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Soy flour versus skimmed milk powder in artificial diets for *Galleria mellonella*, a factitious host for *Exorista larvarum*

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Abstract

Galleria mellonella (L.) (Lepidoptera Pyralidae) is worldwide produced and utilized for different purposes. In the entomology laboratories of the University of Bologna, Italy, this insect has long been maintained in continuous colony, and used as a factitious host for *Exorista larvarum* (L.) (Diptera Tachinidae), a polyphagous, gregarious larval parasitoid, mainly of Lepidoptera, which lays eggs on the host body. The "standard" diet used to feed *G. mellonella* larvae, which contains skimmed milk powder, white and whole wheat flour, maize flour, honey, bee wax, brewer's yeast and glycerine, has long proven to be efficient, but presents the drawback to be rather expensive. We tested the possibility to reduce diet costs by replacing skimmed milk powder (added for its protein and energy intake) with soy flour, an alternative and cheaper protein source. The development times and adult yields of *G. mellonella* were not negatively affected, when the "soy" diet was used to feed the larvae for one generation. Host acceptance by *E. larvarum* (measured as number of parasitoid eggs laid on *G. mellonella*) was higher for the larvae fed on the "soy" than on the "standard" diet. The use of soy flour in lieu of skimmed milk powder, however, proved inappropriate for the rearing of *G. mellonella* in the long run, because some quality traits, e.g. pupal weights and development times were lower in the "soy" than in the "standard" diet and further worsened through generations. Moreover, the number of eggs laid was dramatically lower for the F2 *G. mellonella* females obtained on the "soy" than on the "standard" diet. These results suggested that the costs of the artificial diet currently used for *G. mellonella* may not be lowered by replacing skimmed milk powder with soy flour on a regular basis, possibly due to nutrient imbalance.

Key words: insect rearing, greater wax moth, diet ingredients, soy flour, quality control, parasitoid, factitious host.

Introduction

The greater wax moth, *Galleria mellonella* (L.) (Lepidoptera Pyralidae) is cultured and utilised worldwide in research laboratories as a model species for studies on insect physiology (Tojo *et al*., 2000), toxicology (Moya-Andérico *et al*., 2021), pathology (Marchetti *et al*., 2009; 2012; Salari *et al*., 2015), and as factitious host for parasitoids (Campadelli, 1973a; Martini *et al*., 2019; Çim and Altuntaş, 2021). It has also been used as an alternative infection model to study diseases of human beings and livestock (Champion *et al*., 2016). The production of *G. mellonella* is of interest also in other contexts, since the larvae of this moth are well appreciated insectivore pet feed and fish baits (Finke, 2002; Madero *et al*., 2007) and are also considered good candidates as human food, or feed in animal husbandry, due to their nutrient content (EFSA, 2015). Studies on the possibility that *G. mellonella* larvae may eat polyethylene were also carried out (Bombelli *et al.*, 2017).

The rearing of *G. mellonella*, which lives in beehives and is a pest of honey bees (Kwadha *et al*., 2017), was successfully performed on a variety of artificial diets, most of which were at least partially based on ingredients contained in the larval natural food, i.e., honey and bee wax (Beck, 1960; Campadelli, 1973b; 1987; Hickin *et al*., 2021). The general objective of rearing insects on artificial diets is to obtain high yields of the produced individuals, of good quality and at reasonable price (Leppla, 2014; Cohen, 2015). These parameters, especially quality, are, however, very much related to the production aims of each insect (Grenier, 2009; van Lenteren, 2003; Portilla *et al*., 2014). At a laboratory level, where the produced larvae must be suitable for research aims, higher costs associated with rearing (including diet prize) may be more justified than in other contexts. Yet, in continuous colonies, the reduction of costs, with no adverse effects on insect quantity and quality, is an objective to be pursued.

In the entomology laboratories of the University of Bologna, *G. mellonella* has been used for decades as a factitious host for tachinid parasitoids (Mellini and Coulibaly, 1991; Coulibaly *et al*., 1993; Bratti and D'Amelio, 1994), including, in the last years, *Exorista larvarum* (L.) (Diptera Tachinidae), a polyphagous, gregarious larval parasitoid, mainly of Lepidoptera, which lays eggs on the host body (Dindo *et al*., 2003; 2021; Benelli *et al*., 2017; 2018). The diet utilized for culturing the moth larvae was developed by Campadelli (1973b; 1987) and contains skimmed milk powder, white and whole wheat flour, maize flour, honey, bee wax, brewer's yeast and glycerine (table 1). This diet has long proven to be efficient from the quantitative and qualitative point of view, but it has the inconvenience to be rather expensive. Therefore, in this work we started exploring the possibility to reduce diet costs by replacing expensive ingredients with alternative, cheaper components. We followed the traditional approach of varying only one ingredient (Cohen, 2015), which was chosen among those not included in larval natural food (i.e. bee wax and honey). The most expensive of these "non-natural" ingredients was skimmed milk powder (added for its protein and energy intake, as shown by Campadelli, 1973b; 1987) (table 1). It was replaced with an alternative, cheaper component, i.e., soy flour, which was selected due to its protein content and energy intake, even higher compared with skimmed milk (Bratti

Table 1. Composition and costs of the two diets used for the rearing of *G. mellonella* in the experiments.

Ingredients	Ingredient cost (euros/Kg)	"Standard" diet (control)		"Soy" diet	
		Ingredient amount (Kg)	Cost (euros)	Ingredient amount (Kg)	Cost (euros)
Skimmed milk powder (1)	12.80	1	12.80	-	-
Soybean flour (1)	3.60	-	-	1	3.60
White wheat flour (2)	1.24	2	2.48	2	2.48
Whole wheat flour (2)	0.79	1	0.79	1	0.79
Maize flour (2)	0.97	1	0.97	1	0.97
Brewer's yeast (1)	1.74	0.5	0.87	0.5	0.87
Beeswax (3)	14.80	0.9	13.32	0.9	13.32
Wildflower honey (2)	9.62	2	19.24	2	19.24
Glycerin (4)	3.50	1	3.50	1	3.50
Total diet cost (euros)			53.97		44.77

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and Costantini, 1992). Moreover, despite differences for lipid and carbohydrate contents (table 2), soy flour was successfully used in replacement of skimmed milk powder in food industry (Yeganehzad *et al.*, 2013). The development of *G. mellonella* on the modified vs. the standard diet was preliminary investigated for one generation in the first experiment. Acceptance and suitability for *E. larvarum* of the larvae reared on the two diets were compared in the second experiment, based on the consideration that for parasitoids, including tachinids, both parameters may be influenced by host food (Reitz and Trumble, 1997; Stoepler *et al.*, 2011). Finally, the effects of the two diets on *G. mellonella* quantity and selected quality parameters (i.e., pupal weight, development time, adult sex ratio, number of eggs laid) were studied through three generations in the third experiment.

Materials and methods

Insects

A laboratory colony of *G. mellonella* was maintained at 30 ± 1 °C, 65 ± 5% RH, 0:24 L:D photoperiod. The larvae were kept in plastic boxes (24 × 8 × 8 cm) and reared on the artificial diet developed by Campadelli (1973b; 1987) ("standard" diet, described in table 1).

To obtain eggs, about 100 cocooned mature larvae approaching pupation were placed in oviposition boxes, i.e., plastic boxes (3.5 L volume) with a 6-cm diameter hole on their lids. Holes were covered with filter paper discs which were fixed to the lids with adhesive tape. Adults emerged 5-6 days after pupation, mated and females laid eggs on the paper disc. Eggs were collected 2-3 times a week and placed in new boxes with diet, which was added every 2-3 days until the end of larval development. Adult moths mate soon after emergence (Campadelli, 1973a) and do not feed (Kwadha *et al.*, 2017).

A colony of *E. larvarum* was established in 2004 and augmented in 2010 from adults emerged from *Lymantria dispar* (L.) and *Hyphantria cunea* (Drury) (Lepidoptera Erebidae) larvae field collected in the province of Modena (44°10'49"N 10°38'54"E) and Forlì-Cesena (44°13'21"N 12°2'27"E) (Emilia-Romagna region, northern Italy). The colony was maintained as described by Dindo *et al*. (2003), using *G. mellonella* mature larvae as hosts. Parasitization occurred by exposing the host larvae to *E. larvarum* females for about 30 minutes. The fly adults were kept in Plexiglas cages (40 × 30 × 30 cm with 50-70 individuals per cage) in a rearing chamber at 25 ± 1 °C, 65 ± 5% RH, 16:8 L:D photoperiod. They were fed on sugar cubes, pollen, and distilled water via drinking troughs with soaked cotton (Dindo *et al*., 2019).

Diets

Table 1 reports the composition and costs of the "standard" and "soy" diets used for the rearing of *G. mellonella* larvae in the three experiments described below. The "standard" diet contained skimmed milk powder, which was replaced with an equal amount of soy flour in the "soy" diet. Both ingredients, intended for animal feed, had similar texture. The price of soy flour (= 3.6 euros/Kg) was about ¼ than that of skimmed milk powder (= 12.8 euros/Kg). The overall cost (table 1) was about 9 euros lower for the "soy" than for the "standard" diet, which may result in annual savings of about 100 euros if the diet is prepared about once a month (as in our laboratory). The latter cost for about 10 Kg, would be much more important for a mass production. The diets were prepared as described by Campadelli (1973b; 1987). The nutritional components of skimmed milk powder and soy flour are reported in table 2.

Table 2. Nutritional components (amount per 100 g) of soy flour and skimmed milk powder (CREA, 2019).

Component	Soy flour	Skimmed milk powder
Water (g)	7	5
Energy (kcal)	469	351
Protein (g)	36.8	33.1
Total lipid (g)	23.5	0.9
Available carbohydrates (g)	23.4	56.2
Fibre (g)	11.2	0

Development of *G. mellonella* on the "soy" vs. the "standard" diet (first experiment)

This experiment was aimed at preliminarily testing the capability of *G. mellonella* to reach the adult stage on the "soy" vs. the "standard" diet. A paper disc with newly laid (< 24 hours) eggs was removed from the laboratory colony. Two portions of the disc (of about 2 cm^2 surface each) were separately placed in two 24 × 8 × 8 cm plastic boxes, which were respectively supplied with 50 g of either "soy" or "standard" diet. The boxes with eggs and, upon hatching, larvae were maintained at 30 ±1 °C, 65 ± 5% RH, 0:24 L:D photoperiod. Every two days the boxes were checked, and a small amount of the relevant diet was added. After 15 days the larvae had a body length of 13-14 mm (corresponding to the 4^{th}-5^{th} instar according to Hosemani *et al*., 2017) and could be easily handled without damage (contrary to eggs or younger larvae). Fifty larvae per treatment were selected, transferred to new boxes, and fed *ad libitum* with the relevant diet (soy or standard) until pupation. The newly formed pupae were collected and placed in a separate box until adult emergence. Four replicates were carried out with a total of 200 larvae per diet. Results were evaluated in terms of percentages of emerged adults (based on larvae = 50 per replicate) and development time from egg to adult (in days).

Acceptance and suitability of soy-reared *G. mellonella* larvae for *E. larvarum* (second experiment)

Five *G. mellonella* mature larvae (weight range 250-300 mg), reared from the egg stage either on the "soy" diet (group 1) or on the "standard" diet (group 2), were selected. Each group of 5 larvae was separately exposed to 50-70 *E. larvarum* flies (8-10 days old) in a 40 × 30 × 30 cm Plexiglas cage, and removed after 30 minutes, as in the standard rearing conditions described above. After counting the eggs laid on each larva, the larvae with eggs were placed individually in plastic cylindric containers (4.5 cm diameter, 5.5 cm height) until puparium formation. The newly formed puparia were counted and individually placed in glass vials until adult emergence. The experiment was conducted in the climatic chamber of *E. larvarum*, at 25 ± 1 °C, 65 ± 5% RH, 16:8 L:D. Four replicates were carried out with a total of 20 larvae per group/diet. Host acceptance and suitability of soy-reared vs. standard-reared larvae were respectively evaluated in terms of number of eggs laid by *E. larvarum* females and of number and percentage of eggs producing puparia. The number and percentage of puparia emerged as adults were also calculated.

Rearing *G. mellonella* on the "soy" vs. the "standard" diet for three generations (third experiment)

This experiment was aimed at testing the possibility of rearing *G. mellonella* on the "soy" diet through generations, to reduce the costs associated with diet ingredients by replacing skimmed milk powder with the cheaper soy flour on a regular basis. The two diets (soy and standard) were tested for the rearing of three generations of *G. mellonella*, namely the parental, the F1 and F2 generations. The "parental" generation consisted of individuals obtained from eggs collected from the laboratory colony. The F1 and F2 generations respectively included individuals obtained from eggs laid by the parental and F1 generation. Each generation was started by the same methods used in the first experiment, i.e., two around 2 cm^2 portions of a paper disc with newly laid (< 24 hours) *G. mellonella* eggs were separately placed in two 24 × 8 × 8 cm plastic boxes. The eggs in each box were supplied with 50 g of either "soy" or "standard" diet. The boxes with eggs (and, subsequently, larvae) were maintained at 30 ± 1 °C, 65 ± 5% RH, 0:24 L:D photoperiod and supplied with the relevant diet every two days. After 15 days, 100 larvae (4^{th}-5^{th} instar) per treatment were selected, transferred to new boxes, and fed *ad libitum* with the relevant diet (soy or standard) until pupation. The newly formed pupae were collected, sexed, weighed, and placed singly in plastic cylinders (4.5 cm diameter × 5.5 cm height) until emergence. For the parental and F1 generation, the newly emerged males and females were transferred to oviposition boxes (described above, one per diet) to obtain eggs intended to start the new generation. Three replicates per diet per generation were carried out, each comprising 100 larvae, for a total of 600 larvae. Results were evaluated in terms of the following parameters: percentages of pupae (based on larvae = 100) and of emerged adults (based on pupae), male and female pupal weights (in mg), and development time from egg to adult (in days). The sex ratio, expressed as percentages of adult females (based on emerged adults) was also calculated.

A fecundity trial was performed with the F2 adults, to verify if, already after few generations, the number of eggs laid was affected by diet type. Ten couples of newly emerged F2 adults, either obtained from the "soy" or from the "standard" diet, were placed in plastic cylinders (6 cm diameter and 8 cm height) with a hole on their lids (1 couple per cylinder). Holes were covered with filter paper discs, where oviposition occurred. Filter papers with eggs were removed two days after the couple formation and the eggs were counted under a stereomicroscope. Each couple was considered as a replicate (10 per diet). To evaluate the results, the number of eggs laid were considered.

Statistical analysis

Statistical analysis was performed with the software STATISTICA 10.0 (StatSoft, 2010). In the first experiment, all data were analysed by one-way ANOVA. In the second, one-way ANOVA was used to analyse the eggs laid by *E. larvarum*, while the eggs that produced puparia and the puparia that emerged as adults were analysed by 2 × 2 contingency tables. Yates correction for small numbers (< 100) was applied when necessary. In the third experiment, all data (except the female development times) were analysed by a 2 × 3 factorial analysis of variance (Zar, 1984). The two factors tested were "diet" (soy and standard) and "generation" (parental, F1, F2). For "generation", means were compared using the Tukey's HSD multiple range test where significant difference ($P < 0.05$) occurred. The female development times were not analysed by factorial analysis of variance, because of variance heterogeneity. For this parameter, one-way ANOVA was used separately for the three generations. The number of eggs laid by the F2 *G. mellonella* were also analysed by one-way ANOVA.

In all experiments, variance homogeneity was tested with Levene's test and percentages were arc sine transformed for statistical analysis. When sample size was < 50, the Freeman-Tukey transformation was used, as tabulated by Mosteller and Youtz (1961).

Results

In the first experiment, the percent emergence of *G. mellonella* adults obtained on the "soy" diet was high (mean ± SE = 94.5 ± 2.5%) and not significantly different from that achieved on the "standard" diet (92.5 ± 2.5%) (F = 0.07; df = 1,6; P = 0.805). No significant difference was also found between the two diets for the development times from the placement of eggs to adult emergence (36.6 ± 0.4 days for the "soy" and 37.3 ± 0.4 days for the "standard" diet, F = 1.41; df = 1,6; P = 0.28).

In the second experiment, *E. larvarum* eggs were laid on all *G. mellonella* mature larvae, either reared on the "soy" or on the "standard" diet. The number of eggs laid, an expression of the acceptance of *G. mellonella* as host for the parasitoid, was significantly higher on the larvae reared on the "soy" diet (20.9 ± 3.7) than on the "standard" diet (13.7 ± 2.6) (F = 10.16; df = 1,6; P = 0.019).

The few data on *E. larvarum* eggs that produced puparia in the accepted *G. mellonella* larvae (a measure of host suitability) and of puparia that emerged as parasitoid adults were pooled for the four replicates. The percentage of eggs that produced puparia was significantly lower on the "soy" than on the "standard" diet (χ^2 = 8.46, df = 1, P = 0.004) (figure 1). Conversely, the percentage of puparia that emerged as adults exceeded 85%, with no significant difference between the two diets (Yates corrected χ^2 = 0.01, df = 1, P = 0.92) (figure 2).

In the third experiment (table 3), the female pupal weight was significantly affected by diet. Weights were higher on the "standard" than on the "soy" diet, whereas neither the generation effect nor the interaction were significant. For male development times, both the diet and the generation effects were significant. The time (in days) for the parental generation (grand mean = 34.2 ± 1) was significantly shorter than for the F1 (40.0 ± 3.6) and the F2 (45.5 ± 5.6) generations. The female development times (separately analysed by one-way ANOVA for the three generations) were longer on the "soy" than on the "standard" diet, although a significant difference was found only for the F1 generation (figure 3). The adult sex ratio (expressed as percentage of females) was female-biased in the parental generation (grand mean = 63.1 ± 1.5) and male-biased in the F1 (50.1.1 ± 1.1) and F2 (51.6 ± 1.1) generations. For this parameter, no significant difference was found between the F1 and F2 generation values, which were both significantly lower compared with the parental generation (Tukey HSD test). The percentages of pupae and of emerged adults and the male pupal weight were not significantly affected by either diet or generation (table 3).

The F2 females reared on the "soy" diet laid a significantly lower number of eggs (519.7 ± 66.6) than the F2 females reared on the "standard" diet (976.6 ± 7 5.8) (F = 20.49; df = 1,18; P < 0.001) (figure 4).

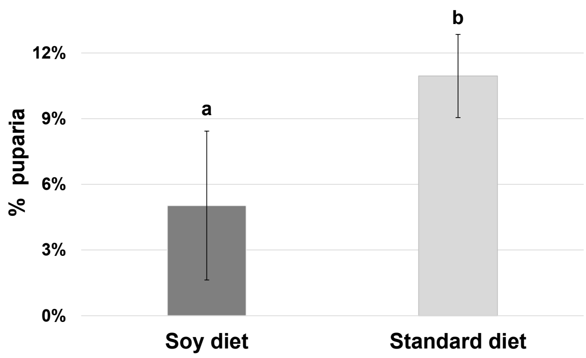

Figure 1. Suitability of soy-reared vs. standard-reared *G. mellonella* larvae for the development of *E. larvarum.* Columns indicate the percentage (± SE) of parasitoid eggs that produced puparia in the accepted larvae. Different letters above the columns indicate differences in the percentages, as determined by 2 × 2 contingency tables. Total number of *E. larvarum* eggs laid on host larvae = 417 (soy diet) and 274 (standard diet).

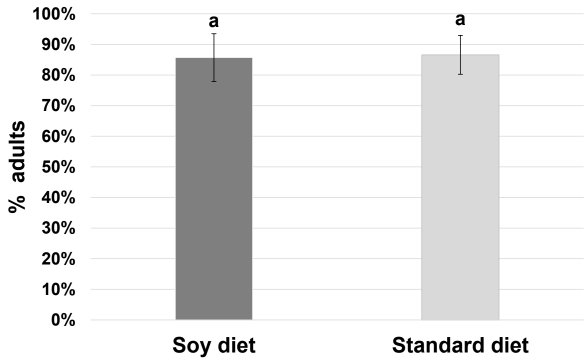

Figure 2. *E. larvarum* puparia that emerged as adults (%), obtained from soy-reared and standard-reared *G. mellonella* larvae. Means (± SE) capped with the same letter are not significantly different as determined by 2 × 2 contingency tables. Total number of *E. larvarum* puparia obtained from host larvae = 21 (soy diet) and 30 (standard diet).

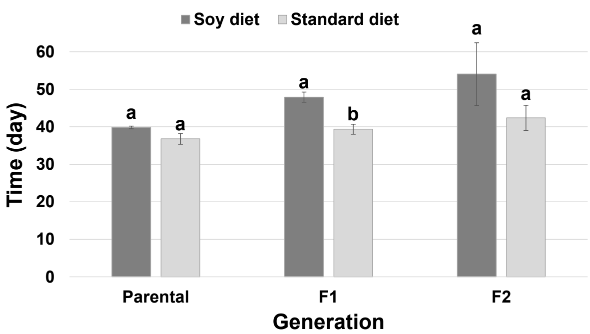

Figure 3. Female development times from egg to adult (days) of *G. mellonella* reared on the "soy" or on the "standard" diet for three generations (Parental, F1, F2). Within each generation, means (± SE) capped with the same letter are not significantly different (one-way ANOVA; P > 0.05). Number of replicates per treatment = 3.

Table 3. Percentages of pupae (based on the original number of larvae = 100), male and female pupal weights (mg), male development times from egg to adult (days) percentages of emerged adults (based on pupae), percentages of females (based on adults) of *G. mellonella* reared on the "soy" or on the "standard" diet for three generations, as related to the combination of the factors "diet" and "generation". Means (± SE). Number of replicates per treatment = 3.

Parameter	Diet	Generation			ANOVA results		
		Parental	F1	F2	Diet effect	Generation effect	Interaction
Pupae (%)	Soy Standard	98 ± 1.0 98.7 ± 1.3	98.3 ± 1.7 98.3 ± 0.7	94.7 ± 0.7 98.7 ± 0.9	F = 1.16 df = 1,12 P = 0.30	F = 1.04 df = 2,12 P = 0.38	F = 1.61 df = 2,12 P = 0.24
Male pupal weight (mg)	Soy Standard	159.8 ± 9.6 171.9 ± 20.9	154.6 ± 9.3 189.0 ± 40.1	138.8 ± 17.2 144.1 ± 4.5	F = 3.19 df = 1,12 P = 0.09	F = 3.68 df = 2,12 P = 0.06	F=0.83 df = 2,12 P = 0.46
Female pupal weight (mg)	Soy Standard	205.3 ± 9.3 225.4 ± 21.1	194.2 ± 6.2 235.8 ± 45.3	176.9 ± 26.1 194.8 ± 12.3	F = 5.49 df = 1,12 P = 0.04*	F = 2.99 df = 2,12 P = 0.09	F = 0.66 df = 2,12 P = 0.65
Male development time (days)	Soy Standard	35.03 ± 0.8 33.3 ± 0.6	43.9 ± 1.9 36.2 ± 1.8	50.2 ± 5.1 40.7 ± 2.7	F = 8.75 df = 1,12 P = 0.01*	F = 9.35 df = 2,12 P = 0.004*	F = 1.21 df = 2,12 P = 0.33
Adults (%)	Soy Standard	91.6 ± 2.1 95.9 ± 1.5	94.6 ± 1.8 89.9 ± 1.9	90.5 ± 5.4 96.6 ± 2.2	F = 0.41 df = 1,12 P = 0.54	F = 0.53 df = 2,12 P = 0.61	F = 1.23 df = 2,12 P = 0.33
Females (%)	Soy Standard	62.5 ± 2.4 63.7 ± 2.4	50.01 ± 2.5 50.2 ± 0.4	50.4 ± 2.2 52.7 ± 0.6	F = 0.43 df = 1,12 P = 0.52	F = 26.08 df = 2,12 P = 0.00004*	F =0.09 df =2,12 P = 0.92

* = $P < 0.05$.

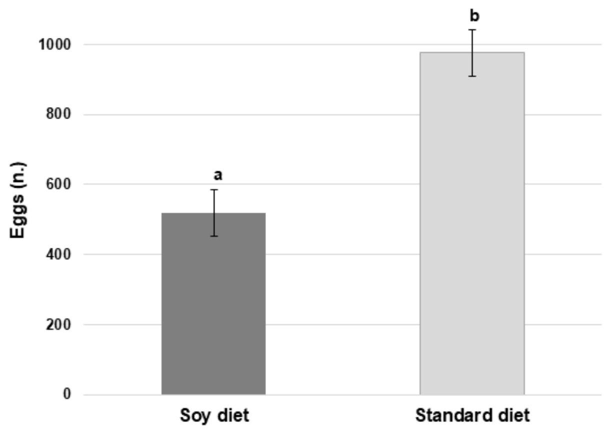

Figure 4. Mean (± SE) number of eggs laid by F2 *G. mellonella* females obtained on the "soy" diet or on the "standard" diet. Means (± SE) capped with the same letter are not significantly different (one-way ANOVA; $P > 0.05$). Number of replicates = 10, each consisting of one couple of adults.

Discussion

Soy flour was positively used as an ingredient of artificial diets for lepidopterous larvae (Brewer, 1981; Blanco *et al*., 2009). In our study, compared with the "standard" *G. mellonella* diet (Campadelli, 1987), the modified diet, containing soy flour in lieu of skimmed milk powder, did not alter either adult emergence or development time, when used to feed the larvae for one generation. Moreover, host acceptance by *E. larvarum* (measured as number of parasitoid eggs laid on *G. mellonella*) was significantly higher for the larvae fed on the "soy" than on the "standard" diet. In tachinids adopting direct oviposition strategies (e.g. laying eggs on or in the host body), short-range host location and acceptance may be mediated by physical and chemical cues (Nakamura *et al*., 2013). Cuticular components were found to elicit oviposition in *Heliothis virescens* (F.) (Lepidoptera Noctuidae) larvae by the tachinid *Eucelatoria bryani* Sabrosky (Burks and Nettles, 1978). For *E. larvarum*, which lays eggs on the host body, Depalo *et al.* (2012) showed that host-induced plant volatiles were crucial for host location, but chemical cues involved in acceptance were not investigated. It may, however, be hypothesised that oviposition was stimulated more effectively by the cuticular components of the larvae fed on the "soy" than on the "standard" diet. Soy flour is rich in lipids, potentially incorporated into cuticle, and this may consolidate this hypothesis. Host food (either plant or artificial diet) was found to influence host acceptance by hymenopterous parasitoids (Braimah and van Emden, 1994; Song *et al*., 1997). Higher host acceptance for the soy-reared larvae did not, however, result in higher suitability, as the percentage of *E. larvarum* eggs producing puparia (reflecting suitability) was lower than in the larvae reared on the "standard" diet. On both diets, however, the puparial yield was very low, probably due to the excessive number of eggs laid per host larva (the optimal number is 3-5) and, as a consequence, to excessive superparasitism, which negatively affects parasitoid development (Mellini and Campadelli, 1997). The puparial yield may increase by exposing a higher number of larvae to female flies, something that needs to be tested in future studies.

Bratti and Costantini (1992) reared *G. mellonella* on an artificial diet also based on the diet developed by Campadelli (1987), but without maize flour and whole wheat flour, and containing both soy flour and skimmed milk powder. The diet proved suitable for the rearing of *G. mellonella* for one generation, on the basis of the adult yields obtained, development times and pupal weights. Moreover, consistent with this study, the larvae obtained on the diet added with soy flour were better accepted by the parasitoid *Archytas marmoratus* (Townsend), a tachinid displaying indirect oviposition strategy whose first instar larvae, laid by females close to host, wait for host larvae to pass by to penetrate their body (Hughes, 1975). The authors suggested including soy flour in the diet for *G. mellonella* larvae intended to be used as laboratory hosts for *A. marmoratus*. However, they did not check the efficiency of the diet on *G. mellonella* development through generations.

In our study, the replacement of skimmed milk powder with the more economical soy flour proved inappropriate for the rearing of *G. mellonella* in the long run. Quantity parameters (i.e. the percentages of pupae and adults) were not affected either by diet or generation, but some quality traits, e.g. pupal weights and development times were worse in the "soy" than in the "standard" diet, and worsened through generations, though the diet effect was significant for female weights, male development times and F1 female times, and the generation effect only for male development times. What is even more important, the number of eggs laid was significantly (and dramatically) lower for the F2 females obtained on the "soy" than on the "standard" diet. These results suggested that the purpose of reducing the overall *G. mellonella* diet costs, while maintaining the same outcomes usually obtained in the "standard" diet, may not be pursued by replacing skimmed milk powder with soy flour. The diet added with soy flour was probably not optimally balanced, something that may have determined the decrease in quality occurred through generations (although skimmed milk powder or soy flour only represented 11% of the whole diet). Nutrient balance is a crucial factor determining the success of an artificial diet for insects (House, 1969; Singh, 1977; Cohen, 2015). Other ingredients in partial/total replacement of skimmed milk, such as whey or whey albumin, previously used in insect diets (Marston and Campbell, 1973; Dussutour and Simson, 2008; Huynh *et al*., 2019) can be studied to develop a cheaper diet supporting several generations of *G. mellonella*. Moreover, a different approach than varying a single ingredient may be followed. As shown by Hickin *et al*. (2021) this traditional approach often fails to show fundamental interactions among components, and different experiments strategies, including multi full factorial designs, are advisable to guide the optimization of insect diets, even when the purpose is to develop suitable substrates at lower price. *G. mellonella*, however, confirmed to be a suitable host for *E. larvarum* on both the diets used in this study, and the use of the "soy" diet for 1-2 generations may be taken into consideration, with the aim of lowering the production costs of both this factitious host and the parasitoid.

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