



# Performance of *Hermetia illucens* (Diptera: Stratiomyidae) larvae reared on organic waste diets and pupal chitin and chitosan yield

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## Abstract

Recently, much research has been oriented towards the influence of different food wastes and agricultural by-products on the final larval biomass and chemical composition of the insect species *Hermetia illucens* L. (Diptera: Stratiomyidae). However, there is a gap in the literature regarding the possible relationship between the feeding substrate of *H. illucens* larvae and chitin. In this context, in the present study, larvae of *H. illucens* derived from two populations (i.e., UNIPI and UTH), were reared on different diets composed of fruits, vegetables, and meat. Based on the results, the larval survival was high for all diets tested. Larval growth in terms of weight gain, larval length, and feed conversion ratio (FCR) depended on the composition of each diet. The chitin and chitosan composition of larvae, reared on different substrates, did not reveal significant differences. Given the fact that the feeding substrate represent a significant cost in the industrial production of insects, its correlation with a high value product (i.e. chitosan) is important. On the other hand, as the prepupal stage of *H. illucens* is currently used as animal feed, the metabolization of chitin by farmed animals when the larvae or prepupae were offered as feed could have adverse effects. Thus, depending on the final product that is to be produced, industries could benefit from the establishment of a suitable diet.

**Keywords** Circularity · Food waste valorization · *H. illucens* · Larval growth · Chitin · Chitosan

## Introduction

The rapid growth of the human population has led to an ever-increasing demand for food and energy resources. An allied outcome of this evolution process was the generation of huge amounts of agro-industrial food waste. The high cost associated with the processing methods is leading modern

societies to discard industrial and household food wastes in landfills (Kumar Awasthi et al. 2022). Indicatively, worldwide one-third of edible parts of food produced for human consumption is wasted (FAO 2011). The quantities of food waste produced globally are estimated to be 1.3–1.4 billion tons per year, while, until 2025, the projections show that it will reach as high as 2.6 billion tons (Sinha and Tripath 2021).

On the other hand, agro-industrial and domestic food waste could constitute a valuable feeding substrate for insects. Thus, the development of the insect sector in Europe is a promising solution to the transition to a more sustainable food system (EC 2020). The valorization of food wastes has been studied for several insect species, such as the yellow mealworm, *Tenebrio molitor* (Coleoptera: Tenebrionidae) (Oonincx et al. 2019; Rumbos et al. 2022); the superworm, *Zophobas morio* (Coleoptera: Tenebrionidae) (Van Broekhoven et al. 2015); the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) (Gianotten et al. 2020);

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Christina Adamaki-Sotiraki and Linda Abenaim participated equally to this study.

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and the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae) (Bava et al. 2019; Bosch et al. 2019).

It is necessary to point out that, in the EU, the main obstacles in insect-rearing development are at the normative level. In particular, the most relevant EU regulatory restrictions are related to the type of insect-rearing substrate since insects reared for feed fall under the definition of ‘farmed animal’ (Article 3.6 of Regulation EC n. 1069/2009), which has several consequences for the choice of the organic resource which are allowed to be used as feeding substrates. In the EU, for instance, the use of several substrates such as ruminant proteins, catering waste, meat-and-bone meal, and manure are not allowed for any farmed animals (insects comprise) (Regulation (EU) No. 767/2009). Currently, the only authorized rearing substrate for insects, as farmed animals, are the animal feed materials according to the EU catalogue (Regulation EU No. 68/2013), the food produced for human consumption (excluding meat and fish), but no longer considered as such (due to expiry date or manufacturing or packaging defects); ex-food of plant origin (EU No. 68/2013); ex-food of animal origin (non-PAP) (EU No. 142/2011); and other types of organic waste of vegetable nature such as gardening and forest material (EC No. 767/2009, Annex III; EC No. 68/2013 Directive 2008/98/EC) (Bosch et al. 2019).

The insect species *H. illucens* is native to the Neotropical region (Brammer and von Dohlen 2007). Nowadays, *H. illucens* has a cosmopolitan distribution and, in some countries, it is utilized to counteract the environmental detriments caused by food waste, by producing organic fertilizers (Sheppard et al. 1994). Thus, much research has been directed to the influence of different food wastes and agricultural by-products on the growth and performance of *H. illucens* larvae (Nguyen et al. 2013; Cammack and Tomberlin 2017; Salomone et al. 2017; Barragan-Fonseca et al. 2018; Gold et al. 2020; Kawasaki et al. 2022;). Most of the studies highlighted the variations in final larval biomass and chemical composition. Researchers also correlate differences in larval growth with chitin content in larvae of *H. illucens* (Diener et al. 2009; Meneguz et al. 2018), which is one of the most abundant polysaccharides in nature that provides insects with the rigid structure of the exoskeleton. Via deacetylation reaction, chitin could be converted to chitosan. Both chitin and chitosan are biocompatible, biodegradable, and non-toxic biopolymers. The importance of the two biopolymers has been brought to light due to their improved functionality and biodegradability compared to synthetic polymers (Rinaudo 2006). Indicatively, chitin and chitosan are utilized in food processing, packaging, cosmetics, textiles, and drug delivery and their production has recently gained considerable economic importance (Park and Kim 2010; Hamed et al. 2016).

As reported by many authors, the *H. illucens* instars currently used as animal feed are prepupae (Panikkar et al. 2022; Kroeckel et al. 2012). However, the metabolization of chitin by farmed animals when the larvae or prepupae were offered as feed could be problematic (Lee et al. 2022). From an industrial point of view, the extraction of chitin and chitosan from *H. illucens* prepupae, pupae, and exuviae, is of considerable economic importance. Thus, both sectors could benefit from the establishment of a suitable diet which, as mentioned above, may alternate the growth and chemical composition of different life stages of *H. illucens*. Therefore, the present study aims to evaluate the growth and performance of *H. illucens* larvae reared on diets composed of organic waste allowed by EU legislation, compared to a meat-based diet reported in the bibliography as unsuitable and not allowed by EU legislation as well as to investigate the possibility of chitin and chitosan production from prepupae to be influenced by the diet.

## Materials and methods

### *Hermetia illucens* rearing

Experiments were performed using two populations of *H. illucens*, one reared at the Laboratory of Entomology at the Department of Agriculture, Food, and Environment of the University of Pisa (Italy) (UNIPI population) and the other one at the Laboratory of Entomology and Agricultural Zoology of the University of Thessaly (Greece) (UTH population). The two populations were maintained under the same laboratory conditions (28 °C, 60% RH, and photoperiod 16:8 = L:D). The protocol utilized for the rearing of both populations was the same. In detail, the eggs were removed after two days from the oviposition support (a groove of wooden support (20 × 3 × 1 cm)) with a wet brush with a fine tip and moved into plastic boxes (29 × 18 × 9 cm) containing an artificial diet composed of chicken feed and water at a ratio of 40:60, respectively. Each plastic box was covered with gauze to ensure humidity. Once the first pupae appeared, the box was positioned inside a cage (47.5 × 47.5 × 93 cm model BugDorm-4M4590DH) for the emergence of the new adults. The cage was provided with water and sugar *ad libitum* and a plant branch to increase the surface area available for adults to lean on and perform their “lekking” behaviour for mating.

### Diet composition and chemical analyses

Both *H. illucens* populations (UNIPI and UTH) were fed with five different diets: (a) control diet, based on poultry feed and water at 40:60 w/w; (b) fruit diet, 75% of fruits (apple, orange,

and banana at a 1:1:1 ratio w/w) and 25% of control diet; (c) vegetable diet, 75% of vegetables (celery, sweet pepper, and potatoes at a 1:1:1 ratio w/w) and 25% of control diet; (d) meat diet, 75% of poultry meat and 25% of control diet; (e) mixed diet, i.e. control diet, fruit diet, vegetable diet, and meat diet at 1:1:1:1 ratio w/w. Fruits and vegetables were packaging waste from the local supermarkets, washed under tap water for 5 min, and left to air dry before use. Diets were ground by a kitchen grinder, operating at 800 W for 4 min.

The different kinds of diets were chemically analysed following the procedures specified in AOAC (2000) (i.e. crude protein (method 984.13) and ether extract (method 920.39) (Tognocchi et al. 2023)). The contents in moisture, proteins, and lipids are reported in Table 1.

### Larval performance

Thirty 6-days-old larvae of *H. illucens* were weighted as a group and introduced in a polyethylene (PE) cup (5 cm diameter × 8 cm height), filled with 25 g of each diet. Five replicates for each diet were performed for a total of 150 larvae per diet. The cups were maintained under the same laboratory conditions described above (28 °C, 60% RH, and photoperiod 16:8 = L:D). Larval survival, weight, and length were recorded every 3 days until the larvae were 18 days old. Each group of larvae was counted and weighed with an analytical balance and then the individual larval weight was calculated. The length of each one of the larvae was measured with a ruler. The larval growth rate was calculated when the larvae were 15 days old. In fact, after that, the mature larvae stop eating to prepare to moult and, from then on, their weight starts to decrease (Bosch et al. 2019; Miranda et al. 2020). For this reason, the feed conversion ratio (FCR) was calculated 15 days after the start of the bioassay using the following formula:

$$\text{FCR (g)} = \text{Total Feed consumed (g)} / \text{Live weight gained (LWG in g)}$$

The FCR indicates the proportion of digested feed assimilated and converted into body mass. The calculation of FCR was performed on as is basis. Low values of FCR indicate

a high efficiency of substrate conversion to biomass, while high values of FCR indicate that the feed has been digested, but having a little nutrient value, a large part was excreted (Nyakeri et al. 2017).

### Determination of chitosan yield

The same described rearing protocol was used for all five different diets to obtain 50 g of prepupae that were frozen, dried at 75 °C until constant weight, grounded and then stored at −20 °C for chitosan production.

Chitin extraction and chitosan production were performed according to Hahn et al. (2020) with some modifications, as follows. The demineralization process of the dried raw powder was carried out by stirring at room temperature with 0.5 M formic acid (1 g DW:10 mL). After 30 min, the demineralized biomass was filtered using non-woven tissues and washed with distilled water to reach pH neutrality, and finally, it was oven-dried until complete desiccation. These washing and drying steps were repeated also after the deproteinization (2 M sodium hydroxide stirred for 2 h at 80 °C; 1 g DW 10 mL) and deacetylation process (12 M sodium hydroxide stirred for 3 h at 90 °C; 60 g DW 800 mL).

The yield of chitin and chitosan (%) was determined according to the following equation:

$$\frac{\text{Chitin or chitosan (g DW)}}{\text{Starting dry raw biomass (g DW)}} \times 100$$

Chitosan production was performed in two replicates for each diet and the two populations.

### Statistical data analysis

To compare the different growth traits (larval weight and length) and the FCR, data were analysed by ANOVA with a model that considered the fixed effect of the different diets (control, fruit, vegetable, meat and mixed), the different populations (UNIPI and UTH), and their interaction. Statistical differences between groups were assessed using Tukey's test; "p" values are presented in order to declare

**Table 1** Diets' composition for *Hermetia illucens* larvae rearing

Diet	Ingredients	Moisture %		Protein %		Lipids %	
		UTH	UNIPI	UTH	UNIPI	UTH	UNIPI
Control	Poultry feed and water at 40:60 w/w	57.21	55.71	5.61	1.42	1.67	1.14
Fruit	75% fruits (i.e. apple, orange, and banana at a 1:1:1 ratio w/w) and 25% of control diet	63.18	67.46	1.22	1.29	1.14	0.59
Vegetable	75% of vegetables (i.e. celery, sweet pepper, and potatoes at a 1:1:1 ratio w/w) and 25% of control diet	72.50	76.31	3.12	1.49	1.23	0.38
Meat	75% of poultry meat and 25% of control diet	70.70	74.67	20.49	20.80	5.63	0.80
Mixed	Control diet, fruit diet, vegetable diet, and meat diet at 1:1:1:1 ratio w/w	70.63	72.77	13.14	19.90	4.51	0.81

the existence or not of statistically significant differences between tested groups. The analyses were conducted with JMP software (SAS Institute, Inc., Cary, NC, USA). The same ANOVA model was used to analyse data relating to chitin and chitosan yield.

The progression of the population survival during the experiments was estimated by Kaplan–Meier survival analyses. Survival differences among treatments were assessed by a log-rank test. The values “ $\chi^2$ ” and “ $p$ ” are represented below and help to determine whether there’s a significant difference in survival rates between different groups or categories in the data. The Kaplan–Meier analyses were performed by the SPSS 22.0 software (IBM SPSS Statistics, Armonk, North Castle, NY, USA).

## Results

The survival analysis showed no overall differences in the larvae mortality depending on the population ( $\chi^2 = 0.043$ ,  $p = 0.835$ ) with survival rates ranging from 94 to 100% (Fig. 1). As for the diets, the highest survival rates were recorded for the control (100% survival) and the vegetable diets (97.3 and 98.6%, for UNUPI and UTH populations, respectively) with no significant difference between control and vegetable diets ( $\chi^2 = 0.043$ ,  $p = 0.835$ ).

The lowest larval survival (94%) was observed in larvae fed on the meat (UNUPI, UTH) and the mixed diets (UNUPI). In general, significant differences compared to the control diet were recorded for the fruit diet ( $\chi^2 = 5.642$ ,  $p = 0.18$ ), meat diet ( $\chi^2 = 12.363$ ,  $p < 0.001$ ), and mixed diet ( $\chi^2 = 6.472$ ,  $p = 0.011$ ), while vegetable diet showed significant differences only compared to the meat diet ( $\chi^2 = 6.748$ ,  $p = 0.009$ ).

The larval weight and length on the different diets for both populations were compared (Figs. 2 and 3). For both populations, larvae ceased feeding and stopped gaining weight after 9 days (15-day-old larvae). Concerning the final individual larval weight and length, the two-way ANOVA showed a highly significant interaction between populations and diet treatments ( $p < 0.001$  and  $p < 0.01$ , for the final individual larval weight and length, respectively) (Table 2). In particular, concerning the average larval weight for the UNUPI population, larvae reared on the control, and the fruit, diets gave better results and were statistically different compared to the vegetable, meat, and mixed diets. In contrast, for the UTH population, the control, meat, and mixed diets gave better results, statistically different, than the vegetable and fruit diets. However, among the two populations, no statistical differences have been evidenced comparing the weight of the larvae fed on the same kind of diet except the weight of the larvae reared on the meat diet. In particular, for these, the larval

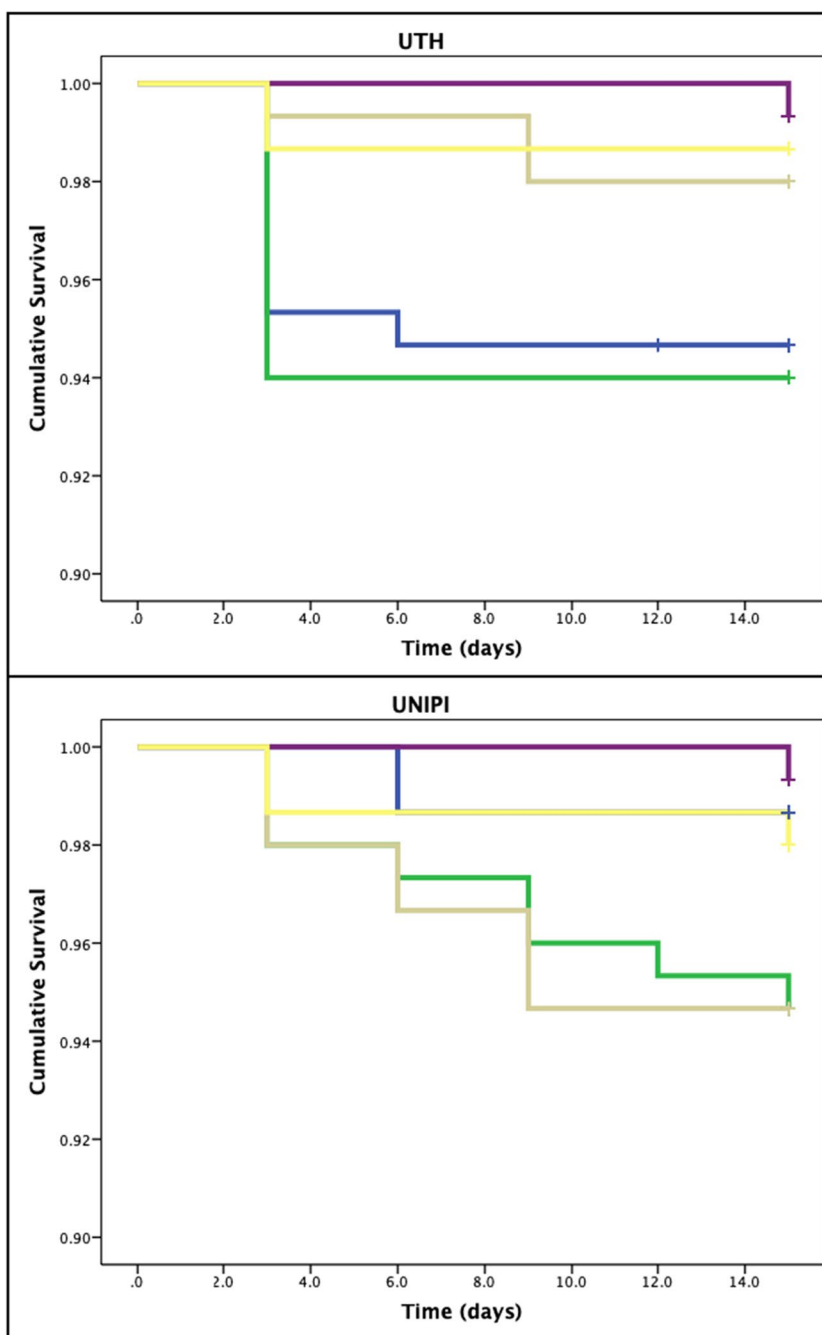
weights differed between the two populations, with the UNUPI population larvae weight being statistically lower than the UTH one. As for larval length, a statistical difference was recorded for the UNUPI larvae reared on the meat diet, shorter than the others. It should be noted that the larvae fed on the control diet had the highest weight ( $p < 0.001$ ,  $0.20 \pm 0.006$  g) and larval length ( $p < 0.01$ ,  $1.76 \pm 0.016$  g) compared to the other diets for both populations. Moreover, our results indicated that for both populations, the highest FCR was recorded for the meat diet. The two-way ANOVA indicated that the interaction between the populations and the dietary treatments was not significant. Both FCRs of the two populations showed significant differences between the meat diet and the other diets ( $p < 0.001$ ) (Table 3).

Finally, the final biomass recovery of chitin and chitosan is reported in Table 4. Our results did not reveal the statistically significant influence of the diet factor on the yield of both chitin ( $p = 0.571$ ) and chitosan ( $p = 0.713$ ). The population factor also showed no significant influence on the final yield ( $p = 0.449$  and  $p = 0.642$  for chitin and chitosan, respectively). The same occurred for the interaction between the diet and the population factors ( $p = 0.641$  and  $p = 0.699$  for chitin and chitosan, respectively).

## Discussion

The present study investigates the survival, growth performance, and food conversion efficiency of larvae as well as the chitin and chitosan composition of prepupae of two populations (UTH and UNUPI) of the insect species *H. illucens*, reared on different organic waste-based diets. From our results, the high survival rates (> 90%) for all tested diets indicate that *H. illucens* larvae can thrive in various organic wastes. Similar results also reported by other authors (Nguyen et al. 2013; Cammack and Tomberlin 2017; Salomone et al. 2017; Barragan-Fonseca et al. 2018; Gold et al. 2020; Kawasaki et al. 2022), including kitchen and vegetable wastes (Oonincx et al. 2019; Chia et al. 2020). On the other hand, our data showed that, while larval survival was high for all diets tested (Fig. 1), larval weight gain, larval length, and feed conversion ratio, strongly depended on the composition of each diet (Table 2; Table 3). Moreover, regarding the larval survival, the results of both populations (UNUPI and UTH) reared on a meat-based diet are in agreement with Kawasaki et al. (2022) and Candian et al. (2023), who reported the lowest larval survival when larvae were fed on a meat-based diet (77% and 88% of larval survival respectively). In another study, Zhou et al. (2013) reported differences concerning populations of different geographical origins in terms of waste conversion efficiency and development time. Similarly, for *T. molitor*, it has also been reported that insect populations from different geographical regions perform

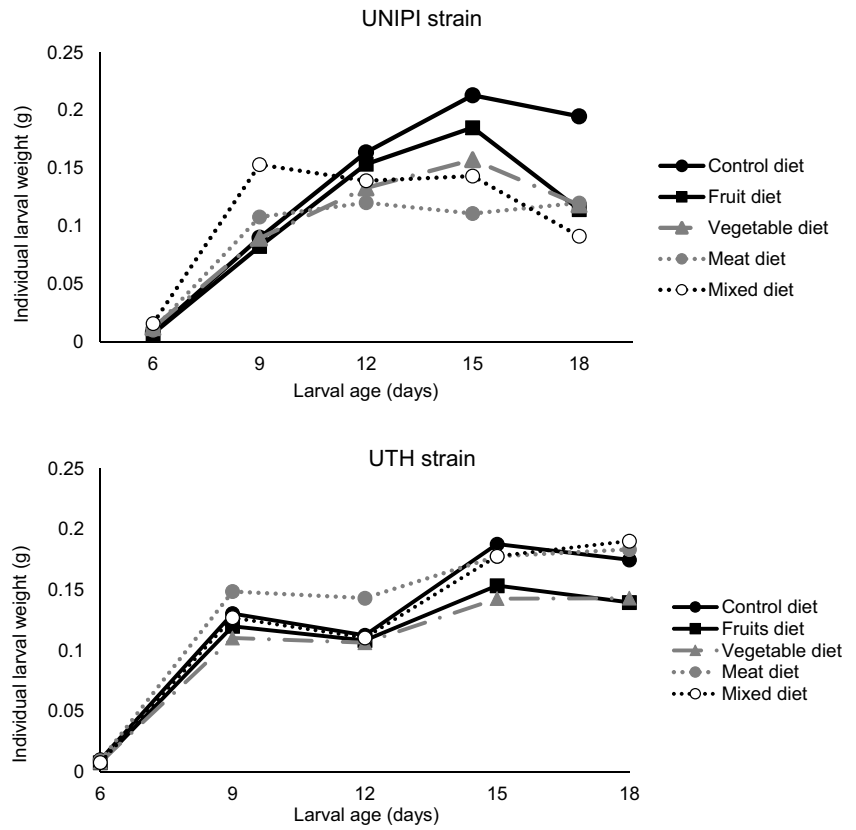
**Fig. 1** Survival of *Hermetia illucens* larvae from two different populations (i.e., UNUPI and UTH population) fed on five different diets (i.e., control, fruit, vegetable, meat, and mixed diet) over a time period of 15 days. More specifically, the purple line represents the control diet, the yellow line represents the vegetable diet, the brown line represents the mix diet, the blue line represents the fruit diet, and the green line represents the meat diet



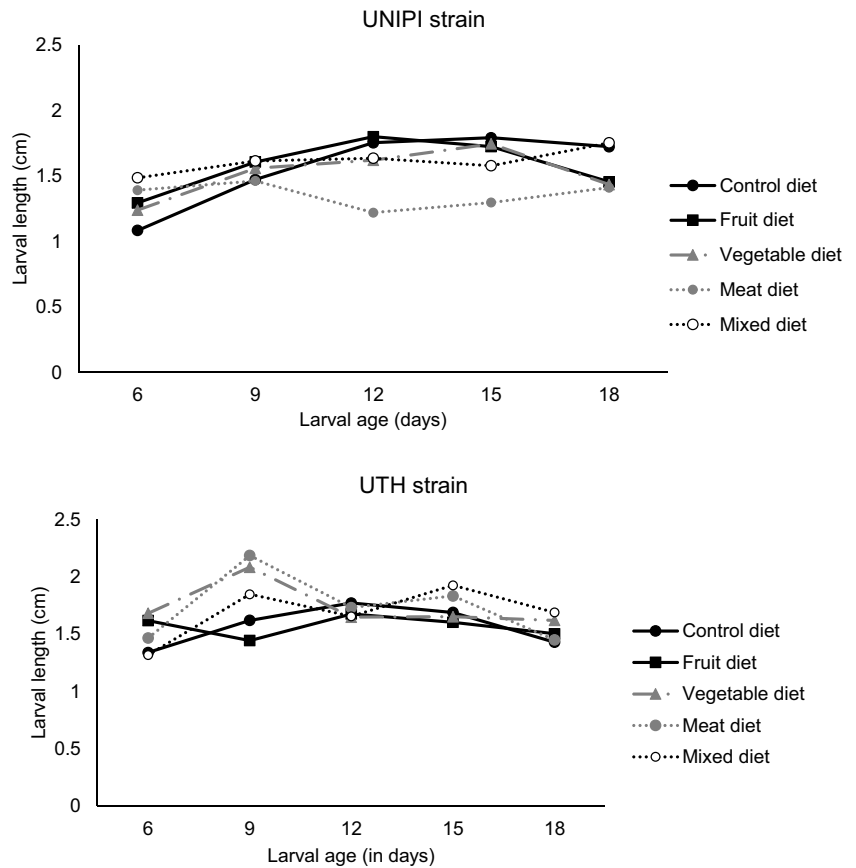
differently when feeding on the same diet (Rumbos et al. 2021; Adamaki-Sotiraki et al. 2022). For most of the tested diets, our results did not reveal statistical differences between the two tested populations which were fed on the same diet, except the meat diet. However, each population utilized in this study (UNUPI and UTH populations), performed better in different diets. For instance, besides the control diet, the UNUPI population gave heavier larvae on the fruit diet ( $0.185 \pm 0.014$  g), while the heavier larvae for the UTH population were produced on meat and mixed diets ( $0.177 \pm 0.003$  g and  $0.177 \pm 0.009$  g, respectively). Based on the above, it is apparent that through

a diet with a variety of different nutrient ingredients, larvae may develop faster and gain more weight than via diets with high protein content. It is possible that the results of our study may have influenced from the fact that the diets were not only organic waste, but also contained 25% poultry feed. This approach was followed in order to reduce the moisture content of the diets, as according to Montevecchi et al. (2023) excessive amounts of moisture increase larval survival. In the present study, the highest final individual larval weight was recorded, in descending order, for control, fruit, meat, and mixed diets. Regarding the larval performance obtained with the control

**Fig. 2** Average weight of *Hermetia illucens* larvae from two different populations (i.e., UNUPI and UTH population) fed on five different diets (i.e., control, fruit, vegetable, meat, and mixed diet) throughout 6–18-day



**Fig. 3** Average length of *Hermetia illucens* larvae from two different populations (i.e., UNUPI and UTH population) fed on five different diets (i.e., control, fruit, vegetable, meat, and mixed diet) throughout 6–18-day





**Table 2** Final individual larval weight (g) and final larval length (cm) of two *Hermetia illucens* populations (i.e., UNUPI and UTH population) fed on five different diets (i.e., control, fruit, vegetable, meat, and mixed diet). Data represent the mean ± standard deviation.

Diet	Population	Populations × diets ( $p < 0.0001$ )	
		Mean individual larval weight (g)	Mean individual larval length (cm)
Control	UNUPI	0.213 ± 0.006 <sup>a</sup>	1.754 ± 0.015 <sup>a</sup>
	UTH	0.187 ± 0.006 <sup>ab</sup>	1.772 ± 0.017 <sup>a</sup>
Fruit	UNUPI	0.185 ± 0.014 <sup>ab</sup>	1.799 ± 0.095 <sup>a</sup>
	UTH	0.153 ± 0.005 <sup>bcd</sup>	1.676 ± 0.018 <sup>a</sup>
Vegetable	UNUPI	0.157 ± 0.008 <sup>bcd</sup>	1.619 ± 0.018 <sup>a</sup>
	UTH	0.142 ± 0.002 <sup>cd</sup>	1.648 ± 0.016 <sup>a</sup>
Meat	UNUPI	0.119 ± 0.01 <sup>d</sup>	1.102 ± 0.102 <sup>b</sup>
	UTH	0.177 ± 0.003 <sup>abc</sup>	1.732 ± 0.025 <sup>a</sup>
Mixed	UNUPI	0.143 ± 0.005 <sup>cd</sup>	1.478 ± 0.015 <sup>ab</sup>
	UTH	0.177 ± 0.009 <sup>abc</sup>	1.656 ± 0.021 <sup>a</sup>

The column represents the average values for all populations and diets tested. Different letters in the column represent the differences between groups. Significant interactions between populations and diet treatments are also presented

diets, the mean individual larval weight ( $0.187 \pm 0.006$  g and  $0.213 \pm 0.006$  g, UTH and UNUPI populations respectively) is in agreement with Bava et al. (2019), who reported  $0.23 \pm 0.2$  g/larva. Our data are also in agreement with Jucker et al. (2017), who reported that larvae reared on fruit diets and vegetable diet had lower weights than those reared on mixed (fruit and vegetable) diet. The meat-based diet appears to be unsuitable, as evidenced by the UNUPI results which are in line with those of Gobbi et al. (2013) and Kawasaki et al. (2022) which show the lowest prepupal rate (23%) and adults' weight (8–9%) if compared with the control diet (32% of prepupal rate and 15–16% of adults' weight, respectively). Along the same lines, Nguyen et al. (2013) investigated larval development on six different feeding substrates (i.e. fruits, vegetables, liver, manure, fish rendering, and kitchen waste). Their results showed that larvae fed on kitchen waste gained the highest weight. The authors highlight that equilibrium between calories, fat, and protein is important for *H. illucens* larvae diets. In a few relevant works, Lee et al. (2014) opine that vegetable diets contain fibres, and Barbi et al. (2020) report that fruits are high in carbohydrates.

However, neither vegetables nor fruits are high in protein and should be combined with proteinaceous sources to produce fast-growing larvae with high body weight. Yet, exceeding proportions of protein in diets may diminish larval growth (Nguyen et al. 2013; Kawasaki et al. 2022). Interestingly, Barbi et al. (2020) reported that larval growth and development time were optimized by a diet composed of a variety of different fruits, but larvae fed on diets with specific fruits, i.e. melon and exotic fruit, performed better. The authors reported that melon contains a high percentage of fibres, and exotic fruit is rich in polyphenols and carbohydrates. Our data also showed a connection between FCR and diet, which is also supported by the statistical analysis which revealed that FCR was not affected by population but was affected by diet. Indicatively, for larvae fed on meat-based diets, the highest FCR was recorded

**Table 3** Feed conversion ratio (FCR) of UNUPI and UTH populations of *Hermetia illucens* larvae fed on five different diets (i.e., control, fruit, vegetable, meat, and mixed diet) ( $n = 5$ ). Data represent the mean ± standard deviation. Different letters in the table represent the differences between diets within the same population

Diets	Feed conversion ratio (FCR)	
	UNUPI population	UTH population
Control	1.10 ± 0.07 <sup>a</sup>	1.00 ± 0.04 <sup>a</sup>
Fruit	1.32 ± 0.14 <sup>a</sup>	1.58 ± 0.29 <sup>a</sup>
Vegetable	1.91 ± 0.39 <sup>a</sup>	1.72 ± 0.14 <sup>a</sup>
Meat	7.6 ± 1.35 <sup>b</sup>	5.53 ± 1.91 <sup>b</sup>
Mixed	2.21 ± 0.16 <sup>a</sup>	1.19 ± 0.06 <sup>a</sup>

**Table 4** Chitin and chitosan (CHT) yield (%) of *Hermetia illucens* prepupae of two different populations (i.e., UNUPI and UTH population) fed on five different diets (i.e., control, fruit, vegetable, meat, and mixed diet). Data represent the mean ± standard deviation

Diets	Populations	Populations × diets	
		( $p = 0.641$ ) % Chitin	( $p = 0.699$ ) % CHT
Control	UNUPI	6.17 ± 1.95	4.25 ± 1.97
	UTH	5.53 ± 1.45	3.41 ± 0.80
Fruit	UNUPI	5.37 ± 0.51	3.69 ± 0.72
	UTH	6.08 ± 0.06	4.32 ± 0.20
Meat	UNUPI	5.01 ± 0.45	3.46 ± 0.56
	UTH	4.96 ± 0.08	3.19 ± 0.48
Vegetable	UNUPI	5.17 ± 1.70	3.11 ± 1.61
	UTH	5.38 ± 1.31	3.71 ± 1.16
Mixed	UNUPI	3.88 ± 0.65	2.67 ± 0.38
	UTH	5.57 ± 0.71	3.60 ± 0.06

for both populations, thus resulting in the least suitable diet. The present study confirms previous studies reporting that the composition of the substrate has an important effect on the final growth performance of *H. illucens* larvae (Nguyen et al. 2013; Jucker et al. 2017; Meneguz et al. 2018; Barbi et al. 2020; Chia et al. 2020; Kawasaki et al. 2022). Interestingly, in contrast to our results, Montevecchi et al. (2023) reported a correlation between larval weight and the protein quantity present in the substrate. The authors utilized a variety of kitchen leftovers. It is evident that, as additional scientific data emerge, it becomes apparent that the growth of *H. illucens* larvae when fed a mixed diet substrate is influenced not only by protein quantity, but also by protein quality.

Apart from feeding substrates, there is a growing interest of scientific and industrial stakeholders on chitin and chitosan in insects due to its various applications (Mohan et al. 2020; Aranaz et al. 2021). Combining these two subjects, in one of the few available studies, Meneguz et al. (2018) investigated the chitin content of *H. illucens* prepupae that derived from larvae reared on fruit and vegetable diets, as well as on brewery and winery by-products. The researchers reported that there is a correlation between the chitin content of prepupae and the development time of larvae but not with the substrate. Similar results were reported by Diener et al. (2009), who observed that the larvae that grew slower, and gained less weight had high chitin content. Our results are in line with the above-mentioned studies, as in both populations (UNUPI and UTH), no influence of the 5 different rearing substrates was found on the chitin content extracted from *H. illucens* prepupae. Given the fact that the feeding substrate represent a significant cost in the industrial production of insects, its correlation with a high value products (i.e. chitosan) should be further investigated. Moreover, potentially, the prospect of biomass increase during *H. illucens* larvae production will also lead to increased chitin derived from insects. Thus, additional research should be focused on the potential correlation of the feeding substrate and the chitin composition of insects. On the other hand, as the prepupal stage of *H. illucens* is currently used as animal feed, the metabolization of chitin by farmed animals when the larvae or prepupae were offered as feed could have adverse effects (Lee et al. 2022). Thus, depending on the final product that is to be produced, industries could benefit from the establishment of a suitable diet.

## Conclusions

In conclusion, the larvae reared on the fruits, vegetables, and mixed diets showed efficient conversion of the substrate to body weight, as indicated by the low FCR indices, with no statistical difference between the diets or between the two populations. The results thus confirm that larvae reared on vegetable and fruit waste, legally permitted in the EU, are suitable for high-performance mass production of BSF prepupae

as animal feed. The present study is among the few highlighting the need to investigate the connection of *H. illucens* larval growth performance on different organic wastes with the chitin and chitosan content of the prepupae. Although, none of the different substrates affected the chitin and chitosan content in either population, in order for the industries to increase the availability of chitin, the correlation of the diet provided to chitin production should be further investigated.

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**Author contribution** Adamaki-Sotiraki C., Abenaim L, Mannucci A., Rumbos I.C., Bedini S., Castagna A., Conte G., Tognocchi M., Dolianitis V., Athanassiou G.C., and Conti B. revised the content and all agreed to submit the manuscript. Prior to submission, Adamaki-Sotiraki C., Abenaim L, Mannucci A., Rumbos I.C., Bedini S., Castagna A., Conte G., Tognocchi M., Dolianitis V., Athanassiou G.C., and Conti B. contributed to the conceptualization of the idea as well as to the design of the work and analysis of data. Moreover, Adamaki-Sotiraki C., Abenaim L, Mannucci A., Rumbos I.C., Bedini S., Castagna A., Conte G., Tognocchi M., Dolianitis V., Athanassiou G.C., and Conti B. approved the publication of this version and declared to be accountable for all aspects of the work in ensuring that inquiries regarding the accuracy or integrity of all parts of this work are investigated and resolved appropriately.

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**Data availability** Data will be made available on request.

## Declarations

**Ethical approval** Research activities in the present study involved invertebrate animals, in particular insects. Insect mass rearing was performed taking into consideration insect welfare, treating insects as sentient beings and ensuring insect well-being. Particularly, trials were conducted with the following insect species: *Hermetia illucens* (Diptera: Stratiomyidae). No ethical restrictions are applied for experimental and practical tests having as subject the above cited species. All the trials that involved insects were conducted by UNUPI and UTH.

**Consent to participate** No human subjects were involved; thus, this part is not applicable.

**Consent for publication** No human subjects were involved; thus, this part is not applicable.

**Competing interests** The authors declare no competing interests.

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