

# Development and Biomass Composition of *Ephestia kuehniella* (Lepidoptera: Pyralidae), *Tenebrio molitor* (Coleoptera: Tenebrionidae), and *Hermetia illucens* (Diptera: Stratiomyidae) Reared on Different Byproducts of the Agri-Food Industry

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## Abstract

The aim of this study was to evaluate five agro-industrial byproducts (apricots, brewer's spent grains, brewer's spent yeast, feed mill byproducts including broken cereal grains, and hatchery waste including eggshell debris, fluff, infertile eggs, dead embryos, and egg fluids) or mixtures thereof as food diets of *Ephestia kuehniella* (Zeller), *Tenebrio molitor* (L.), and *Hermetia illucens* (L.). Eleven out of 26 tested combinations allowed the first instar larvae to reach the adult stage. Results showed that bioconversion parameters and biomass composition can vary depending on the diet composition, especially in the case of *E. kuehniella* and *H. illucens*, whose nutritional requirements seem more complex than those of *T. molitor*. *Tenebrio molitor* was able to develop in almost all byproducts. However, only when *T. molitor* was fed with suitable mixtures of byproducts the development parameters were similar to those obtained with the standard diet. The best results in terms of bioconversion parameters were obtained by feeding *H. illucens* with a diet including dried brewer's spent grain, feed mill byproducts and brewer's spent yeast. The larvae of these three species can be considered interesting from a nutritional point of view, because of their high protein and fat content. However, the fatty acids profile of *H. illucens* larvae, with high proportions of saturated fatty acids, seems less healthy for human consumption compared with those of *E. kuehniella* and *T. molitor*.

**Key words:** bioconversion, food diets, yellow mealworm, black soldier fly, Mediterranean flour moth

Humanity faces the serious challenge of supplying enough food for its growing world population in an efficient and sustainable way, limiting the generation of wastes that may contaminate the environment. Furthermore, the high demand and competition for raw materials between the production of feedstuff, as well as foodstuff, when in some countries the population suffers malnutrition, has triggered the search for new and alternative sources of proteins and fat (Tao and Li 2018). Insects have been used as a food source by many civilizations since they have high contents of biologically valuable protein, fatty acids (FAs), and micronutrients (Rumpold and Schluter 2013, Nowak et al. 2016, Payne et al. 2016, Sun-Waterhouse et al. 2016, Gravel and Doyen 2020). However, the nutritional value of the insect biomasses depends on several factors, i.e., species, climate and season, life stage, diet, rearing conditions, and processing before consumption (Ghosh et al. 2017).

It has been reported that the protein content of insect biomass from different orders ranged from 13 to 77% of their dry matter, with a large variation between and within insect orders (Jonas-Levi and Itzhak Martinez 2017, Xiaoming et al. 2010). Furthermore, insect protein has high nutritional value, because it contains high levels of essential amino acids, including lysine, tryptophan, and threonine, which are frequently deficient in cereals (Kouřimská and Adámková 2016). Insects are also a considerable source of FAs. Womeni et al. (2009) analyzed the fat content of several insect species and found that it can be highly variable, between 9 and 67% on a dried matter basis. Moreover, some insect species are rich in polyunsaturated and essential FAs that are nutritionally relevant (Michaelsen et al. 2009, Paul et al. 2017). As for other compounds, the FAs profile of insects appears to be influenced by several factors, among which the diet is one of the most important (Bukkens 2005). For example, a poor-protein

content diet affects both protein and lipid accumulation in fat body cells, leading to a lower abundance of protein granules, as well as a larger and more abundant lipid droplets (Pimentel et al. 2017).

Insects have also been studied for waste management from the food industry (Ramos-Elorduy 2009, Rumpold and Schluter 2013). These byproducts can be converted very efficiently into biomass with a relatively low energy input (van Huis et al. 2013), and with much less land and emissions of greenhouse gases and ammonia than in the case of conventional livestock (Oonincx et al. 2010). When rearing an insect species, there is a relationship between the quality and quantity of food offered and their larval growth rate (Grandison et al. 2009, Borzoui et al. 2018, Suits et al. 2017). Moreover, larval diet has a considerable impact on adult fecundity and egg fertility (Zhang et al. 2011, Majd-Marani et al. 2017). Eating a low-quality food has been associated with high mortality, reduced growth, delayed development, and low reproductive output in insect herbivores (Awmack and Leather 2002, Behmer 2006, Suits et al. 2017). Therefore, to select which insect species can be reared on industrial residues to obtain protein, fat, and other commercially important compounds, it is essential to assess if they can properly develop in the selected byproducts.

Many of edible insect species belong to the orders Lepidoptera, Coleoptera, and Diptera, and they are usually eaten as larvae (Yi et al. 2013). The yellow mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), and the black soldier fly, *Hermetia illucens* (L.) (Diptera: Stratiomyidae), are among the most studied species used for mass rearing to produce food and feed products (van Huis et al. 2013). These two species have been reported by the European Food Safety Authority (EFSA) in the list of insects with the biggest potential to be used as food and feed in the European Union (EFSA 2015). The Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) is a cosmopolitan stored product pest that feeds on cereal byproducts such as flour and semolina, but also infests a wide range of other dried commodities. For its nutritional properties, the eggs of this species are used as a factitious food source for the mass rearing of insect pest predators and parasitoids in mass production factories (Riudavets et al. 2020).

The aim of this study was to evaluate the preimaginal development of *E. kuehniella*, *T. molitor*, and *H. illucens* reared with different agro-industrial byproducts, as well as the nutritional composition of the resulting insect biomasses. In order to evaluate the feasibility and sustainability of the bioconversion process and to check the suitability of the larvae biomasses as feed or food sources, some diets including different combinations of byproducts from the agri-food industries were considered. Their main parameters related to the biomass development and composition were compared with those obtained from standard diets.

## Materials and Methods

### Insects

Individuals of the three species tested were obtained from stock cultures maintained in a climatic chamber at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and with a photoperiod of 16:8 (L:D) h at IRTA. *Ephestia kuehniella* was originally collected in a flourmill near Barcelona, Spain. *Tenebrio molitor* was purchased at Reptimerca (Purias, Murcia, Spain) and *Hermetia illucens* at Entosur (San Isidro de Nijar, Almería, Spain). They were reared on the following standard diets: *E. kuehniella* on whole wheat flour and yeast (2:1) (Jacob and Cox 1977), *T. molitor* on whole flour wheat, wheat bran, and pet food (Ultima dog food with chicken, Affinity Petcare) (3.3:2.5:1) (Ribeiro et al. 2018), and *H. illucens* on wheat bran, rabbit feed

(Cryspy Muesli, Versele-Laga), yeast, and water (10:6:1:24) (Nakamura et al. 2016) (Table 1).

### Byproducts

Five industrial byproducts were tested from different food processing companies in Spain, namely: i) apricots from a fruit marketing cooperative, ii) brewer's spent grains and malt bagasse, and brewer's spent yeast from a brewery, and iii) feed mill byproducts (mainly broken cereal grains) and hatchery waste (eggshell debris and fluff, infertile eggs, dead embryos, and egg fluids) from a chicken farm (Table 1). The presentation of some of these byproducts was modified to adapt them to the type of diets that these three insect species feed upon. Thus, apricots and malt bagasse were tested raw and dried, and yeast was lyophilized to be used dry, since none of the selected species is able to develop on a liquid substrate.

### Comparison of Byproducts for Insect Growth

In a first step, a small quantity of 50 g of each byproduct and mixtures thereof combined were offered to the three insect species in ventilated plastic containers (polyethylene terephthalate, 710 ml capacity,  $12 \times 10.5$  cm) (Garcia de Pou, Ordis, Spain). Between 25 and 30 first-instar larvae were added to the containers and kept for some weeks until they become adults. Percentage of mortality, weight of the last instar larvae, and developmental time to reach pupae and adult stages were recorded. Three to 10 replicates per insect species and selected byproducts or mixtures thereof were analyzed (Table 2).

### Bioconversion of Agro-Industrial Byproducts by Insects

Based on the results obtained in the first trial, five byproducts or mixtures thereof were selected to produce larval biomasses of the three insect species (Apricots (AP-R), Feed mill byproducts (FM) and the Mixtures *Ek2*, *Tm1*, and *Hi1*). We started with a variable number of individuals of each insect species and a variable amount of initial substrate. This was based on the different larval sizes and mortality rates during the preimaginal development when reared on

**Table 1.** Moisture and total protein and fat content of the agro-industrial byproducts and standard diets tested

Diets	Code	Moisture (%)	Total fat (% dry matter)	Total protein (% dry matter)
<i>E. kuehniella</i> standard diet		11.4	2.6	16.4
<i>T. molitor</i> standard diet		12.1	6.0	17.2
<i>H. illucens</i> standard diet		61.2	1.8	7.5
Apricots (raw)	AP-R	88.5	0.2	0.6
Apricots (dried)	AP-D	30.7	0.8	3.7
Brewer's spent grain (raw)	BG-R	75.3	2.4	7.6
Brewer's spent grain (dried)	BG-D	6.7	8.6	23.4
Brewer's spent yeast (raw)	BY-R	90.1	0.8	3.8
Brewer's spent yeast (dried)	BY-D	2.0	2.3	44.7
Feed mill byproducts	FM	8.7	2.9	11.3
Hatchery waste	HW	37.7	6.0	10.5

**Table 2.** Byproducts or mixtures thereof offered to the three insect species tested

Diets	Composition	<i>Ephestia kuehniella</i>	<i>Tenebrio molitor</i>	<i>Hermetia illucens</i>
Apricots	AP-R	X	X	X
	AP-D	X	X	X
Brewer's spent grain	BG-R	X	X	X
	BG-D	X	X	X
Brewer's spent yeast	BY-D	X	X	X
Feed mill byproducts	FM	X	X	X
Hatchery waste	HW	X	X	X
Mixture <i>Ek1</i>	BG-D + BY-D (2:1)	X		
Mixture <i>Ek2</i>	FM + BY-D (2:1)	X		
Mixture <i>Tm1</i>	BG-D + FM + BY-D (3.5:5:1)		X	
Mixture <i>Tm2</i>	BG-D + AP-D + BY-D (3.5:5:1)		X	
Mixture <i>Hi1</i>	BG-D + FM + BY-R (2:1:5)			X

their standard diets, and the availability of larvae from the rearings, but always maintaining a proportion of 0.4–0.5 larva per g of substrate. Then, 2-liter aerated plastic containers (polypropylene) were prepared with 453, 93, and 192 first-instar larvae and 910, 223 and 410 g of substrate for *E. kuehniella*, *T. molitor*, and *H. illucens*, respectively. Percentage of mortality, weight of the last instar larvae, and substrate weight reduction caused by the reared larvae were calculated. Nine replicates were made for each combination of byproduct and insect species, including their standard diets.

### Biomass Samples Pretreatment

At the end of the growing period, larvae biomasses were collected, separated from the substrate residues, and stored at  $-80^{\circ}\text{C}$ . Before starting the analyses, biomasses were homogenized with a blender-mixer R401 (Robot Coupe, Isleworth, United Kingdom) in the presence of dry ice. Each sample was split in two aliquots, one was packed in multilayer (Al-PE) flexible bags under vacuum, stored at  $-80^{\circ}\text{C}$  and used to calculate moisture. The second was lyophilized with a Lyomicron 55 (Coolvacuum Technologies, Barcelona-Spain), packed in multilayer (Al-PE) flexible bags under vacuum, stored at  $-20^{\circ}\text{C}$  and used for the other analyses.

### Proximate and FAs Analyses

Moisture was assessed gravimetrically by measuring the weight loss after drying at  $105^{\circ}\text{C}$  in a convection oven, total protein content was calculated following the Kjeldahl method, and total fat were estimated by Soxhlet extraction (AOAC 1999). FAs profile was assessed by adapting the protocol described by Mach et al. (2006). Samples (250 mg) were extracted with a mixture of chloroform: methanol (2:1, v/v), derivatized with a mixture of toluene and HCl 3N in methanol (1:4, v/v) at  $80^{\circ}\text{C}$  for 1 h and added with NaCl 10 % in water and hexane (10:3, v/v) (Christie 1993). FAs methyl esters were recovered in the organic phase and then separated on a gas chromatograph Agilent 6890 Series II (Hewlett Packard SA, Barcelona, Spain) equipped with a capillary column DB23 (30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$ ; Agilent, Santa Clara, USA), a split-splitless injector, and a FID detector. Identification of the single methyl esters were done by comparing retention time of the peaks with those of pure standards; quantification was carried out by using tripentadecanoic (Merck KGaA, Darmstadt, Germany), as internal standard.

### Statistical Analysis

To compare the effect of byproducts on insect growth, larval weight was analyzed with a Kruskal–Wallis test ( $\alpha=0.05$ ), and further

pairwise comparisons between the different food commodities and the control diet were conducted with Dunn's multiple comparisons. To compare bioconversion by insects of byproducts, data on the percentage of mortality, the weight of larvae, the substrate weight reduction, the production of larvae per unit of substrate, and the percentage of larvae moisture content, fat, and protein for each insect species were analyzed by Kruskal–Wallis test ( $\alpha=0.05$ ). Then, Dunn's multiple comparisons were used for further pairwise comparisons between the different diets. Analyses were performed using JMP 14.2.0 (SAS Institute 2018).

## Results

### Comparison of Byproducts for Insect Growth

Of all tested combinations of insect species and byproducts (Table 2), only some of them allowed the first instar larvae to reach the adult stage. In the case of *E. kuehniella* larvae, they survived in four out of the 10 combinations tested, including the standard diet. However, most of the larvae died before they reached the pupal stage in two of these combinations: dried apricots (AP-D) and feed mill byproducts (FM). When the larvae of *E. kuehniella* were fed with the Mixture *Ek2*, a high number of them reached the adult stage, even higher than when they were fed with the standard diet (Table 3). In contrast, larvae had a lower weight feeding this mixture than the standard diet ( $\chi^2 = 9.82$ ,  $\text{df} = 1$ ,  $P < 0.01$ ), and developmental time was extended by three extra weeks. Since this was the only byproduct diet which allowed a complete development of *E. kuehniella*, it was selected for the bioconversion experiment.

No larvae reached adulthood feeding *T. molitor* with raw apricots (AP-R) and raw brewer's spent grain (BG-R). When they were offered dried apricots (AP-D), dried brewer's spent grain (BG-D) or hatchery waste (HW), mortality was very high, and for this reason these byproducts were also discarded from the bioconversion experiment. Lyophilized yeast (BY-D) and the Mixture *Tm2* were also discarded since the mortality of *T. molitor* was higher when the larvae were fed with these diets, larval weight was significantly reduced ( $\chi^2 = 18.76$ ,  $\text{df} = 4$ ,  $P < 0.01$ ) and development time was longer than when they were fed with the standard diet (Table 3). When *T. molitor* larvae were fed with feed mill byproducts (FM), mortality and development time increased, but the weight of the produced larvae was similar than the weight of the larvae obtained with the standard diet. Finally, lower mortality, and similar larval weight and developmental time than with the standard diet were observed when offering the Mixture *Tm1*. Therefore, the diet of FM and the Mixture *Tm1* were finally selected for the bioconversion experiment.

Regarding *H. illucens*, the larvae of this species completed their development only when fed with raw apricots (AP-R) and the Mixture *Hi1* (Table 3). Larvae were heavier ( $\chi^2 = 8.38$ ,  $df = 2$ ,  $P < 0.01$ ) than those grown on the standard diet, and mortality was higher when they were offered only raw apricots (AP-R). Both diets were finally selected for the bioconversion because no *H. illucens* larvae survived when feeding on the other byproducts tested.

### Bioconversion of Agro-Industrial Byproducts by Insects

The larvae of *E. kuehniella* were able to efficiently bioconvert the diet with the selected mixture of byproducts (Mixture *Ek2*). Mortality was slightly, but significantly higher, and the weight of the larvae

at the end of their development was lower than when they were fed with the standard diet (Table 4). Therefore, the weight of the larvae produced per unit of substrate (Biovalorization) was significantly lower with the selected Mixture *Ek2* than with the standard diet. However, these larvae reduced a similar amount of the mixture of byproducts and, consequently, the bioconversion measured as the reduction of the substrate per larvae was higher than the obtained with the standard diet.

The larvae of *T. molitor* were able to efficiently bioconvert one of the two tested diets (Mixture *Tm1*). Mortality was higher, and the weight of the larvae at the end of their development was lower than with the standard diet (Table 4). However, the reduction of the diet and the bioconversion were similar than those obtained when offering the standard diet to the larvae. In comparison, when

**Table 3.** Mortality (%) (mean  $\pm$  SEM), larval weight (mg) (mean  $\pm$  SEM), and time needed to reach pupae and adult stage (weeks) of the three insect species tested when reared with the selected byproducts

	Mortality		Weight		Dev. time (weeks)	
	<i>n</i>	%	<i>n</i>	mg	Pupae	Adults
<i>Ephestia kuehniella</i>						
Standard diet	10	60.4 $\pm$ 2.42	7	43.5 $\pm$ 2.52	3	4
Mixture <i>Ek2</i>	10	38.4 $\pm$ 7.50	7	18.3 $\pm$ 2.47*	6	7
<i>Tenebrio molitor</i>						
Standard diet	10	8.0 $\pm$ 5.81	7	156.5 $\pm$ 4.45	11	13
BY-D	10	58.0 $\pm$ 4.77	7	125.9 $\pm$ 3.10*	19	20
FM	10	39.7 $\pm$ 3.28	7	142.4 $\pm$ 5.52	16	18
Mixture <i>Tm1</i>	10	22.8 $\pm$ 2.31	7	140.1 $\pm$ 12.07	11	13
Mixture <i>Tm2</i>	10	75.2 $\pm$ 4.61	7	99.0 $\pm$ 9.66*	16	18
<i>Hermetia illucens</i>						
Standard diet	10	21.6 $\pm$ 6.60	7	225.7 $\pm$ 10.33	3	5
AP-R	10	83.9 $\pm$ 2.87	7	309.9 $\pm$ 31.81*	3	8
Mixture <i>Hi1</i>	10	32.1 $\pm$ 7.68	7	292.0 $\pm$ 11.94*	3	5

The initial quantity of byproducts was 50 g and the number of individuals per replicate (first instar larvae) ranged between 25 and 30.

Data of larval weight followed by an asterisk (\*) within each insect species denotes significant differences between the different food commodities and the control diet (Dunn's multiple comparisons,  $P < 0.001$ ).

**Table 4.** Mortality (%) (mean  $\pm$  SEM), weight of each larvae (mg) (mean  $\pm$  SEM), substrate weight reduction (%) (standard and selected byproducts diets), reduction of substrate (mg) per larvae (Bioconversion), and production of larvae (mg) per g of substrate (Biovalorization) (mean  $\pm$  SEM) of the three insect species tested and reared with selected food commodities from the first to the last instar larvae ( $n = 9$ )

	Mortality (%)	Weight per larvae (mg)	Substrate weight reduction (%)	Bioconversion (mg)	Biovalorization (mg)
<i>Ephestia kuehniella</i>					
Standard diet	40.3 $\pm$ 3.74b	28.3 $\pm$ 3.05a	7.8 $\pm$ 1.68a	269 $\pm$ 64.9b	9.4 $\pm$ 1.29a
Mixture <i>Ek2</i>	57.6 $\pm$ 3.21a	16.4 $\pm$ 2.81b	10.8 $\pm$ 3.54a	497 $\pm$ 128.3a	3.9 $\pm$ 0.82b
	$\chi^2 = 7.74$	$\chi^2 = 6.79$	$\chi^2 = 0.63$	$\chi^2 = 4.68$	$\chi^2 = 7.99$
	$P < 0.01$	$P < 0.01$	$P = 0.43$	$P < 0.05$	$P < 0.01$
<i>Tenebrio molitor</i>					
Standard diet	9.1 $\pm$ 2.17b	187.4 $\pm$ 3.56a	13.2 $\pm$ 0.83a	349 $\pm$ 24.2a	321 $\pm$ 8.3a
FM	41.0 $\pm$ 6.63a	104.3 $\pm$ 8.05b	6.3 $\pm$ 5.56b	252 $\pm$ 213.0b	123 $\pm$ 18.9b
Mixture <i>Tm1</i>	33.8 $\pm$ 3.22a	122.2 $\pm$ 8.34b	10.1 $\pm$ 3.59ab	346 $\pm$ 103.5ab	154 $\pm$ 15.9b
	$\chi^2 = 17.23$	$\chi^2 = 17.73$	$\chi^2 = 11.69$	$\chi^2 = 8.77$	$\chi^2 = 17.55$
	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.05$	$P < 0.001$
<i>Hermetia illucens</i>					
Standard diet	16.7 $\pm$ 1.84b	220.7 $\pm$ 7.31b	57.5 $\pm$ 5.22a	1473 $\pm$ 132.3b	209 $\pm$ 4.9a
AP-R	83.6 $\pm$ 2.89a	303.0 $\pm$ 21.86a	53.9 $\pm$ 1.57a	8527 $\pm$ 1262.9a	57 $\pm$ 10.3b
Mixture <i>Hi1</i>	42.4 $\pm$ 9.85ab	312.0 $\pm$ 15.87a	14.2 $\pm$ 5.89b	396 $\pm$ 164.8c	202 $\pm$ 31.6a
	$\chi^2 = 16.97$	$\chi^2 = 13.25$	$\chi^2 = 17.29$	$\chi^2 = 21.87$	$\chi^2 = 13.77$
	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Data followed by different letters within each insect species denotes significant differences between them (Dunn's multiple comparisons,  $P < 0.001$ ).

*T. molitor* larvae were fed with feed mill byproducts (FM), mortality obtained was higher and the weight of the larvae, the substrate weight reduction, and the bioconversion per larvae at the end of their development were significantly lower than when fed with the standard diet. None of both diets performed better than the standard diet in terms of biovalorization, since they yielded a significantly lower larval weight per unit of substrate offered.

The larvae of *H. illucens* were very efficient in the bioconversion of raw apricots (AP-R). Although mortality obtained was high and comparable to the first experiment, the few surviving larvae were able to reduce a similar amount of substrate than the larvae fed with the standard diet (Table 4). Consequently, the bioconversion obtained was very high. When *H. illucens* larvae were fed with the Mixture *Hi1*, their survivorship was similar, and the weight of the larvae was higher than the one obtained with the standard diet. However, the substrate weight reduction and the bioconversion per larvae were much lower than when they were fed with the standard diet. The weight of larvae per unit of substrate was very similar with the Mixture *Hi1* and with the standard diet, and both were much higher than when larvae were fed with only apricots.

### Larvae Biomass Proximate Analysis and FAs Profile

In the case of *E. kuehniella*, the alternative diet (Mixture *Ek2*) generated a biomass with significantly less moisture than the standard diet (Table 5). Other compositional parameters, as total fat and total proteins were not affected (dry weight basis). Even if this alternative diet did not modify the total FAs content of the biomass, its FAs profile was influenced by causing the accumulation of more saturated and polyunsaturated FAs (SFA and PUFA, respectively), and less monounsaturated FAs (MUFA) than with the standard diet (Table 6). As a global effect of these changes in FAs profile, the ratio SFA/MUFA+PUFA was also slightly, but significantly higher with the alternative diet. When *T. molitor* was fed with the alternative diets, no significant effects were observed on the compositional parameters of the biomasses when compared with the standard diet. Feeding *H. illucens* larvae with raw apricots (AP-R) increased the moisture and decreased the PUFA

content in the biomass, but did not significantly change the fat and protein content in the dry matter when compared with the standard diet. The use of a byproduct mixture (Mixture *Tm1*) reduced the total protein content when compared with the standard and apricot diets, while other compositional parameters were not significantly affected.

### Discussion

Results reported in the present study illustrate how different diets had a strong influence on the preimaginal development of the three insect species tested and, consequently, how the inclusion of different agroindustrial byproducts in the diet can affect the effectiveness of the bioconversion and the composition of the larval biomass.

Of the three insects studied, only *T. molitor* was able to develop in almost all byproducts when they were offered separately, except in raw apricots (AP-R) and brewer's spent grain (BG-R). However, larvae mortality and developmental time increased greatly with many byproducts, and the weight of the larvae decreased in comparison to the larvae fed with the standard diet. In addition, the larvae of *T. molitor* were able to complete their preimaginal development when they were fed with the two mixtures of selected byproducts offered. According to the review conducted by Ribeiro et al. (2018), larvae developmental time can be very variable in this species, being on average between 112 and 203 d under controlled conditions, and the species can have between 11 and 19 instars depending on environmental conditions and food sources. For both byproduct-based diets selected in the present study to produce larval biomass, larval development time was fast, being 16 wk (112 d) (feed mill byproducts, FM) or even faster 11 wk (77 d) (Mixture *Tm1*).

In comparison to *T. molitor*, *H. illucens* was only able to complete its preimaginal development when fed with raw apricots (AP-R), but not with the other byproducts when they were offered separately. Although *H. illucens* is among the most studied insect species for bioconversion of agroindustrial byproducts (van Huis et al. 2013), in the present study it performed worse than *T. molitor* regarding their capacity to complete its preimaginal development with the byproducts tested. In addition, when the larvae of *H. illucens* were fed with raw apricots (AP-R), larvae mortality and total development time was higher than expected, being 84% and 8 wk, respectively. However, if they were fed with the standard diet and the mixture of selected byproducts, both mortality and developmental time (larvae + pupae) was slightly higher as expected for this species at temperatures of 25–30°C, 20–30%, and 5 wk, respectively. However, most studies start with larvae of 4–7 d old, whereas in the present study experiments were started with first-instar larvae. According to Tomberlin et al. (2009), the mortality of the larvae of *H. illucens* reared on a grain-based diet was between 3 and 26% in average, and the development time was around 20 d. According to Jucker et al. (2017), *H. illucens* development ranges between 5 wk when fed with a mixture of fruits and vegetables, and more than 7 wk when fed with these products separately.

*Ephestia kuehniella* was not able to complete its development or only a low proportion of individuals survived in all byproducts when offered separately. Even, in one of the mixtures of byproducts it was not able to survive neither. This species was only able to complete its development with the mixture of feed mill byproducts (mainly broken cereal grains) and brewer's spent yeast, which is compositionally very similar to the standard diet used in the present study. It is known that artificial diets are designed for a high performance of the insects and they are considered nutritionally better than natural food products (Hari et al. 2007, Borzoui et al. 2018). The

**Table 5.** Composition of protein and fat (% of dried matter) (mean  $\pm$  SEM) and moisture (% of fresh weight) of insects reared in the selected diets

	Protein (%)	Fat (%)	Moisture (%)
<i>Ephestia kuehniella</i>			
Standard diet	38.6 $\pm$ 0.57a	29.0 $\pm$ 1.81a	58.6 $\pm$ 2.50a
Mixture <i>Ek2</i>	37.3 $\pm$ 1.60a	30.2 $\pm$ 1.39a	45.2 $\pm$ 2.15b
	$\chi^2 = 2.33$	$\chi^2 = 1.19$	$\chi^2 = 3.86$
	$P = 0.13$	$P = 0.28$	$P < 0.05$
<i>Tenebrio molitor</i>			
Standard diet	48.2 $\pm$ 0.28a	36.4 $\pm$ 2.08a	42.5 $\pm$ 0.49a
FM	46.9 $\pm$ 0.40a	38.8 $\pm$ 1.31a	44.3 $\pm$ 0.61a
Mixture <i>Tm1</i>	46.3 $\pm$ 1.62a	38.1 $\pm$ 0.48a	43.7 $\pm$ 0.89a
	$\chi^2 = 5.42$	$\chi^2 = 2.22$	$\chi^2 = 5.96$
	$P = 0.06$	$P = 0.34$	$P = 0.051$
<i>Hermetia illucens</i>			
Standard diet	44.3 $\pm$ 0.21ab	31.1 $\pm$ 1.00a	61.3 $\pm$ 0.31b
AP-R	46.5 $\pm$ 0.51a	25.9 $\pm$ 1.08a	71.7 $\pm$ 3.67a
Mixture <i>Hi1</i>	39.4 $\pm$ 2.18b	27.3 $\pm$ 1.11a	64.1 $\pm$ 0.71ab
	$\chi^2 = 7.20$	$\chi^2 = 6.49$	$\chi^2 = 7.20$
	$P < 0.05$	$P = 0.051$	$P < 0.05$

Data followed by different letters within each insect species denotes significant differences between them (Dunn's multiple comparisons,  $P < 0.05$ ).



**Table 6.** Mean ( $\pm$  SEM) of total, saturated, mono-unsaturated (MUFA), and poly-unsaturated (PUFA) FAs (mg/100 mg), and proportion of saturated FAs versus MUFA plus PUFA of insects reared in selected diets

	Total FAs mg/100 mg	Saturated mg/100 mg	MUFA mg/100 mg	PUFA mg/100 mg	Saturated / MUFA+PUFA
<i>Ephestia kuehniella</i>					
Standard diet	27.92 $\pm$ 1.543a	8.63 $\pm$ 0.513b	14.70 $\pm$ 0.751a	4.59 $\pm$ 0.308b	0.45 $\pm$ 0.003b
Mixture <i>Ek2</i>	28.53 $\pm$ 1.951a	9.72 $\pm$ 0.420a	11.98 $\pm$ 1.494b	6.83 $\pm$ 0.297a	0.52 $\pm$ 0.024a
	$\chi^2 = 0.05$ $P = 0.83$	$\chi^2 = 3.86$ $P < 0.05$	$\chi^2 = 3.86$ $P < 0.05$	$\chi^2 = 3.86$ $P < 0.05$	$\chi^2 = 4.09$ $P < 0.05$
<i>Tenebrio molitor</i>					
Standard diet	31.89 $\pm$ 1.598a	8.58 $\pm$ 0.314a	16.91 $\pm$ 0.977a	6.41 $\pm$ 0.329a	0.37 $\pm$ 0.009a
FM	37.86 $\pm$ 2.712a	9.24 $\pm$ 0.841a	21.01 $\pm$ 1.320a	7.62 $\pm$ 0.564a	0.32 $\pm$ 0.009a
Mixture <i>Tm1</i>	34.36 $\pm$ 1.206a	8.91 $\pm$ 0.347a	19.08 $\pm$ 0.811a	6.38 $\pm$ 0.301a	0.35 $\pm$ 0.017a
	$\chi^2 = 6.49$ $P = 0.051$	$\chi^2 = 1.42$ $P = 0.49$	$\chi^2 = 6.45$ $P = 0.051$	$\chi^2 = 5.42$ $P = 0.07$	$\chi^2 = 5.85$ $P = 0.05$
<i>Hermetia illucens</i>					
Standard diet	27.15 $\pm$ 2.034a	20.08 $\pm$ 2.271a	3.16 $\pm$ 0.062a	3.90 $\pm$ 0.273a	2.85 $\pm$ 0.435a
AP-R	27.21 $\pm$ 0.526a	21.44 $\pm$ 0.334a	3.86 $\pm$ 0.246a	1.71 $\pm$ 0.044b	3.86 $\pm$ 0.178a
Mixture <i>Hi1</i>	23.78 $\pm$ 2.067a	17.76 $\pm$ 1.459a	3.29 $\pm$ 0.376a	2.74 $\pm$ 0.305ab	2.96 $\pm$ 0.166a
	$\chi^2 = 5.60$ $P = 0.06$	$\chi^2 = 3.82$ $P = 0.15$	$\chi^2 = 5.60$ $P = 0.06$	$\chi^2 = 7.20$ $P < 0.05$	$\chi^2 = 5.60$ $P = 0.06$

Data followed by different letters within each insect species denotes significant differences between them (Dunn's multiple comparisons,  $P < 0.05$ ).

mortality observed for this species during its larval development was higher in comparison to the other two insects, being between 40 and 60%. However, larval mortality rates reported in the literature for this species are also very variable and relatively high in some cases depending on the diet, moisture content, and temperature (Jacob and Cox 1977). The same happens to other species from the same family, such as the Indian meal moth *Plodia interpunctella* (Hübner) (Johnson et al. 1992).

In the present study, *E. kuehniella* and *T. molitor* reduce the food substrate between 250 and 500 mg per larvae. *Ephestia kuehniella* effectively consumed the offered mixture of byproducts, even in a higher amount than the standard diet. *Tenebrio molitor* was also effective in reducing the offered mixture of byproducts, but less effective in reducing the feed mill byproducts. Regarding *H. illucens*, reduction of the mixture of byproducts per larvae (400 mg approx.) was much lower than the standard diet (1,500 mg approx.). A remarkable reduction of the substrate was also observed when this species was fed with apricots (more than 8,500 mg per larva), but with a very high larvae mortality. A significant part of the reduction might be explained by the high initial water content of the substrate (almost 90% in raw apricots), of which most of it evaporated during the experiment conducted at 28°C. According to Veldkamp et al. (2012), one *H. illucens* larva can consume between 25 and 500 mg/day of substrate, depending on the food quality and moisture, as well as the larval age. Nyakeri et al. (2017) reported similar feeding rates of 100 mg of food per *H. illucens* larva per day. This represents more than 2,000 mg of food per larvae in optimal conditions during its development, with a larvae development time of 3 wk. Although consumption of byproducts per larva was not very different between species, it must be taken into account that the development of *T. molitor* was much longer, and, therefore, if a quick reduction of the byproduct is necessary, this species could be in disadvantage with the other two.

The weights of *E. kuehniella* and *T. molitor* larvae obtained when they were fed with the byproducts were much lower than with the standard diets. According to Rodríguez-Menéndez et al. (1988), at the end of the development the weight of *E. kuehniella*

varies between 22 and 30 mg per larvae when feed with different cereal grain diets. These values are in the same range to those obtained in the present study with the standard diet (28 mg). Kim et al. (2015) observed individual weights of *T. molitor* larvae in the range of 150–200 mg, both in optimal laboratory and mass rearing conditions, which is also a very similar value than the obtained in the present study with the standard diet (187 mg). Regarding *H. illucens*, larvae were heavier when they were fed with the byproducts than with the standard diets. A high range of weight values have been reported in the literature for this species, being from 117 to 158 mg per larvae when they were fed with different mixtures of bread and aquaculture wastes (Lopes et al. 2019); 299 mg with fresh human feces (Banks et al. 2014); and 550 mg with banana peeling; 780 mg with brewer's waste (Nyakeri et al. 2017). Consequently, considering the production of biomasses as amount of byproduct, the mixture of byproducts offered to *E. kuehniella* was much less efficient than the standard diet. The same was observed for *T. molitor* with both the mixture of byproducts and the feed mill byproducts, as well as for *H. illucens* with apricots. Nevertheless, bioconversion was similarly effective with the mixture of byproducts offered to *H. illucens* than with the standard diet. However, the production of biomass is generally variable, and can be optimized very much when scaling up depending on several factors, such as the density of larvae per food substrate or the provision of refuges to avoid cannibalism (Weaver and McFarlane 1990). For example, Salomone et al. (2017) reported a production of 300 kg of *H. illucens* larvae per 10 tonnes of food-waste from different sources in a pilot plant. According to Oonincx et al. (2015), *H. illucens* converted feed more efficiently than conventional production animals.

Insect biomasses have been pointed out as an interesting alternative source of protein and fat for human consumption as well as to improve the nutritional characteristics of livestock and aquaculture feeds (Govorushko 2019, Imathiu 2020). If we consider insects as a potential source of alternative protein and fat, the effects of the different byproducts-based diets on the nutritional parameters were not significant for *T. molitor* and significant for

some parameters for *H. illucens* and *E. kuehniella*. These results confirm that *T. molitor* has a good adaptive capacity to grow on different byproducts, while *E. kuehniella* and *H. illucens* seem more restricted in terms of diet composition. The proportion of protein and fat obtained for the three species are among the expected values in comparison to the values reported in the literature for these species and for other insect species of interest, being around 37–48% protein and 26–39% fat on dry weight basis (Newton et al. 1977, Yi et al. 2013, Zielińska et al. 2015, Nyakeri et al. 2017). Therefore, the larvae of these three species can be considered a good energy source because of their high protein and fat content.

The values of the ratios SFA/(MUFA+PUFA) observed in *H. illucens* biomasses (ranging from 2.85 to 3.86) indicated a much higher proportion of saturated fatty acids in comparison to *E. kuehniella* and *T. molitor* (ranging from 0.32 to 0.52), in agreement with the results of Barroso et al. (2017). According to the American Heart Association (<https://www.heart.org>), the ideal ratio of SFA/(MUFA + PUFA) in a fat source should be around 0.5 in order to be considered beneficial for human health (Hayes 2002). Moreover, there is evidence of the protective role of unsaturated fatty acids intakes against obesity and coronary heart diseases (Reis 2014, Tan 2014, Vannice and Rasmussen 2014, Shatha and Peter 2017). So, even if the percentage of unsaturated fatty acids in the larvae can be slightly modulated by modifying the diet composition (Ewald et al. 2020), *H. illucens* seems nutritionally of scarce interest as human food.

In conclusion, feeding *E. kuehniella*, *T. molitor*, and *H. illucens* with different agro-industrial byproducts and mixtures of them indicated that the results in terms of bioconversion parameters and biomass composition can vary dramatically depending on the diet composition, especially in the case of *E. kuehniella* and *H. illucens*, whose nutritional requirements seems more complex than those of *T. molitor*. *Ephestia kuehniella* provided low production of larvae per unit of substrate when fed with the agro-industrial byproducts included in this study, due to the high mortality and low weight per larvae. Nonetheless, its protein content and FAs profile suggest that the biomass could be suitable for human consumption. Good results in terms of bio-valorization parameters were obtained with *H. illucens* fed with a diet of dried brewer's spent grain, feed mill byproducts, and brewer's spent yeast (2:1:5). The biomass composition of this species with this diet was very similar to that obtained with a standard diet, and based on its FAs profile, it could be recommended for feed formulation. In the case of *T. molitor*, diets based on feed mill byproducts or mixture of dried brewer's spent grain, feed mill byproducts, and dried brewer's spent yeast (3.5:5:1) did not modify the larval biomass composition compared with a standard diet, and confirmed the suitability of this specie for human nutrition (Gasco et al. 2018). Notwithstanding, high larvae mortality and a clear decrease of the production of larvae per unit of substrate were observed with these byproducts-based diets, indicating that further studies should be carried out to guarantee their industrial viability.

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