0		28.0
Pea by-product (class I)										
Pea by-product (class II)		9.0		25.5			38.0		39.0	
Vetch by-product (class I)		36.0			28.0		30.5			62.0
Vetch by-product (class II)			36.0	25.5				43.0	31.0	
Yeast						10				

Table 3. Proximate composition as a % of dry weight basis and energy content (kJ/g DM) of two sets of isonitrogenous diets (A and B).

Diet	Dry matter	Nitrogen	Lipids	Ash	Energy
	%	% DM	% DM	% DM	kJ/g
A1	86.7	2.7	2.2	4.6	16.7
A2	89.2	2.7	1.5	6.5	15.8
A3	91.1	2.7	1.3	11.8	15.1
A4	90.9	2.7	0.9	10.5	15.0
A5	89.6	2.7	1.9	7.4	16.1
B1	87.7	3.2	2.2	4.8	17.0
B2	89.8	3.2	1.6	6.2	15.9
B3	91.4	3.2	1.8	10.8	15.6
B4	91.1	3.2	1.0	9.2	15.3
B5	89.9	3.2	2.8	10.3	15.7

Efficiency of Digested Food Conversion (ECD) =

$$\frac{\text{Weight gained}}{\text{Feed ingested}-\text{faeces}} \text{ x } 100\% \tag{3}$$

Specific Growth Rate (SGR) =

$$100 \text{ x } \frac{(\ln \text{Final Body weight} - \ln \text{Initial body weight})}{\text{days}} \tag{4}$$

Nitrogen efficiency (N-ECI) =

$$\frac{(\text{Amount of N in the insect at harvest})}{(\text{Dietary N provided during the experiment})} \text{ x } 100\% \tag{5}$$

The FCR and SGR were based on fresh weight, whereas ECI, N-ECI and ECD were based on dry matter (DM). The N-ECI was utilised as an indicator of protein retention. Nitrogen content and efficiency are reported in favour of crude protein because the commonly used nitrogen-to-protein conversion factor (Kp) of 6.25 is incorrect for most feed materials (Mariotti *et al.*, 2008). Furthermore, the correct Kp of *T. molitor* larvae is currently under debate (Belghit *et al.*, 2020; Boulos *et al.*, 2020; Janssen *et al.*, 2017).

The feed costs per kilogram of live larvae, named the Economic Conversion Ratio (ECR), was calculated by multiplying the FCR with the cost per kg of feed (Psofakis *et al.*, 2020).

In the third bioassay this ECR was calculated based on the inclusion level per by-product per diet. The expected N-ECI per diet in the third bioassay was calculated by multiplying the inclusion level per by-product with their N-ECI (calculated in the second bioassay) and summing these values per diet.

Significant differences between treatments ($P<0.05$) for survival, FCR, ECI, N-ECI, ECD, SGR and ECR were determined via the Kruskal-Wallis H test, followed by Dunn multiple comparisons for post-hoc testing. The Kaplan-Meier method was used to analyse development time and a Mantel-Cox test was used to detect differences between treatments. The Pearson correlation test was used to determine correlations between development time and survival rates, feed conversion parameters (FCR, ECI, ECD, N-ECI and SGR), larval lipid, DM and N content, as well as between development time and feed conversion parameters. All data were analysed using SPSS 26.0 (IBM Corporation, Armonk, NY, USA).

3. Results

Composition and cost of by-products

All by-products had a high dry matter content (87-94%), but varied in nitrogen (1.3-4.4% DM), lipid (0-14% DM) and ash content (3-23% DM; Table 1). Most by-products had a high nitrogen content, with the exception of both barley by-products (1.3-1.9% DM) and one pea by-product (class I; 1.7% DM). The latter consisted mainly of bean pods and other non-seed materials which explains the lower nitrogen content compared to the class II pea by-product (4.1% DM). Cotton seed meal had the highest lipid content (14% DM) followed by cotton cake (7% DM) and oat by-product (3% DM). Ash level, which is indicative of mineral content, was high in the class II pea by-product (23% DM), which consequently had a low energy content (11 KJ/g). The other by-products had a higher energy content (15-20 KJ/g) with the highest value found for the lipid rich cotton seed meal. Their prices varied between 100 and 350 €/ton, with the lowest costs for the class I pea, class I vetch and class I barley by-products.

Bioassay I: Single by-products without a moisture source

In the first bioassay, *T. molitor* growth and survival after 4, 8 and 12 weeks was similar on the two barley by-products (class I and II), the oat by-product and the wheat bran control (Figure 1, Table 4). Larvae also survived on the vetch by-product (class I), but their final larval weight (week 12) was approximately 65% lower than the control. On the other seven by-products larvae grew and survived poorly. Chemical analysis of the larvae was possible for seven of the dietary treatments. These had similar dry matter contents, some variation in nitrogen content (8.4-9.4% DM) and a wider variation in lipid content (23-38% DM; Table 5).

Bioassay II: Single by-products with a moisture source

In the second bioassay, the weight gain of larvae provided with a moisture source and either cotton cake, oat by-product, sugar beet pulp meal, barley by-product (class II)

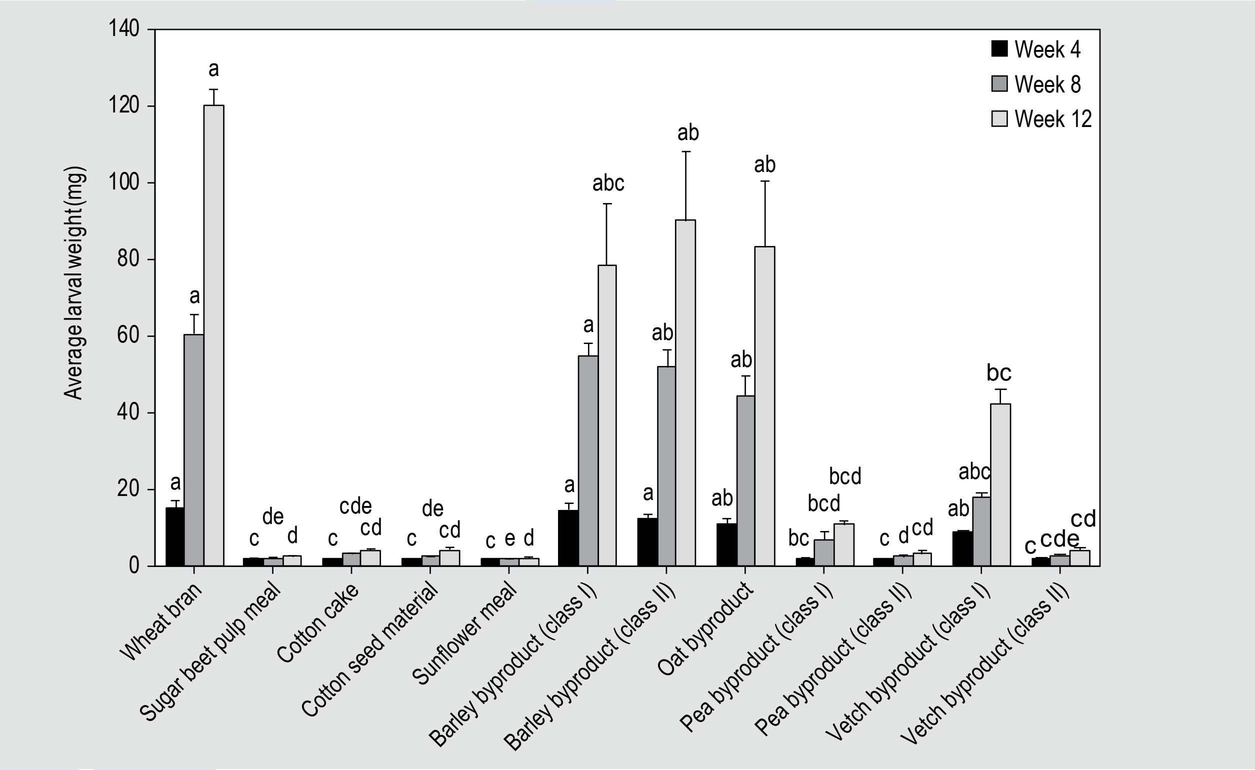

Figure 1. Average larval weight (mg) after 4, 8 and 12 weeks of development of *Tenebrio molitor* larvae reared on an agricultural by-product or wheat bran (control) without moisture source (Bioassay I). Within each evaluation interval (Week 4, 8 and 12), means followed by the same lowercase letter are not significantly different. In all cases, values represent means ± SEM (n=6; *P*=0.05).

Table 4. Survival (%) after 4, 8 and 12 weeks of development of *Tenebrio molitor* larvae reared on an agricultural by-product or wheat bran (control) without moisture source (Bioassay I).[1]

By-products	Week 4	Week 8	Week 12
Wheat bran (control)	100.0±0.0^{a}	98.3±1.1^{a}	90.0±2.2^{abc}
Sugar beet pulp meal	20.8±5.1^{c}	5.0±2.6^{e}	2.5±1.7^{e}
Cotton cake	44.2±4.2^{c}	31.7±3.8^{bc}	27.5±2.1^{bcd}
Cotton seed meal	58.3±6.4^{bc}	29.2±4.7^{bcd}	19.2±5.5^{cde}
Sunflower meal	48.3±6.7^{c}	6.7±2.1^{de}	2.5±1.1^{e}
Barley by-product (class I)	99.2±0.8^{a}	98.3±1.1^{a}	96.7±3.1^{a}
Barley by-product (class II)	96.7±1.7^{ab}	91.7±2.1^{ab}	90.0±3.4^{abc}
Oat by-product	99.2±0.8^{a}	99.2±0.8^{a}	95.8±1.5^{ab}
Pea by-product (class I)	41.7±4.2^{c}	19.2±2.4^{cde}	17.5±1.7^{cde}
Pea by-product (class II)	27.5±5.3^{c}	7.5±1.7^{cde}	2.5±1.1^{e}
Vetch by-product (class I)	100.0±0.0^{a}	98.3±1.7^{a}	96.7±2.1^{a}
Vetch by-product (class II)	40.0±3.4^{c}	20.0±4.0^{bcde}	8.3±3.1^{de}

[1] Values are presented as means ± SEM (n=6; *P*=0.05). Within each column, means followed by the same lowercase letter are not significantly different.

Table 5. Dry matter content (%), and nitrogen and lipid content (% DM) of *Tenebrio molitor* larvae reared on an agricultural by-product or wheat bran (control) without moisture source (Bioassay I).[1]

By-products	Dry matter %	Nitrogen % DM	Lipids % DM
Wheat bran	43.8	8.5	29.8
Sugar beet pulp meal	41.1	8.6	30.4
Cotton cake	42.2	8.4	31.9
Cotton seed meal	n.d.	n.d.	n.d.
Sunflower meal	n.d.	n.d.	n.d.
Barley by-product (class I)	39.1	9.1	27.1
Barley by-product (class II)	41.9	8.4	37.5
Oat by-product	42.3	8.4	34.4
Pea by-product (class I)	n.d.	n.d.	n.d.
Pea by-product (class II)	n.d.	n.d.	n.d.
Vetch by-product (class I)	37.5	9.4	22.6
Vetch by-product (class II)	n.d.	n.d.	n.d.

[1] n.d. = not determined due to insufficient sample. Values are presented as means (n=3).

or sunflower meal, was similar to larvae on the wheat bran control, attaining over 100 mg of live weight (Figure 2). Survival generally followed a similar pattern, with two exceptions; barley by-product class I resulted in high survival (80%), but slow growth and a low final weight (92 mg). The opposite was true for sugar beet pulp meal with low survival (39%) and a high (110 mg) final weight (Figure 3).

The larval development time was influenced by by-product (Mantel-Cox χ^2=162.5, df=11, *P*<0.001) and varied between 68 and 183 d over treatments (Table 6). On sunflower meal larvae grew faster than on the other by-products. This was followed by the control diet, sugar beet pulp meal, cotton cake, barley (class II) and oat by-product. Shorter development times correlated with a lower FCR (r=0.705, *P*<0.001) and a higher ECI (r=-0.653, *P*<0.001), ECD (r= -0.432, *P*<0.001), N-ECI (r=-0.496, *P*<0.001), and SGR (r= -0.944, *P*<0.001) indicating a better feed utilisation, allowing faster growth. A shorter development time also correlated with lower ECR values (r=0.546, *P*<0.001), which in turn correlated with N-ECI (r=-0.662, *P*<0.001), indicating that a higher nitrogen efficiency decreases feed costs and rearing time. Considerable plasticity of the composition of *T. molitor* larvae was observed regarding nitrogen (7.0-10.1%), lipid (15.0-40.9%) and dry matter content (26.7-36.9%) (Table 7). Larvae with a higher lipid content, consequently had a lower N content (r=-0.980, *P*<0.001).

Bioassay III: Isonitrogenous diets composed of by-products

Larval growth and development time varied considerably between the composed diets (Figure 4; Table 8). This variation was greater in series A, with the lower nitrogen level (2.7% DM), than in series B, with the higher nitrogen level (3.2% DM).

The larval development time was influenced by diet (Mantel-Cox χ^2=151.7, df=9, *P*<0.001) and varied between 44 and 114 d over treatments (Table 8). The shortest development time was found for larvae on diets A3 and A5 and on the control diets (A1 and B1; Table 8). Furthermore, these shorter development times correlated with higher

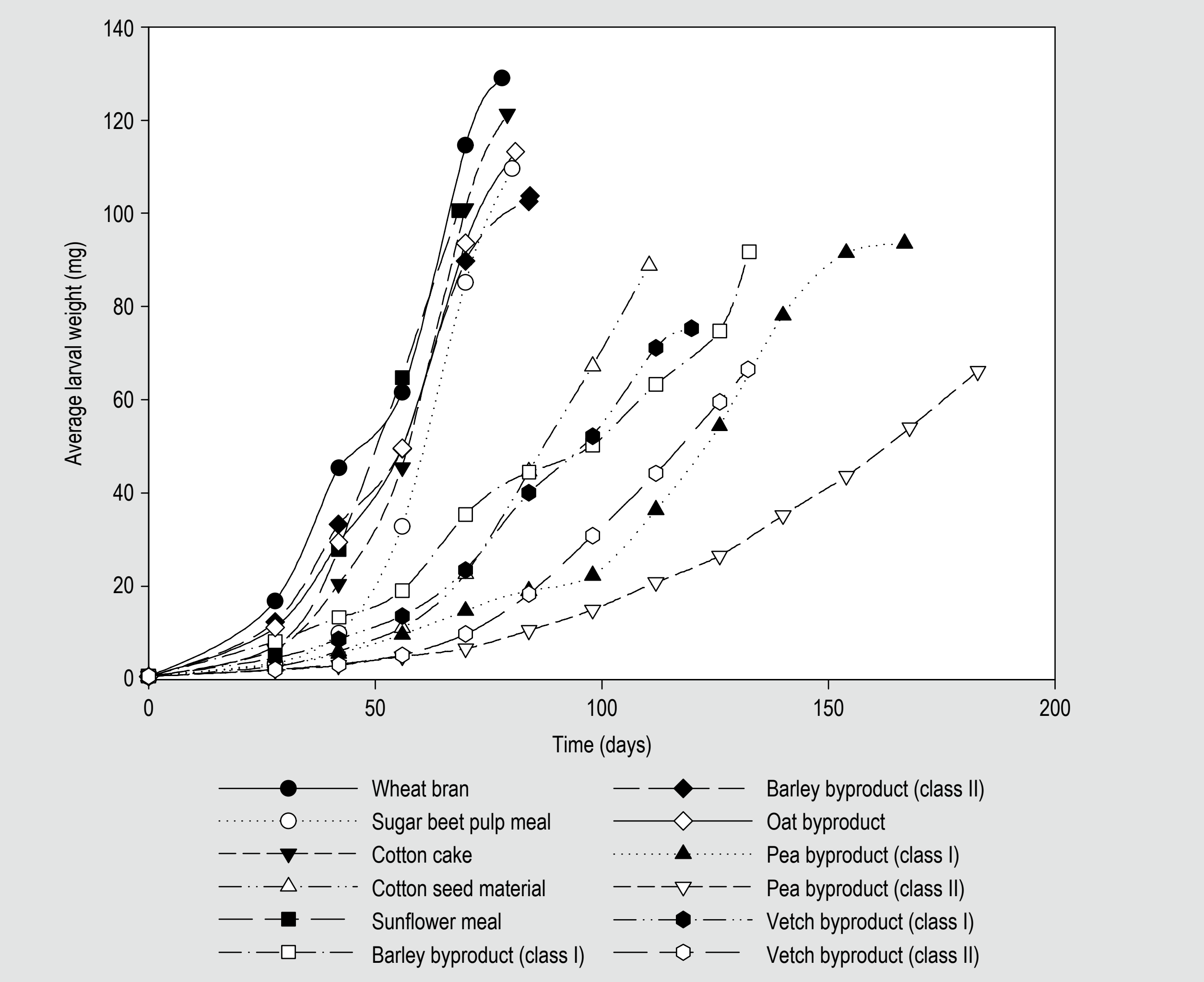

Figure 2. Average larval weight (mg) of *Tenebrio molitor* larvae reared on an agricultural by-product or wheat bran (control) with provision of carrots (Bioassay II) (n=6).

survival rates (r=-0.611, $P<0.001$). Overall, high survival rates were recorded (>83%; Figure 5), except for B2 (67%). Feed utilisation parameters varied greatly between dietary treatments. In the A series the control diet (A1; wheat bran) resulted in an FCR of 2.3, which was similar to diets A3 (2.5) and A5 (2.3), and lower than A2 (3.0) and A4 (3.9) (Table 8). Within the B series the lowest FCR was calculated for the control (wheat bran with yeast; 1.9), followed by B3 (2.8) and B5 (2.7), and then B2 (5.2) and B4 (5.9). Lower FCRs, that is a more efficient use of feed, led to shorter development times (r=0.904, $P<0.001$). As would be expected, lower FCRs correlated strongly with higher ECIs (r=-0.906, $P<0.001$), higher N-ECIs (r=-0.886, $P<0.001$), higher ECDs (r=-0.479, $P<0.001$) and a higher SGR (r=-0.954, $P<0.001$). Although these feed utilisation parameters greatly affect the feed costs, in certain cases this was offset by differences in ingredient costs. The lowest ECR was found for diet B5 (209 €/ton of live larvae), which was just over half the feed costs of the wheat bran control diet (387 €/ton of live larvae). The other control diet (B1 wheat bran with yeast diet), which had the lowest FCR, also had the highest ECR (1,852 €/ton of live larvae).

Dietary treatment had an effect on the larval DM content (30-39%), and lipid content (25-36% DM), but not nitrogen content (Table 9). The two control diets numerically resulted in the highest dry matter (38-39%) and lipid contents (35-36%). Higher DM contents correlated with lipid content (r=0.877, $P<0.001$), but also with development time (r=-0.813, $P<0.001$) and feed conversion parameters such as FCR (r=-0.794, $P<0.001$), ECI (r=0.336, $P=0.009$) and N-ECI (r=0.763, $P<0.001$).

4. Discussion

This study shows that several agricultural by-products from Greece are suitable as feed for yellow mealworm larvae. The first bioassay indicated the suitability of the oat by-product, and the two barley by-products (class I and II) with respect to survival, and weight gain, without

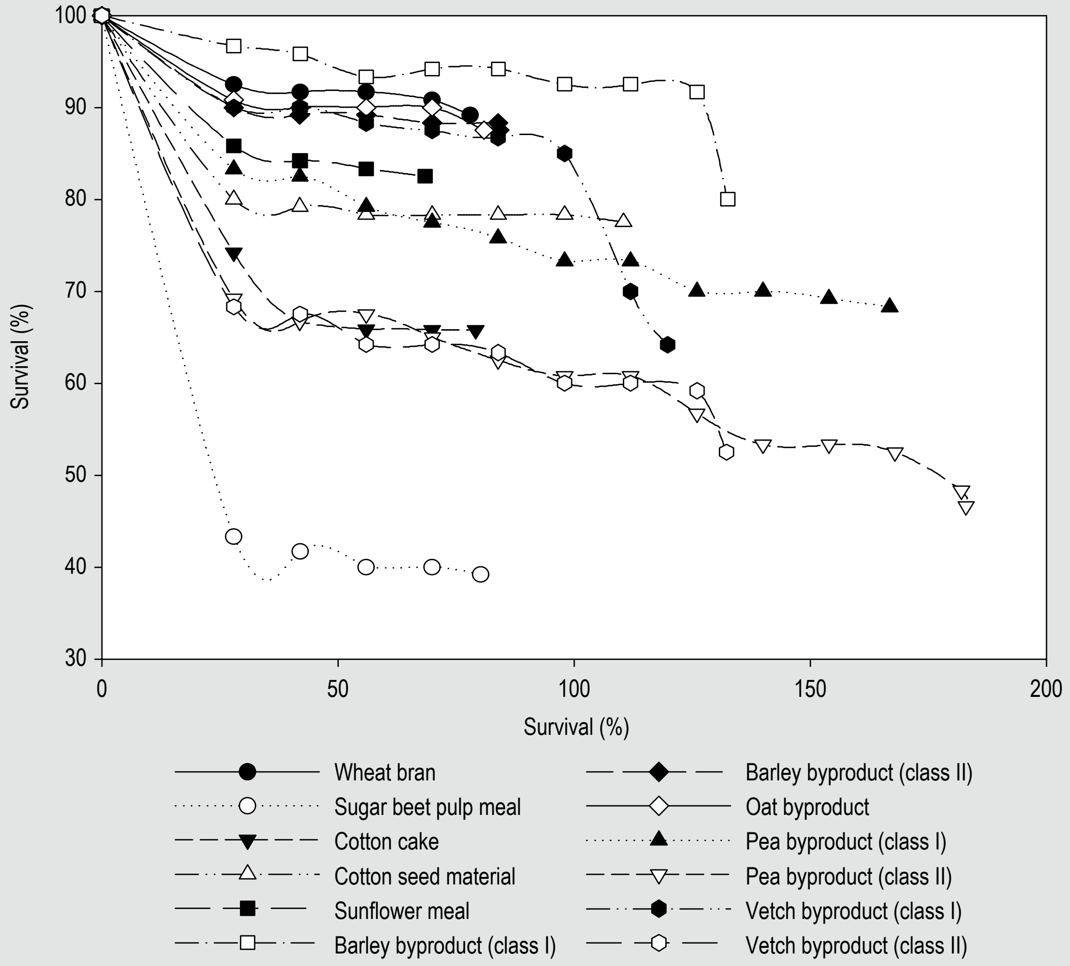

Figure 3. Survival rate (%) of *Tenebrio molitor* larvae reared on an agricultural by-product or wheat bran (control) in the presence of a moisture source (Bioassay II). Values presented as means ± SEM (n=6).

Table 6. Development time (days), feed conversion ratio (FCR), dry matter efficiency of ingested food conversion (ECI, %), nitrogen conversion efficiency (N-ECI, %), dry matter efficiency of digested food conversion (ECD, %), specific growth rate (%) and economic conversion ratio (ECR, (€/ton larvae) of *Tenebrio molitor* larvae reared on agricultural by-product or wheat bran (control) in the presence of a moisture source (Bioassay II).[1]

Substrate	Development time	FCR	ECI	N-ECI	ECD	SGR (%/day)	ECR (€/ton larvae)
Wheat bran (control)	78.0±1.9^{d}	2.3±0.2^{def}	18.9±1.3^{abc}	57.2±4.1^{a}	50.0±2.9^{abc}	6.9±0.2^{a}	396.4±29.6^{def}
Sugar beet pulp meal	80.3±1.9^{d}	4.8±0.6^{bcd}	8.4±1.6^{cdef}	18.4±3.4^{bcd}	64.5±10.8^{a}	6.4±0.1^{ab}	1,017.8±128.1^{abc}
Cotton cake	79.2±2.6^{d}	4.0±0.2^{cde}	9.2±0.5^{abcde}	18.3±1.1^{bcd}	22.6±1.7^{de}	6.6±0.1^{a}	952.1±58.4^{abc}
Cotton seed meal	110.5±3.5^{c}	4.6±0.4^{bcd}	6.8±0.6^{def}	16.7±1.4^{bcd}	25.5±3.9^{cde}	4.6±0.0^{bcd}	1,619.3±141.2^{ab}
Sunflower meal	68.5±0.6^{e}	2.8±0.1^{def}	18.6±3.0^{abcd}	38.7±6.3^{ab}	162.1±28.0^{a}	7.1±0.3^{a}	611.0±244.1^{cdef}
Barley by-product (class I)	132.5±8.7^{bc}	6.0±0.4^{abc}	5.9±0.5^{efg}	35.5±2.8^{ab}	62.0±6.7^{a}	3.8±0.3^{de}	597.3±38.8^{bcde}
Barley by-product (class II)	84.2±3.9^{d}	1.9±0.2^{ef}	23.0±1.8^{ab}	82.9±6.4^{a}	58.1±8.7^{ab}	6.2±0.3^{abc}	261.9±21.4^{ef}
Oat by-product	81.0±1.1^{d}	1.6±0.1^{f}	24.6±0.8^{a}	58.9±1.9^{a}	42.8±3.0^{abcd}	6.4±0.1^{abc}	193.8±6.2^{f}
Pea by-product (class I)	166.8±9.6^{a}	7.8±0.6^{ab}	5.3±0.4^{efg}	22.2±1.6^{bc}	36.0±5.7^{bcd}	3.0±0.2^{de}	779.8±54.8^{bcd}
Pea by-product (class II)	183.0±12.0^{a}	19.8±1.4^{a}	1.5±0.1^{g}	3.3±0.2^{d}	4.0±0.5^{e}	2.6±0.1^{e}	4,363.4±300.7^{a}
Vetch by-product (class I)	119.8±3.2^{c}	4.2±0.4^{bcde}	8.2±0.7^{bcdef}	21.8±1.9^{bc}	44.5±10.7^{abcd}	3.9±0.1^{cde}	416.8±36.9^{cdef}
Vetch by-product (class II)	132.3±4.7^{b}	13.7±1.9^{a}	2.9±0.5^{fg}	6.0±1.0^{cd}	7.3±1.4^{e}	3.6±0.1^{de}	3,705.2±507.9^{a}

[1] Within each column, means followed by the same lowercase letter are not significantly different. In all cases, values represent means ± SEM (n=6; df=11; *P*=0.05).

Table 7. Dry matter, nitrogen and lipid content of *Tenebrio molitor* larvae reared on eleven (11) agricultural by-products and wheat bran (control) in the presence of a moisture source (Bioassay II).[1]

Substrate	Dry matter (%)	Nitrogen (% DM)	Lipids (% DM)
Wheat bran (control)	37.2**	8.1	34.6
Sugar beet pulp meal	33.7**	9.7*	20.8
Cotton cake	32.5**	8.4	28.0
Cotton seed meal	27.9	10.1	15.0
Sunflower meal	32.4**	9.2	22.7
Barley by-product (class I)	31.0	7.7	33.9
Barley by-product (class II)	36.9**	7.0	40.9
Oat by-product	36.0**	7.4	36.8
Pea by-product (class I)	36.5	7.3	35.3*
Pea by-product (class II)	26.7*	8.7*	n.d.
Vetch by-product (class I)	29.7**	9.2	23.7
Vetch by-product (class II)	32.3	8.4*	29.7*

[1] n.d. = not determined due to insufficient sample. Values are presented as means [n=2 unless indicated with * (n=1) or ** (n=3].

carrot supplementation. While the second bioassay, in which diets were supplemented with carrots, confirmed the suitability of the oat by-product, and barley by-product (class II), the barley by-product class I seemed less suitable. Moreover, the second bioassay also indicated the suitability of sunflower meal, which was not apparent from the first bioassay. This discrepancy could be due to the carrot supplementation, which led to a differently balanced diet, or it could be that it reflects a difference in requirements between newly eclosed larvae and larger (4th to 6th instar) larvae. Whereas the composed diets in the third bioassay showed good potential, the pure oat by-product yielded better results. This indicates the added value of first screening by-products singly, prior to mixing them into composed diets.

Isonitrogenous diets

To our knowledge, this is the first study in which isonitrogenous diets were evaluated for the growth and development of *T. molitor* larvae. Most other studies evaluated by-product-based diets for *T. molitor* with variable nitrogen and fat content (Harsányi *et al.*, 2020; Mancini *et al.*, 2019; Oonincx *et al.*, 2015; Ruschioni *et al.*, 2020; Stull *et al.*, 2019; Van Broekhoven *et al.*, 2015). Whereas those report higher survival and faster development on diets with a higher nitrogen content, our results also show large differences within the isonitrogenous diets. Moreover, several low nitrogen diets (A2, A3 and A5) in the current study performed better regarding larval weight, growth rate, survival and feed conversion efficiency, than some of the high nitrogen diets (B2 and B4). This indicates the need to consider other factors when designing optimal diets. This pertains to aspects of the nutrient profile such as lipids and carbohydrates, but also the amino acid profile, and the vitamin, mineral and sterol contents (Cohen, 2003). Moreover, the digestibility of the diets and thereby the nutrient availability is an essential factor to fulfil the

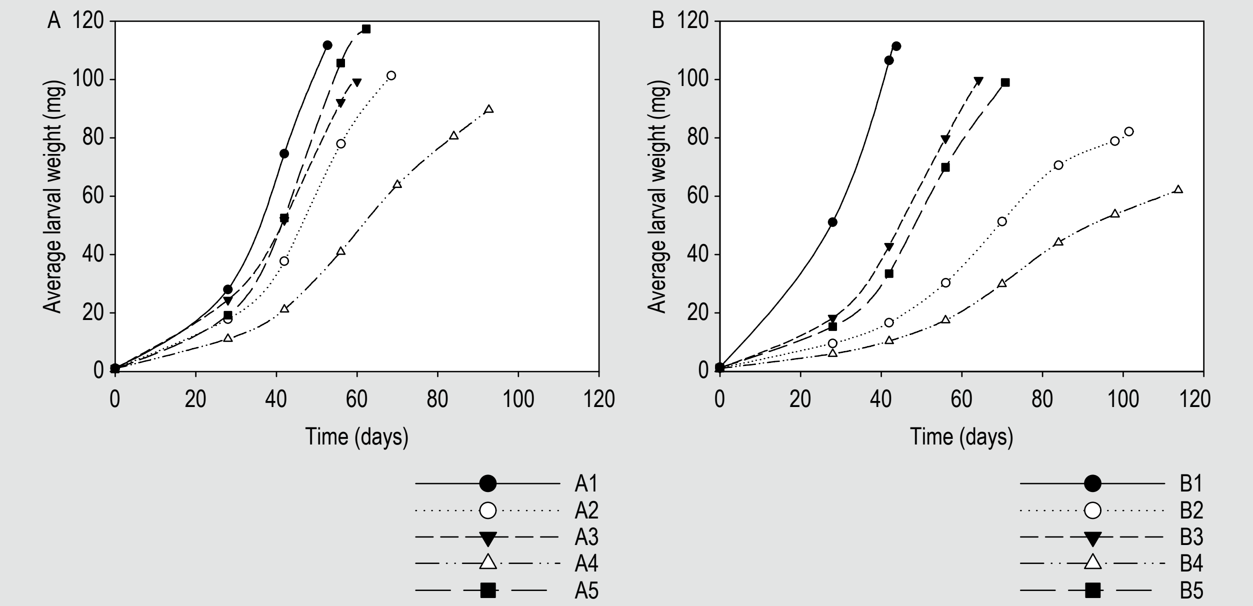

Figure 4. Average larval weight (mg) of *Tenebrio molitor* larvae reared on two groups of isonitrogenous diets (A=2.7% N, B=3.2% N). Values presented as means ± SEM (n=6) (Bioassay III). Refer to Table 2 for the composition of diets A1-A5 and B1-B5.

Table 8. Development time (days), feed conversion ratio (FCR), dry matter efficiency of ingested food conversion (ECI, %), observed nitrogen conversion efficiency (N-ECI, %) as well as expected N-ECI, dry matter efficiency of digested food conversion (ECD, %), specific growth rate (SGR, %) and economic conversion ratio (ECR, (€/ton larvae) of *Tenebrio molitor* larvae reared on two groups of isonitrogenous diets (A=2.7% N, B=3.2% N) (Bioassay III). Refer to Table 2 for the composition of diets A1-A5 and B1-B5.

Diet	Development time	FCR	ECI	N-ECI$_{observed}$	N-ECI$_{expected}$	ECD	SGR (%/day)	ECR (€/ton larvae)
A1	52.7±1.1^{f}	2.3±0.1^{d}	19.4±0.8^{ab}	57.4±2.5^{a}	57.2	51.6±2.8^{a}	8.9±0.2^{ab}	386.4±15.5^{bcd}
A2	68.5±2.9^{cd}	3.0±0.2^{bc}	14.1±0.7^{cde}	44.8±2.3^{abc}	53.7	28.5±2.0^{cd}	6.8±0.2^{cde}	393.2±22.2^{bcd}
A3	60.0±2.0^{e}	2.5±0.1^{cd}	16.4±0.7^{abc}	53.0±2.2^{ab}	30.3	57.0±4.8^{a}	7.6±0.2^{abc}	409.7±17.9^{bcd}
A4	92.7±4.5^{b}	3.9±0.3^{ab}	9.5±0.9^{def}	29.7±2.9^{cde}	19.8	42.3±4.9^{ab}	5.0±0.3^{d}	680.4±60.1^{abc}
A5	62.3±1.3^{de}	2.3±0.1^{cd}	18.1±1.0^{abc}	56.3±3.0^{a}	59.2	41.2±4.6^{abc}	7.8±0.2^{abc}	246.8±15.8^{de}
B1	43.8±0.7^{g}	1.9±0.1^{e}	23.0±0.7^{a}	56.9±1.8^{a}	n.d.	53.7±2.2^{a}	10.1±0.2^{a}	1,851.6±65.1^{a}
B2	101.5±4.3^{ab}	5.2±0.4^{a}	7.2±0.5^{ef}	19.5±1.4^{de}	34.0	23.0±2.4^{d}	4.3±0.1^{e}	824.3±57.5^{ab}
B3	64.2±1.6^{de}	2.8±0.1^{bcd}	14.7±0.6^{cde}	38.3±1.6^{cd}	32.7	42.9±2.3^{ab}	7.1±0.1^{bcd}	363.1±14.9^{cde}
B4	113.7±5.8^{a}	5.9±0.2^{a}	5.6±0.2^{f}	14.8±0.4^{e}	13.8	34.7±3.2^{bcd}	3.7±0.1^{f}	1,255.2±38.3^{a}
B5	70.8±2.1^{c}	2.7±0.3^{bcd}	15.2±1.1^{bcd}	40.0±2.9^{bcd}	38.3	48.5±3.5^{a}	6.3±0.2^{cde}	208.9±19.1^{e}

[1] n.d. = not determined. Within each column, means followed by the same lowercase letter are not significantly different. In all cases, values represent means ± SEM (n=6; df=9; *P*=0.05).

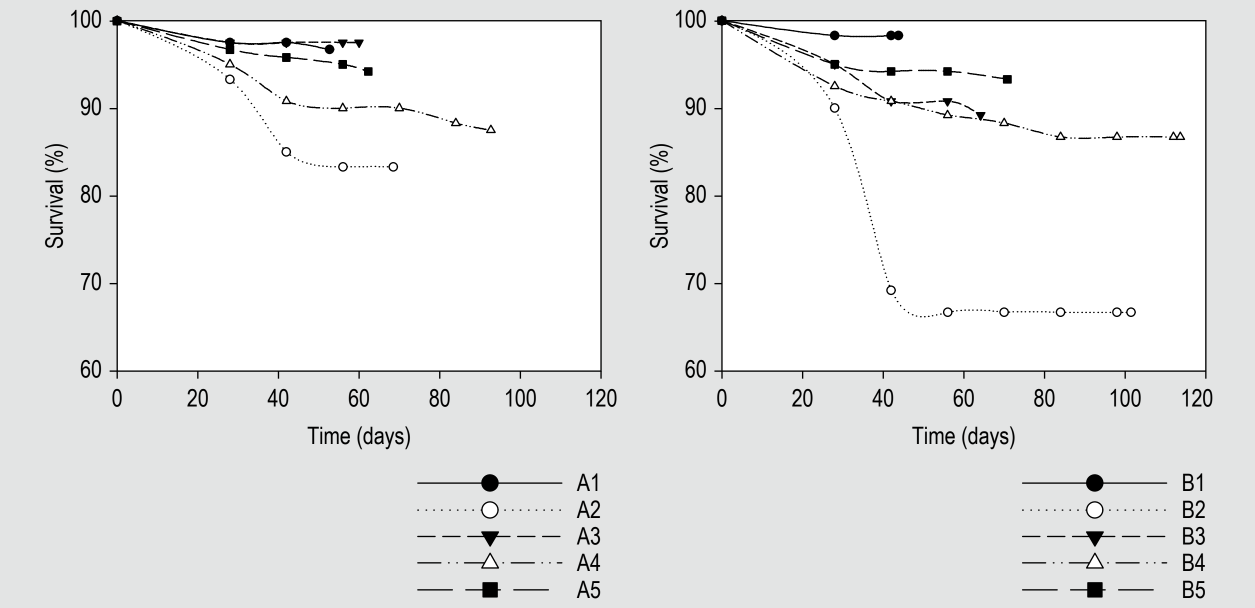

Figure 5. Survival rate (%) of *Tenebrio molitor* larvae reared on two groups of isonitrogenous diets (A=2.7% N, B=3.2% N). In all cases, values represent means ± SEM (Bioassay III) (n=6).

nutrient requirements of *T. molitor*. Lastly, the presence of antinutrient factors can be of relevance. The latter aspect might explain the poor performance of larvae on diets B2, B4 and A4. These contained 26-39% of pea by-product (class II), which can hold saponins that exert phagodeterrent and insecticidal effects (De Geyter *et al.*, 2007; Singha and Kaur, 2018; Taylor *et al.*, 2004).

Feed utilisation

The oat by-product resulted in the lowest feed conversion ratio (1.6) of all by-products and diets tested. This by-product contained 3.1% nitrogen (DM), which was lower than the concentration (3.4-4.0% DM) chosen by *T. molitor* larvae given the choice between a wide range of ingredients (Morales-Ramos *et al.*, 2020). Apparently, this protein is highly digestible and has a suitable amino acid profile as

Table 9. Dry matter, nitrogen, and lipid content of *Tenebrio molitor* larvae reared on two groups of isonitrogenous diets (Group A and B: 2.7 and 3.2% nitrogen, respectively) (Bioassay III).

Diet	Dry matter (%)	Nitrogen (% DM)	Lipids (% DM)
A1	37.8	7.9	35.6
A2	36.6	8.5	31.3
A3	36.6	8.6	29.7
A4	32.5	8.3	25.4
A5	37.3	8.3	32.5
B1	39.0	7.9	34.7
B2	33.0	8.6	27.0
B3	37.0	8.3	31.5
B4	29.9	8.5	28.2
B5	36.3	8.4	31.3

indicated by the N-ECI (59%). This nitrogen efficiency is in the higher range of reported values for yellow mealworms (22-58%; Oonincx *et al.*, 2015). An even higher N-ECI (83%) was calculated for barley product class II and warrants investigation of the amino acid profiles of these two by-products in future studies. This could provide insight in matching the amino acid requirements of growing mealworms with locally available by-products (John *et al.*, 1979).

As stated in the previous paragraph, other nutrients should also be considered when trying to optimise feed utilisation. Water is a very important, and often insufficiently considered nutrient. Its relevance for larval performance, including feed utilisation, is apparent from the results of bioassay I and II. The addition of carrot slices in the second bioassay substantially improved growth and survival of larvae provided with cotton cake, cotton seed meal, and sunflower meal. This concurs with a previous study providing carrots as a source of moisture to *T. molitor* larvae, increasing their feed utilisation (ECI and N-ECI) by approximately 50% (Oonincx *et al.*, 2015). Even though other nutrients are present in carrots, it seems highly likely that water availability was the primary cause of the improved performance and high feed utilisation rates in the current study. The importance of water availability for *T. molitor* is also apparent from studies in which larvae were provided with free water or increased relative air humidity (Murray, 1968; Urs and Hopkins, 1973). It seems that under dry conditions larvae utilise feed to produce metabolic water and hence cannot efficiently use this feed for growth (Fraenkel, 1950).

Data from the second bioassay was used to predict the N-ECI of the composed diets in the third bioassay. Except for diets B3 and A4, this approach had good predictive value, indicating its' usefulness for designing composed diets. However, from a commercial perspective, feed utilisation is only one side of the coin.

Feed costs

Within the composed diets, the best larval growth and performance and lowest FCR was achieved in the wheat bran-yeast mixture control. However, the feed costs per ton of larvae were the highest (1,851 €/ton of larvae) on this substrate, making it uneconomical for commercial and industrial rearing systems. When only wheat bran was used as a substrate the ECR was between 387 €/ton (diet A1; Bioassay III) and 397 €/ton (Bioassay II). The lowest ECR was found for the oat by-product (194 €/ton) tested in the second bioassay. This can be explained by its low price (120 €/ton of feed) and its efficient utilisation by the larvae. Also composed diet B5 could greatly decrease the ECR (209 €/ton of feed) compared to the wheat bran control, using the cheap vetch by-product as the main ingredient and supplementing it with oat and barley (II) by-products. The same ingredients in other proportions were used in diet A5 leading to an ECR of 247 €/ton of larvae. While in the current study the focus is on feed costs, differences in development time, and thereby production time should be considered, when conducting a full economic analysis.

Clearly, several of the tested by-products hold potential to decrease the feed costs associated with *T. molitor* rearing. Besides the economic potential, exploitation of locally available organic by-products can likely decrease the environmental impact of insect farming (Madau *et al.*, 2020; Van Huis and Oonincx, 2017). Further studies could include quantification of the environmental impact of the utilisation of by-products to determine the optimal dietary composition from this perspective. Such an approach would be fully aligned with circular economy strategies as promoted by the European Union (European Commission, 2014, 2020).

Chemical composition of the larvae

Diet composition influences insect development, but also strongly affects the chemical composition of the insect body (Mancini *et al.*, 2019; Oonincx and Finke, 2021; Oonincx *et al.*, 2015; Ramos-Elorduy *et al.*, 2002; Van Broekhoven *et al.*, 2015). In the first bioassay, the larval DM content was high on all tested by-products (38-44%), whereas the same by-products in the second bioassay led to a lower DM content (27-37% DM). This can be explained by the addition of carrots in the second bioassay providing moisture (Oonincx *et al.*, 2015). Overall, larval nitrogen content (7.0-10.1%) was in accordance with the findings of previous reports (Ramos-Elorduy *et al.*, 2002; Van Broekhoven *et al.*, 2015). While the by-products tested differed in their nitrogen content

(1.3-4.4%), this did not affect the larval nitrogen content in the first bioassay. These parameters did however correlate in the second bioassay (r=0.782, *P*=0.003), whereas such correlation was absent for the two groups of isonitrogenous diets in the third bioassay.

Variation in larval lipid content due to dietary composition was apparent in all three bioassays. This variation fell within the ranges previously reported for *T. molitor* (Finke, 2002; Oonincx *et al.*, 2015; Van Broekhoven *et al.*, 2015; Yi *et al.*, 2013). Generally, larval fat content was higher in the second bioassay than in the first bioassay. This could be because some dietary groups were not yet approaching pupation at the end of the first bioassay and hence had not yet gathered fat reserves for pupation (Oonincx and Finke, 2021). Alternatively, moisture availability could have increased energy efficiency, allowing a higher accumulation of fat reserves (Fraenkel, 1950).

5. Conclusions

This study approaches insect farming from the perspective of a circular economy at a local level. It indicates the suitability of several agricultural by-products locally produced in Greece for *T. molitor* larvae. These by-products can be utilised singly or in composed diets and can greatly decrease the feed costs of mealworm production. Furthermore, this study shows that besides nitrogen content, other factors need to be considered when formulating optimal diets.

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Conflict of interest

The authors declare that they have no conflict of interest. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the authors.

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