Journal of Entomological and Acarological Research

Feeding in the adult of *Hermetia illucens* (Diptera Stratiomyidae): reality or fiction?

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Key words: black soldier fly, longevity, starvation, nutritional needs

Acknowledgement

The research was supported by the Project 'Insect Bioconversion: from vegetable waste to protein production for fish feed (INBIOPROFEED)' funded by Fondazione Cariplo (grant n° 2014-0550) and by the grant of the University of Milan Project "PROENTO - Insects: an alternative source of proteins". The Authors are also grateful to Nasim Amiresmaeili and to Giuseppe Russo for their support.

Abstract

Hermetia illucens (L.) (Diptera Stratiomyidae) is a promising species as alternative protein source for animal feed, able to convert a wide range of organic materials. The knowledge on larval biology, development, nutritional needs, and nutritional composition is rich, while few information is available on adult traits. The aim of the present study was to investigate the influence of the adult nutrition on the survival, the longevity and the ovaries development of *H. illucens*. In detail, three food sources have been compared to starvation. Water, a sucrose solution and a protein solution were given to new emerged adults and data on longevity and ovary development were acquired. Trials were conducted on single specimen and on a cohort of adults. In all the trials, starved adults survived significantly shorter than all other thesis. When adults were maintained isolated, the survival was significantly influenced by the nourishment: longevity was longer when adults were feed with a sucrose solution, while the supply of a protein source provided a lifespan significantly higher than starvation but similar to water or to sucrose solution. In cages longevity was always shorter than in isolated adults for both males and females and the overall trend was similar to single individual trials with the exception of protein solution. Ovary development of females under different nourishment did not show differences. More studies are necessary to identify a correct nutrition considering the integration of different chemical compounds to obtain optimal adult performance in terms of longevity and reproduction.

Introduction

The increasing interest in new sources of proteins has risen the attention towards different species of insects for their ability to convert rough biomass into high quality proteins for feed and food (Van Huis, 2013; Makkar *et al.*, 2014; Surendra *et al.*, 2016; Van Raamsdonk *et al.*, 2017). *Hermetia illucens* (L.) (Diptera Stratiomyidae), commonly known as the black soldier fly (BSF), is one of the most promising species as alternative protein source for animal feed due to its bioconversion ability and high protein content. This Diptera is native to the United States, but is now considered cosmopolitan for its settlement in different continents. Larvae are saprophagous and can be reared on numerous organic materials ranging from fruits, vegetables, plant material and market waste (Kalová & Borkovcová 2013; Cičková *et al.*, 2014; Jucker *et al.* 2017), to cadavers, manure, and garbage (Lord *et al.*, 1994; Myers *et al.*, 2008; Lalander *et al.*, 2013). Mature larvae have a high content of protein (40-44%), while the fat amount vary from 25 to 49% depending from the rearing diet (Makkar *et al.*, 2014). Adults of this species are not considered a pest as they do not damage agricultural crops, do not cause illness or unproductivity in cattle, they do not cause nuisance with bites and are not able to transmit human diseases (Cičková *et al.*, 2014). Moreover, *H. illucens* is a good candidate for mass rearing systems as it presents more generation per year, does not enter reproductive diapause (Myers *et al.*, 2008), and can be reared in high number as does not evidence a cannibalistic behaviour.

Literature is rich of information related to larvae and in detail to its biology, development, nutritional needs, chemical composition and the efficiency of bioconversion of the rearing substrates (Paz *et al.*, 2015; ur Rehman *et al*., 2017; Barragan-Fonseca *et al.*, 2018). On the contrary, few information is available on adult survival and performances during its lifetime. While it is well known that the quality of preimaginal feeding strongly affects the size, survival and biological traits of adult flies, some studies report that adults are incapable of feeding (Roper *et al.*, 1996; Blackmore & Lord, 2000; Tomberlin *et al.*, 2002; Gobbi *et al.*, 2013; Jucker *et al.*, 2017). However, Tomberlin *et al.* (2002) evidenced that both males and females of *H. illucens* lived longer when provided with water and Nakamura *et al.* (2016) reported that a supply of water and sugar positively extends the adult longevity.

The aim of the present study was to evaluate the influence of different nutritional sources, through an unidimensional approach (one single alimentary source) on the survival and longevity of *H. illucens* adults and to give indication about foods that can be exploited by them. In detail, the effect of nutrition

was evaluated comparing the survival of starved adults with those fed on a protein solution, a sucrose solution, or only water. Effects on single specimens (males or females) or on a cohort of males and females have been evaluated. Moreover, the influence of the different diets on ovary development was studied.

Methods

The influence on adult longevity and fertility of different nourishments provided during the adult stage was studied comparing starvation (no food), water, and a sucrose solution (1:1 volume) (trial 1). This trial was followed by a second one (trial 2) in which the comparison was extended to a protein source (meat broth), in addition to the previously tested ones. In both trials, adult survival was evaluated on isolated specimens to track single individuals. Moreover, the influence of the diet on a cohort of adults (males plus females) was set up to evaluate the combined influence of nutrition, movement and mating on survival and fertility.

Hermetia illucens Stock Culture
A laboratory rearing system for *Hermetia illucens* was started from field-collected larvae as reported in Jucker *et al.* (2017). Once emerged, males and females were placed in a cage (100 × 80 × 60 cm) at 25 ± 0.5°C, 50 ± 5% RH and natural photoperiod to mate and oviposit. Strips of corrugated cardboard (2 mm in width; three flute openings per cm) were placed in a plastic container with the bottom covered with hen feed as attractant and were provided for oviposition according to Booth and Sheppard (1984). Newly hatched larvae were then reared on hen feed mixed with water (500 g/800 ml water) and transferred in a climate chamber [T 25° ± 0.5°C, RH 60 ± 0.5%, photoperiod 12:12 (L:D)] until pupation. Pupae were then transferred into a container with wood shaving to prevent desiccation and to facilitate adult emergence.
Pupal containers obtained in the stock culture were checked daily until new adult emergence. Adults with less than 6 hours were used for all the trials. Adults were selected to be of near uniform size (mean adult length ±SE = 14.58±0.05mm). All experiments were conducted at 25±0.5°C, 50 ± 5% RH, and summer photoperiod 15:09.

Single specimen survival. To evaluate individual survival, males and females were singularly inserted into 180 mL aerated plastic container (height 12 cm, diameter 6 cm). 20 replicates per sex and thesis were prepared. Excluding no food thesis, to provide water/nutritional source *ad libitum*, a 5 mL plastic vial was inserted in each container and changed every 2 days. These vials were filled respectively with water, sucrose solution or meat broth and closed with cotton to allow the feeding of the insects and avoid the loss of liquid. Longevity was checked daily and containers with dead adults were discarded.

Cohort survival. 50 females and 50 males were inserted into each cage, a polyethylene cube with a base of 28 cm (one cage per thesis). Food or water source were provided in each cage that necessitated it, with 50 mL plastic tube containing cotton to adsorb nutritive solution and to allow insect nutrition avoiding them to die into the solution. All tubes were changed every 2 days. Longevity was checked daily and dead adults were removed from the cages and sexed.

Ovary development. The influence of the nourishment on the fertility was studied. 40 males and 80 females were inserted into four cages (one per thesis) as in cohort survival and fed *ad libitum*. Ten females were extracted from each cages at precise intervals (1, 3, 5, 7, 10, 14 days) and dissected under the stereomicroscope to evaluate the development of ovaries. The length and the width of the ovaries were measured under an optical stereoscope provided with graduated oculars (Wild Heerbrugg M5A, Leica Geosystems GmbH, Heerbrugg, Switzerland) according to Gobbi *et al.* (2013).

Statistical analyses

SPSS® Statistic (Version 24 for Windows, SPSS Inc. Chicago, IL, USA) was used to analyse all data. To test differences, prior to analyses all data were examined with the Levene's test for homogenous distribution, and with Shapiro-Wilk test for normal distribution. Kaplan-Meier survival curve analysis was also used to compare the death rate among different experimental groups in males and females. Log rank, Breslow and Tarone-Ware tests were then applied to compare survival curves.

As data on mortality were not normally distributed and Levene's test did not show variance homogeneity ($P<0.05$), they were analysed using non-parametric ANOVA Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal & Rohlf, 1995). Dunn's Post Hoc test was then applied to test for each significant factor ($P<0.05$).

Data on ovarian development were normally distributed and Levine's test showed variance homogeneity ($p>0.05$), thus they were analysed through a two-way ANOVA to evaluate the effect of the diet and of the timing from emergence. Where significant differences occurred, Tukey–Kramer's Honestly Significant Difference multiple comparisons test was applied for mean separation ($P < 0.05$) between tested diets. Considering that ovaries dimension can be influenced also by adult dimension variability (Blay & Yuval, 1999), the measure of the ovaries was related with the body length to exclude the effect of intraspecific variability.

The two-tailed Student's t-test was used to determine significant differences between male and female survival in single and cohort trials ($P < 0.05$).

Results

Single specimen survival

Adult survival rate in males, varied from 6.60±0.76 days in specimens under starvation to 32.70±1.93 in specimens fed with the sucrose solution; similarly, females leaved from 8.05±0.70 when no food was provided to 20.85±1.13 in specimens fed with the sucrose solution (Table 1).

The Scheirer-Ray-Hare test showed that the survival was significantly influenced by the nourishment (trial 1: df = 2; SS = 2419.303; $p <0.001$; trial 2: df=3; SS=2171.82; $p<0.001$), the sex (trial 1: df = 1; SS = 143; p=0.038; trial 2: df=1; SS=357; $p<0.001$), and the interaction of the two factors (trial 1: df=2; SS= 504.237; $p<0.001$; trial 2: df=3; SS=352.17 $p<0.001$). Also the Kaplan-Meier survival curves analysis followed by log rank, Breslow, and Tarone-Ware statistics evidenced significant differences among the survival according to the nutritional source provided (log-rank $P<0.001$; Breslow $P<0.001$; Tarone-Ware $P<0.001$ for all data). In both trials, longevity was significantly longer for both males and females when a sucrose solution was provided, compared to starved adults. Protein source provided a lifespan significantly higher than starvation and, according to the trial, similar to water or to sucrose solution.

Comparing the longevity between adults of the two sexes in trial 2, where also protein diet was present, Kaplan-Meier curve showed that males lived significantly longer than females only when fed with sucrose (Fig. 1) (log-rank $P<0.001$; Breslow $P<0.001$; Tarone-Ware $P<0.001$). In all the other cases, no statistical differences were observed (log-rank $P>0.001$; Breslow $P>0.001$; Tarone-Ware $P>0.001$).

Cohort Survival

Adult survival rate in the cohort varied, in males, from 7.74±0.70 days in specimens under starvation to 15.98±0.71 days in specimens fed with the sucrose solution and, in females, from 8.3±0.19 days in specimens under starvation to 18.3±1.02 days in specimens fed with the sucrose solution (Table 1).

Scheirer-Ray-Hare test showed that the adult survival was influenced by the nourishment (df=3; SS=1600.35; $p<0.001$), the sex (df=3; SS=71.458; $p<0.001$) and the interaction of the two factors (df=3; SS= 71.458; p=0.018). Also in cohort, Kaplan-Meier survival curves analysis followed by log rank, Breslow, and Tarone-Ware statistics evidenced significant differences among the survival

according to the nutritional source provided (log-rank P<0.001; Breslow P<0.001; Tarone-Ware P<0.001 for all data).
Dunn post hoc test (table 1) evidenced, both in single and cohort experiments, that starved adults survived significantly less than all other thesis. Adults fed with sucrose solution always ranked in the group with major survival. *H. illucens* fed with the protein solution, lived significantly more than specimens under starvation, but in two cases the survival ranked as the thesis with water (single males in trial 2; and females in cohort survival) while the remaining two ranked as sucrose solution (single females in trial 2 and males in cohort survival).
Kaplan-Meier curve showed that in cages males lived fewer days than females when sucrose was provided (log-rank P<0.001, Breslow P<0.001, Tarone-Ware P<0.001) (Fig 1).
Longevity in cages was always shorter than in isolated specimen for both males and females (Males: F=62.35, df=1, 280, P<0.001; Females: F= 22.54, df=1, 270, P<0.001).

Ovary development

Table 2 reports the mean value of ovary area measured in females under different nutritional sources. Overall, in all trials ovaries were visible from the first day after emergence (dimensional variability: from 1.31±0.18 mm^2 in females fed with the protein solution, to 1.61±0.15 mm^2 in females fed with sucrose solution). Ovaries reached major dimension after 3 days (from 6.6±0.95 mm^2 in females fed with sucrose solution to 4.7±0.66 mm^2 in females under starvation) and then the dimension progressively lowered down as represented by the trend lines in figure 2.
Two-way ANOVA evidenced significant differences between days (F= 8.492; df= 4, 180; p<0.005), but not between thesis (F=0.817; df=2, 180; p=0.445); nor the interaction of the two factors (F=1.902; df=5, 179; p=0.102).

Discussion

The insights acquired by this study may be of contribution to the recent literature, since they suggest the presence of a functional digestive system in *Hermetia illucens* adults. In fact, in all experiments, insects provided with any source of food or water lived longer than starved ones. Adults could still live without food for more than a week. Starvation provides an important dietary stress that tests *H. illucens* ability to mobilize stored nutrients for survival. As showed by other author (e.g Newton *et al.*, 2005; Tomberlin *et al.*, 2009), *H. illucens* adults, without food, take advantage from the fat bodies they have stored during larval instar. However, it is not a simple survival, as the adults continued to move and perform normal physiological functions, as demonstrated by the growth of ovaries for oogenesis. It is known (Keeley, 1985; Arrese & Soulages, 2010) that the fat body is a dynamic tissue that plays a role in multiple metabolic functions, among which the store and release energy in response to the demands of the insect. It is also involved in the production of storage proteins used as an amino acid reservoir for morphogenesis, lipophorins (responsible for the lipid transport in circulation), or vitellogenins for egg maturation. This last aspect is also confirmed by our results that did not attribute any influence of the diet to egg maturation in gonads. In all tested conditions, ovaries have similar dimensions, but there are significant differences in adult survival, according to the nutrition provided. Therefore, differences in egg laying during lifetime could be observed. Further researches should be focused to detail this aspect. Our data show that all tested substrates significantly extend adult survival. In particular, it is confirmed that water plays an important role in adult performances as reported by Sheppard *et al.* (2002). The longest survival was observed in presence of sucrose solution. The availability of an energy source seems to impact more on males than on females. In particular, males individually held in tubes, without possibility to fly, live significantly more than females in same conditions, while in the cohort, where adults can fly and mate, no differences were observed among male and female survivals. Therefore, the energy source seems to be mainly used by males for flight and mating, while in test tubes it is exploited only for survival.

On the contrary, the survival with protein did not furnish the same results of sucrose solution, as it was similar to the one of insects provided only with water. The protein intake therefore seems less important for the adult life, even in females who need proteins for the ovary maturation and egg development. Our data suggest that protein requirement is satisfied by the stocks accumulated during larval development. Anyway, we are aware that the animal metabolic systems require numerous nutrients simultaneously, at its individual optimal level (Raubenheimer and Simpson, 1997), and that the approach in our research is unidimentional, in order to verify the effect of a specific nutrient source.

According to Prabhu *et al.*, (2008) there are limits to the dietary composition that can be accommodated such that, beyond a certain point, performance is reduced because of deficiencies in some nutrients or the costs of processing large excesses of other nutrients. Also Cangussu & Zucoloto (1995) found that unbalanced diet has effects on performances of the females of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann).

For optimal performance insects do not only need the right amount of food, but also an equilibrium among nutrients. However, working with unbalanced diets is the first approach through the knowledge of the alimentary needs of *H. illucens* adult nutrition. The choose of a correct food, which consist of different chemical compounds (that can balance the uptake and losses of numerous compound at the same time), is necessary to study the metabolic answers in *H. illucens* similarly to other insects (Cohen, 2015). Artificial diets for insect generally contain different components that should be balanced, in detail a nitrogen source - proteins (but sometimes free amino acids) - lipids, carbohydrates, vitamins, and minerals - and also stabilizers, preservatives, and often fillers or bulking agents (Cohen, 2015).

In conclusion, our data evidenced the presence of a functional gut in *H. illucens* adults and the importance of a nutritional source to extend insect lifespan. Although a direct effect on the female fertility was not evidenced, is possible to speculate that an alimentary source could have a positive influence on the general performances of the mass rearing.

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Table1. Mean survival rate in males and females (±SE) (days) fed with different diets. Different letters in the same column mean significant differences among food sources (Dunn post-hoc test, $p<0.05$).

	Mean Survival (in days)					
	Single specimens survival				Cohort survival	
	Males (trial 1)	Males (trial 2)	Females (trial 1)	Females (trial 2)	Males	Females
Starvation	6.60±0.76 a	8.80±0.44a	8.05±0.70a	9.30±0.28a	7.74±0.70a	8.30±0.19a
Water	14.74±1.40 b	19.65±0.70 b	16.10±1.17 b	19.85±0.50 b	13.60±0.80 b	14.40±0.60 b
Sucrose solution	28.05±8.7c	32.70±1.93 c	18.65±1.26 c	20.85±1.13 c	15.98±0.71 c	18.30±1.02 c
Protein solution	-	21.10±0.76 b	-	20.30 ±1.92c	15.56±0.59 c	12.98±0.46 b

Table 2. Mean area ± SE (mm^2) of *H. illucens* ovaries under different diets. Different letters in the same column mean significant differences among days from adult emergence (Tukey's test, $p<0.05$).

Days after emergence	Starvation	Water	Sucrose solution	Protein solution
1	1.54±0.16a	1.49±0.19a	1.61±0.15 a	1.31±0.18a
3	4.70±0.66c	6.60±0.95c	5.30±0.44d	4.80±0.49c
5	3.01±0.45bc	4.40±0.84bc	3.70±0.45c	3.51±0.85c
7	4.25±0.94bc	2.52±0.58b	3.40±0.44c	3.52±0.76c
10	-	2.46±0.46b	2.16±0.23b	1.89±0.13a
14	-	2.27±0.30b	2.05±0.11b	-

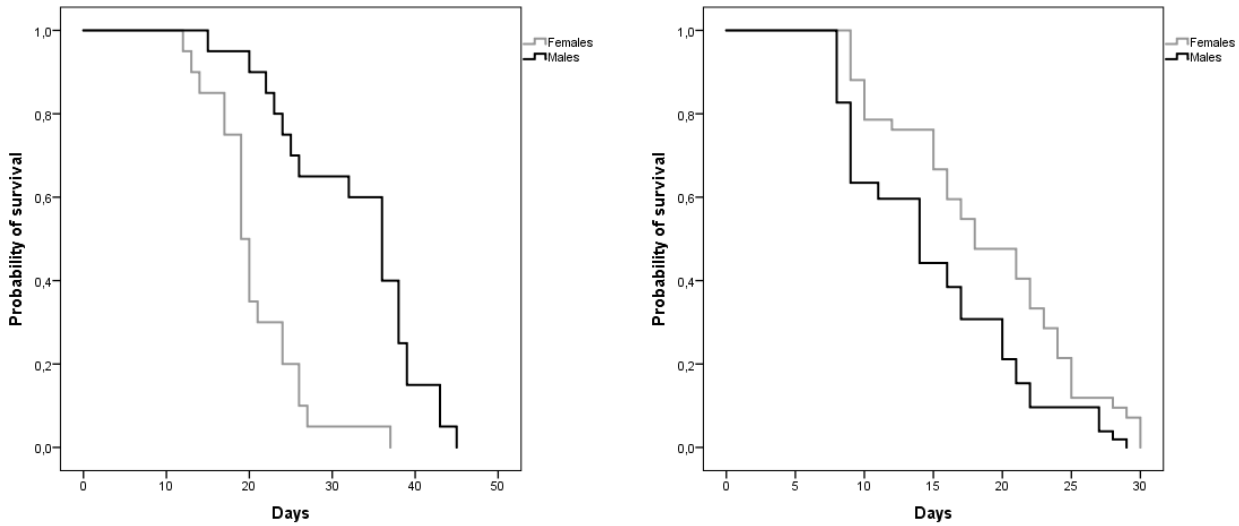

Figure 1. Kaplan-Meier curve for sex survival with sucrose solution as nourishment in single specimen trials (left) and cohort trials (right).

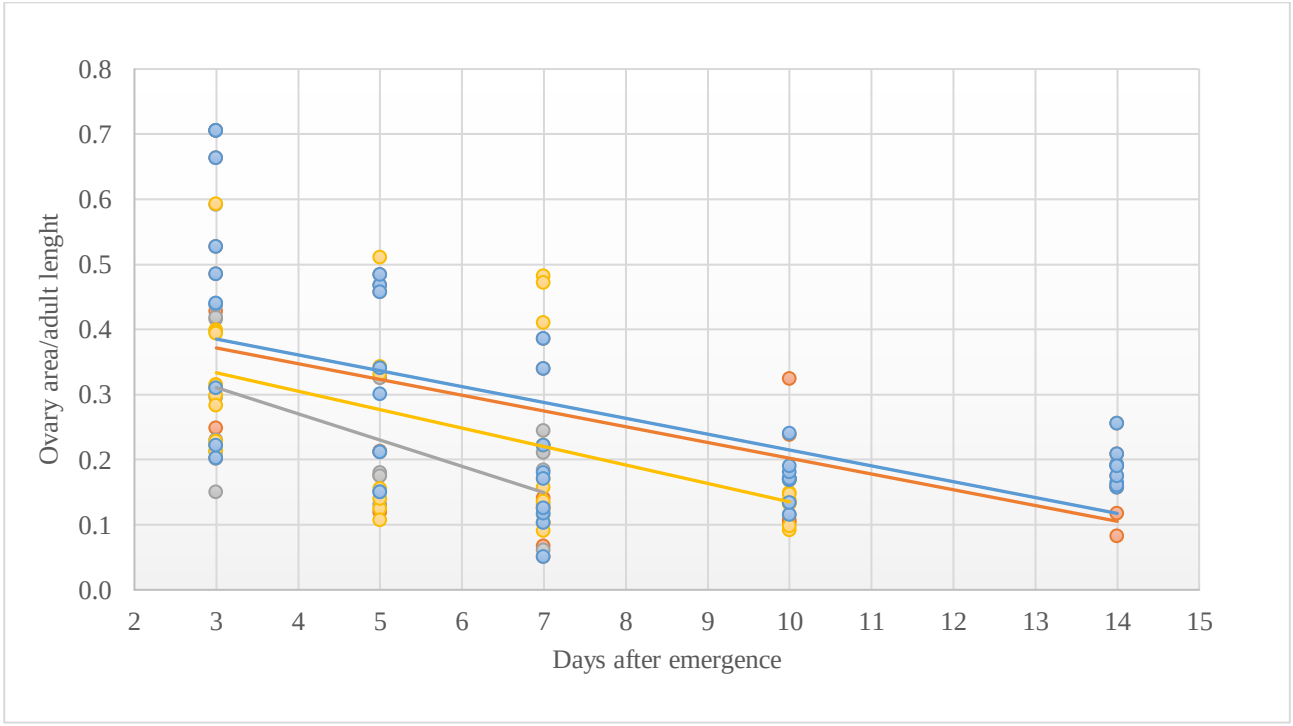

Figure 2. Trend lines of ovary/adult dimension ratio of females fed on different diets (no food=light grey; water= blu; sucrose=orange; protein=yellow) starting from the third day after emergence.