EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **122**: 392–403, 2025
doi: 10.14411/eje.2025.044
ORIGINAL ARTICLE

Evaluation of the efficacy of novel, meat-free and easily sterilisable artificial diet for mass rearing of *Lucilia sericata* (Diptera: Calliphoridae) larvae for medical applications

Izabela KUBASIK*, Alicja MICHALCZUK*, Jaspreet Kaur JANDOO*, Tomasz SKALSKI, Artur GÓRA and Katarzyna PAPAJ**

Tunneling Group, Biotechnology Centre, Silesian University of Technology, Krzywoustego 8, 44-100 Gliwice, Poland; e-mails: izabkub901@student.polsl.pl, alicmic468@student.polsl.pl, k_jaspreet59@yahoo.com, tomasz.skalski@polsl.pl, a.gora@tunnelinggroup.pl, katarzyna.papaj@polsl.pl

Key words. Artificial rearing, meat-free diet, medical maggot, blowfly, development, green bottle fly

Abstract. Medically significant *Lucilia sericata* larvae lack a standardised, reproducible, animal tissue-free, and sterilisable diet for rearing. In this study, we developed and evaluated a diet made from dried milk, protein powder, cholesterol, amino acids, and vitamins with a defined macronutrient composition. We demonstrated that this formulation supports larval development comparable to that of control liver diet. This diet composition was also tested with a deficiency in each ingredient to determine their role and influence on larval development. Specifically, the effects of these diets on the following traits of the fly were measured: third instar larvae length and weight, pupal weight, pupation percentage, eclosion percentage, adult longevity, and egg-laying capacity across two generations. Based on performed studies, we demonstrated that *Lucilia sericata* did not successfully develop on a diet lacking vitamins. Moreover, reduced third instar larval survivorship was observed in diets deficient in protein and cholesterol, whereas higher eclosion failure occurred in diets lacking milk, protein, and Ω-acids. These results indicate that each ingredient is important in the larval development. Overall, larvae reared on liver-based diets showed similar characteristics to those reared on artificial diets. Furthermore, subsequent generations of *Lucilia sericata* reared on this diet exhibited normal development and reproductive capacity, and their eggs produced viable offspring. This represents a potentially novel formulation, with sterilisable and readily available ingredients, and a meat-free composition that can be easily produced and used for medical applications.

INTRODUCTION

As early as the 1930s, medical flies, such as *Lucilia sericata* (*Ls*) (Meigen) (Diptera: Calliphoridae), have become one of the most effective therapeutic approaches for the treatment of chronic wounds using their wound healing activity (maggot therapy) (Baer, 1931). Maggots have the potential to fully cover the TIME acronym of successful wound healing (Schultz et al., 2004; Georgescu et al., 2016; Pritchard et al., 2016), including T – dead tissue debridement (physical and enzymatic), I – inflammation and infection – reduction of pathogenic microbes from the wound, M – moisture – creating the watering condition inside the wound, mostly from the salivary glands, and E – edging – promoting cells proliferation stimulated by growth factors produced by maggot secretions. As a result, even hard to heal wounds can be successfully treated after two months of maggot application (Pritchard et al., 2016).

In recent years, the ageing of the population has led to an increase in chronic diseases, which are the main risk factors for the development of chronic wounds. Consequently, wound treatment has become an important global socio-economic challenge. Effective management often requires a combination of therapeutic approaches, which is further complicated by the growing problem of antibiotic resistance caused by the inappropriate and excessive use of antibiotics (Liu et al., 2022). It is therefore essential to promote alternative therapies such as larval therapy, which offers multifaceted benefits, and support their broader adoption and practical implementation by healthcare professionals (Pritchard et al., 2016).

Ls larvae/maggots feed on zoonotic soft tissues; therefore, rearing and maintenance of sterile populations is a major challenge for the medical community involved in this type of therapy. *Ls* is a holometabolous insect. From oviposition to eclosion, the developmental stages of the fly involve three larval instars, proceeded by a post-feeding/wandering phase before the final moult into the pupa and finally emerging as an imago/adult. The duration from egg

* These authors contributed equally to this work.

** Corresponding author; e-mail: katarzyna.papaj@polsl.pl

to pupa is 10–14 days, where each instar is about 1 day long, and post-feeding and pupation last 4–5 days at 22°C incubation (Grassberger & Reiter, 2001). When rearing the *Ls* maggots, it is crucial to provide adequate food for the larvae from the first stage of their development and suitable environmental conditions, both for therapeutic and epidemiological reasons. Although larvae of these insects can be reared on a variety of natural foods, including beef liver, poultry liver, poultry waste, fish waste, meat, minced meat with fat, carrion, and manure, alternative foods (without non-sterile organic animal residues) are being sought for rearing (Pascacio-Villafán & Cohen, 2023). The development of an alternative food is important because the natural diet of these insects is based on decomposing meat containing pathogenic microorganisms, which can further infest the wound with serious infections. Such food tends to have an unpleasant odour, and the composition of the diet is difficult to control accurately. Moreover, the lack of information about macronutrient content of the diet makes it difficult to verify results obtained in individual laboratories (Sherman & My-Tien Tran, 1995; Shefa et al., 2013). A review of different diets, which were developed by researchers over the years, and the results of growth of medical maggots are presented in the Table 1.

A desirable artificial diet should allow optimal larval growth and viability comparable to that of larvae grown on liver while also fulfilling the following criteria: (i) Meat-free composition. The diet, which does not contain any meat or meat products, helps expunge harmful microbes from the larvae's environment. This is of utmost importance regarding patient's safety during the medical procedure; (ii) Simplicity and reproducibility. The diet should be easy to prepare, consist of well-defined, readily available, and cost-effective ingredients, and allow a standardised

Table 1. Different diets proposed by researchers for rearing fly maggots, with particular focus on the maggots of *Lucilia sericata*.

Species of fly	Diet composition	Result	Authors
Lucilia sericata	Sterile 1:1 mixture of liver and 3% bacto-agar	Proper development of larvae	Sherman & My-Tien Tran, 1995
Lucilia sericata	Purified casein, brewer's yeast powder or Fleischmann's yeast, butterfat or cod liver oil, salt mixture, cystine and 1.5% agar solution	Proper development of larvae	Michelbacher et al., 1932
	Agar containing peptone (Bacto Peptone) or bovine extract (Lemco)	Not suitable for larval growth – maggots died after few days	
	Yeast (Fleischmann's yeast and baker's yeast) and eggs (including coagulated chicken egg white), NaCl, $CaCO_3$, K_2HPO_4 K_2SO_4; KI, $FeSO_4 \cdot 7H_2O$	Not suitable for larval growth – time of developing of maggots was longer than in the control; adult flies were small	
	Technical casein mixed with a 1.5% solution of agar	Not suitable for larval growth – not many larvae reached adult stage; time of development of maggots was longer than in the control	
Lucilia sericata	Blood agar (3% sheep blood) and nutrient agar (consisting of, among other things, peptone, beef extract, and agar)	Not suitable for larval growth – time of development of maggots was longer than in the control; too low nutritional values	Borkataki et al., 2019
Musca domestica L.	Powdered milk, dried yeast and agar	Proper development of larvae	Sawicki & Holbrook, 1961
Lucilia sericata	Agar with 20% added horse blood and dried brewer's yeast	Proper development of larvae	Daniels et al., 1991
Lucilia sericata *Megaselia scalaris*	Raw chicken eggs, nutritional agar and whole milk powder	Proper development of larvae	Khatun et al., 2018
Lucilia cuprina	Whole milk powder, beef blood, chicken eggs and wheat bran	Proper development of larvae	Shefa et al., 2013
Lucilia sericata	Agar, sheep blood, liver powder, brain-heart broth, BHI, sodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, glucose and distilled water	Proper development of larvae	Rueda et al., 2010
Lucilia sericata	Whole milk powder, dried yeast, wheat germ, agar, propionic acid	Proper development of larvae	Tachibana & Numata, 2001
Lucilia sericata	Fish food	Proper development of larvae	Dehghan et al., 2020
Lucilia sericata	Lamb meat (used as control)	Proper development of larvae	Daniels et al., 1991
Lucilia cuprina	Tilapia fish (used as control)	Proper development of larvae	Shefa et al., 2013
Lucilia sericata	Liver (used as control)	Proper development of larvae	Tachibana & Numata, 2001
Lucilia sericata	Sheep meat (used as control)	Proper development of larvae	Borkataki et al., 2019

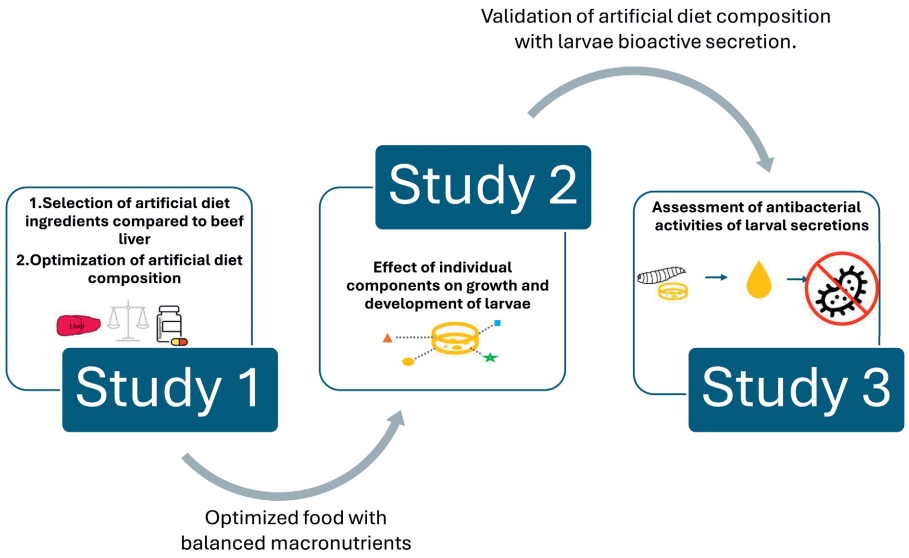


Fig. 1. Schematic representation of studies performed in this paper.

macronutrient composition; (iii) Optimal consistency. If the food is excessively liquid, the larvae may drown. In contrast, if the food has insufficient moisture content, the eggs can fail to hatch due to desiccation; and (iv) Sterility and storage. The food should remain sterile and can be safely stored at 4°C without risk of cross-contamination. Inspired by the existing studies of non-animal source ingredients, we developed a diet consisting of combinations of Mueller-Hinton Agar or agar supplemented with dried whole milk, protein, vitamins, Ω-acids, and cholesterol, which are the major components of bovine liver (traditionally used for larval feeding). The developed diet fulfils all the previously mentioned criteria. A schematic diagram in Fig. 1 shows studies performed to achieve such objective. As part of this study, we evaluated whether larvae reared this diet would develop comparably to those reared on beef liver as a control. The results obtained are presented in this study.

MATERIAL AND METHODS

Fly rearing conditions

Flies (*Lucilia sericata*) obtained from Biollab (Kędzierzyn-Koźle, Poland) were kept in rectangular cages (29.5 cm × 37 cm × 35.5 cm) covered with nylon mosquito net under 24.8 ± 1.3°C average temperature, 61.6 ± 4.3% relative humidity, and 12 h photoperiod. The adult flies were given sugar, water, and a muscle growth supplement (FitMax MASS ACTIVE 20) as food. Flies grown on such conditions and diet were able to produce eggs after three to four weeks from eclosion.

Bacteria

Bacteria used in this study include *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, and *Escherichia coli* ATCC 25922 obtained from BioMaxima (Lublin, Poland).

Egg collection and surface sterilisation

To collect eggs, a fresh beef liver trap was placed in the cages to induce oviposition in adult female flies (Fig. S1A). The trap comprises two compartments: the lower chamber contains a small piece of beef liver covered with polyamide gauze (mesh size 42 µm; Surtex, Łódź, Poland), after which the upper chamber is inserted. This upper section is lined with paper towel moistened with distilled water or PBS (phosphate-buffered saline; Eurx, Gdańsk, Poland) to maintain humidity and prevent desiccation of the eggs. Traps were retrieved after 3–4 h, once oviposition had occurred.

Laid eggs were gently removed from the mesh to sterile plastic containers. As eggs were clumped, to separate them, sterile 5 mL of 1× PBS was added to the containers and gently mixed using a 2 mL sterile Pasteur pipette until evenly separated. After separation, eggs were allowed to settle to remove debris and non-viable floating eggs from the supernatant. Then 1× PBS was replaced with 7 mL of 5% chloramine-T (Sigma-Aldrich, St. Louis, MO, USA) and the eggs were thoroughly washed for seven minutes (Wolff & Hansson, 2005). This procedure was repeated twice. The chloramine was then replaced by sterile 5 mL of 1× PBS and mixed for approximately two minutes. After that, the eggs were resuspended in a fresh portion of sterile PBS (0.5 mL eggs in 1 mL solution). Using a sterile Pasteur pipette, the desired quantity of eggs was placed in sterile plastic containers with the respective test food.

Study 1: Selection and optimisation of artificial diet composition based on larval growth and development

Material

The following ingredients were used to prepare the artificial diets: milk powder (Whole milk powder, containing 26% fat, OSM Siedlce, Poland), a high-protein supplement Protifar (Nutricia, Warsaw, Poland), vitamins B complex (MultiVit, Activlab Pharma, Cracow, Poland), Ω-3 (Activlab Pharma, Cracow, Poland), black cumin oil (source of Ω-6, GAL, Poznań, Poland), cholesterol (Sigma-Aldrich, St. Louis, MO, USA), Mueller Hinton Agar (VWR International, Leuven, Belgium), agar (Oxoid Ltd, Basingstoke, United Kingdom).

Composition of larvae diet

Five different diets (Table 2) were tested for their effects on larval development of *Ls*. The compositions were named as follows:

Control – autoclaved beef liver purée mixed with 3% agar, autoclaved at 130°C for 20 min as described by Sherman & My-Tien Tran (1995).

MHA – Mueller Hinton Agar – ready-to-use medium contains beef extract, casein hydrolysate, starch, and agar.

Table 2. Detailed composition of tested diets.

Ingredients [g]	MHA	MHA+milk +sup	MHA+milk +prot+sup	A+milk +prot+sup
Beef infusion solids	0.13	0.13	0.13	–
Casein acid hydrolysate	1.15	1.15	1.15	–
Starch	0.1	0.1	0.1	–
Agar	1.12	1.12	1.12	0.7
Ω-acids	–	0.67	0.29	0.29
Fat	–	2.6	3.6	3.6
Carbohydrates	–	3.8	5.0	5.0
Protein	–	2.6	22.4	22.4
Cholesterol	–	1.21	0.41	0.41
Vitamins B complex	–	0.07	0.033	0.033
Water	100	100	100	100

MHA+milk+sup – Mueller Hinton Agar with milk and supplements such as vitamins, Ω-acids, and cholesterol. This diet contains whole milk powder which is often included in the artificial diets described in previous publications (Sawicki & Holbrook, 1961; Daniels et al., 1991; Tachibana & Numata, 2001) as it is enriched with vitamins and cholesterol which are needed for normal larval growth (Hobson, 1935; Kadner & LaFleur, 1951; Beenakkers et al., 1985).

MHA+milk+prot+sup – Mueller Hinton Agar with milk, protein and supplements (same as above). This food contained less milk compared to MHA+milk+sup, but the protein content is similar to 100 g of beef liver.

A+milk+prot+sup – agar with milk, protein and supplements (same as above). This composition contains agar instead of MHA which allows exclusion of beef extract.

Experimental procedure

The collected eggs (approximately 50 eggs) and 40 g of food were placed in a sterile container. For ventilation purposes, a hole was made and covered with an autoclaved net (polyamide gauze, mesh size 42 μm) and sealed with industrial glue. Eggs were incubated to the pupal stage in the dark at 32°C. For each diet, five replicates were prepared for each measurement point. Samples from days 1, 2, and 5 for each type of food were flooded with 96% ethanol, and the length of the dead larvae was measured. After day 5, one dry and one-half moistened tissue with 1.5 mL PBS was added to the remaining containers as a substrate for pupation. From day 7, pupae were collected and transferred to cages.

Optimisation of the final composition of food

In the next stage of the experiment, a base medium composition (A+milk+prot+sup) was selected to ensure proper larval development, which was then optimised by modifying the content of milk, protein preparation, and agar to achieve an equilibrium between medium consistency and larval growth. The final composition of the food is presented in Table 3 (complete) and named in the further part of the experiment as A+milk+prot+sup II artificial diet (AD).

Study 2: Effect of individual components on growth and development of larvae

Composition of artificial larval diet

Six different compositions of ingredients of AD with each composition omitted one ingredient (Table 3) were tested for their effects on the larval development of *Ls*. The compositions were named as: artificial food without vitamin B complex (-vit), artificial food without whole milk powder (-milk): a diet significantly reduced in fat and carbohydrate and containing less protein than complete diet, artificial food without cholesterol (-chl), artificial food without high-protein supplement (-prot): a diet significantly reduced in protein, artificial food without Ω-acids (-Ω). As a control, complete diet, containing all the basic components of AD, i.e. fat, carbohydrate, protein, Ω-acids, cholesterol, and B vitamins was used. The ingredients for the complete diet were prepared according to the procedure described in patent application number P.448853.

Table 3. Composition of deficient diets based on selected A+milk+prot+sup II.

Ingredients [g][a]	A+milk+prot+sup II					
	-vit	-milk	-chl	-prot	-Ω	complete
Agar	1.5	1.5	1.5	1.5	1.5	1.5
Ω- acids[b]	0.40	0.35	0.40	0.40	0.05[c]	0.40
Fat	2.2	0.1[d]	2.2	2.1[c]	2.2	2.2
Carbohydrates	3.1	0.1[d]	3.1	3.0[c]	3.1	3.1
Protein	9.1	7.0[d]	9.1	2.1[c]	9.1	9.1
Cholesterol	0.41	0.4	0.01[c]	0.41	0.41	0.41
Vitamins B complex	0.0004[c]	0.0264	0.0264	0.0264	0.0264	0.0264
Water	100	100	100	100	100	100

[a] In addition, the artificial foods contained ingredients such as sodium, potassium, chloride, calcium, phosphorus, magnesium, iron, zinc, and other vitamins, among others, which were present in the milk, protein, and vitamin preparations used to prepare them. [b] Ω-3 acid (mainly EPA, DHA): 0.12 g and Ω-6 acid (mainly LA): 0.28 g. [c] These ingredients are present in milk. [d] These ingredients are present in high-protein supplement.

Briefly, agar was added to autoclaved distilled water and microwaved until completely dissolved. Then powdered milk, cholesterol, Ω-acids, and protein powder were gradually added, ensuring proper mixing and microwaving to achieve a uniform mixture. Vitamins were added to the cooled-down media, and 25 mL was poured into sterile Petri dishes. The diet was prepared under aseptic conditions. For the experiment, one ingredient was omitted, and the diet was prepared in the same manner.

Experimental procedure

50 (± 5) eggs and 10–11.5 g of the test food were placed in a sterile container bottle to enable larval growth and development until pupal stage (Fig. S1B). Eggs were incubated to pupae stage in the dark at 32°C. Three independent replicates were prepared for each diet.

On the following day 3, ten larvae were collected from each replicate. The collected larvae were transferred in 96% ethanol (Stanlab, Lublin, Poland) to kill them. The length of the dead larvae was measured using callipers. To the surviving larvae, fresh food was added along with a clean paper towel as a substrate for pupation. The moisture in the bottle was maintained using sterile 1× PBS. From day 6, pupae were collected, their length was measured using callipers, and they were transferred to larger cages. These cages were maintained under previously described conditions. Following day 14, the number of pupae, larvae, and flies in the bottles was counted and subsequently moved to larger cages.

On day 30 of the experiment, as the flies had reached sexual maturity, a beef liver trap was placed in each cage to check whether the matured flies were able to lay eggs. The collected eggs were weighed, sanitised as indicated earlier, and placed in a container with the same food treatment as the prior generation. The experiment lasted 14 days as well. The results were recorded as described above.

Study 3: Assessment of antibacterial activities of larval secretions

Collection of maggot secretion

The collected eggs were surface sterilised as described earlier, and 100 μL of eggs were dispensed onto sterile bottle (Fig. S1B)

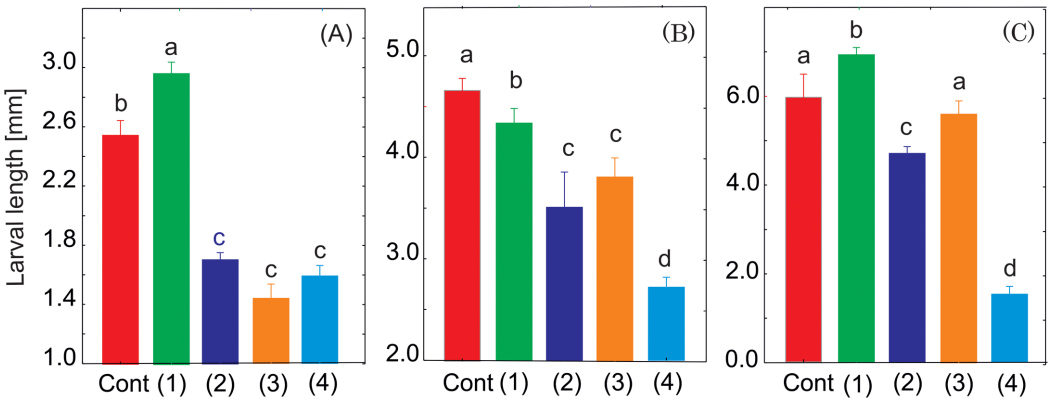


Fig. 2. Mean values of larval length of *Ls* growing on different artificial foods in consecutive days of postembryonic development. A – first day, B – second day, C – fifth day. Same letter indicates no pairwise differences according to Tuckey post-hoc test. Cont. – autoclaved beef liver; 1 – A+milk+prot+sup, 2 – MHA milk+prot+sup, 3 – MHA+milk+sup, 4 – MHA.

containing autoclaved puréed liver mixed with agar and on our formulated artificial diet AD. The bottles were prepared in five replicates. Crude secretion extract was collected by incubating maggots between second-third instar stage in sterile distilled water (0.5 mL of water per 1 g of larvae) for 3 h at 32°C in darkness. The collected extract was siphoned from replicates and centrifuged at 11,000 rpm for 10 min at 4°C to collect the supernatant, which was then filter sterilised using a sterile 0.45 µm syringe filter, followed by sterile 0.22 µm PES (Polyethersulfone, VWR International, Radnor, USA) syringe filter. The secretion extract was concentrated by vacuum evaporation and was further used for testing. The protein concentration from each secretion was estimated using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Antibacterial radial diffusion assay

20 µL of each extract was spotted onto the Mueller-Hinton-II agarose (0.75 w/v %, VWR International, Leuven, Belgium) plate seeded with normalised reference bacteria adjusted to 0.5 McFarland, taken from overnight-grown cultures in Tryptic Soy Broth (TSB, Sigma-Aldrich, St. Louis, MO, USA). The plates were allowed to dry and were transferred to an incubator for overnight incubation at 32°C. The next day, plates were observed for halos, indicating a zone of inhibition.

Statistical analysis

To assess the relationship between body length and artificial food type, a generalised linear model (GLM) with a gamma distribution and log link function was applied. The gamma distribution was chosen because the body length data were positively skewed and did not meet the assumptions of normality required for linear regression. This distribution is appropriate for continuous, strictly positive data with right skewness. The model selection was based on minimising overdispersion, confirmed by a total deviance per degree of freedom (df) always below one, and choosing models with the lowest Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) values (O'Hara, 1997). The Wald statistic was used to test the significance of the model parameters, comparing estimated coefficients against the null hypothesis that they are equal to zero. The pairwise differences in mean body length between artificial food types were assessed using Tukey's test with Bonferroni correction for multiple comparisons. GLM analyses were performed using Statistica version 13.3 software (TIBCO Software Inc.). Finally, the normalised data relative to the control (expressed as percentages) were visualised through heatmaps, non-metric multidimensional scaling (NMDS), and Similarity Percentage (SIMPER) analysis, which were conducted using PAST software (Hammer et al., 2001).

RESULTS

The results of Study 1: "Selection and optimisation of artificial diet composition based on larval growth and development" are shown in Fig. 2. From these results, it can be concluded that larvae growth response was significantly influenced by the composition (GLM, $P < 0.001$) (Table S1). On day 1, larvae fed on A+milk+prot+sup exhibited the greatest mean length, significantly surpassing the control group fed on autoclaved beef liver (larvae fed on A+milk+prot+sup were approximately 15% larger than those in control group), while other diets resulted in markedly reduced lengths (larvae were approximately 33–44% smaller than those in control group). By Day 2, larval length remained highest in control and A+milk+prot+sup. Larvae reared on liver and A+milk+prot+sup were approximately 15–22% larger than larvae reared on MHA+milk+prot+sup and approximately 40% larger than those reared on MHA. Similar trend was also observed on Day 5. These findings indicate a time-dependent effect of diet composition on larval development, with A+milk+prot+sup support superior growth and diet with MHA significantly impairing it. Larvae reared on A+milk+prot+sup were 4.5 times larger than larvae reared on MHA.

The GLM modelling was performed using larval length measurements collected on days 1, 2, and 5 from larvae reared on five different diet treatments, including our developed agar-based diet (A+milk+prot+sup) and control autoclaved beef liver purée. Larval length was modelled as a function of diet composition using a gamma distribution with log link to accommodate the data distribution. Model selection was guided by minimising overdispersion and selecting models with the lowest AIC and BIC values. The analysis revealed a highly significant effect of diet on larval growth ($P < 0.001$), with larvae on the A+milk+prot+sup diet exhibiting significantly greater mean lengths than those on other diets, including the control. These findings were further supported by pairwise Tukey tests with Bonferroni adjustments (Fig. 2).

Table 4. Comparison of food and liver nutrient composition.

Ingredients [g]	A+milk+prot+sup II (content per 100 g)	Liver (content per 100 g)
Fat	2.2	3.6–5.3
Carbohydrates	3.1	2.0–5.0
Protein	9.1	20.4–29.1
Ω-3 acid (mainly EPA, DHA)	0.12	0.01–0.23
Ω-6 acid (mainly LA)	0.28	0.17–0.44
Cholesterol	0.41	0.28–0.40
Vitamins B complex	0.026	0.024–0.300

Although the agar-based diet supported larval growth, pupation, and development into normally functioning adults, its consistency was not suitable. The use of large amounts of protein or milk made it difficult to achieve a homogeneous consistency of the feed, which tends to form lumps. Prepared food has a semi-liquid consistency and when eaten, the larvae became stuck in it, reducing their survival rate. In addition, mould growth or contamination was observed on the food when incubated with for more than 4 to 5 days with larvae at 32°C, due to distinctive odour of lactic acid fermentation. To prevent such situation fresh sterile food was added every 2 to 3 days.

As a result of the difficulties described before, work continued to develop artificial diets that would not only ensure adequate growth and development of the larvae, but would also be easy to prepare, easy to handle and have the right texture. Consequently, modified composition of tested diet namely as A+milk+prot+sup II (AD) was developed. The developed food consists of omega acids, vitamin B complex, cholesterol and carbohydrates in similar values to those found in fresh beef liver (Brust & Fraenkel, 1955; Enser et al., 1996, 1998; Williams, 2007; USDA, 2025), which is often used as larval food. However, the amount of fat and protein in the proposed diet was lower than in liver (Table 4).

The developed diet was used in another experiment to compare the growth and development of larvae fed on artificial diet and those fed on beef liver at 28°C and 32°C incubation temperatures. From this experiment, it was observed that larvae reared on AD and beef liver at 32°C did not differ significantly in size, with mean body lengths of 9.8 ± 0.94 mm and 10.2 ± 0.84 mm, respectively (n = 10). In contrast, at 28°C, larvae reared on the AD diet were smaller than those in the control group, with mean body lengths of 9.0 ± 0.71 mm and 10.4 ± 0.55 mm, respectively (n = 10).

From this experiment, it was observed also that larvae reared on an AD at 32°C started to pupate faster (within 5 days) than larvae reared on the liver (within 6 days). In addition, the number of pupae were reduced in larvae reared on the liver compared to those reared on AD. In the case of larvae reared on beef liver at 28°C, a high mortality of larvae at the third instar was observed; in addition, the larvae pupation was delayed compared to AD (2 days later) and the number of pupated individuals was reduced by half. The larvae reared on AD at 28°C pupated slower than at 32°C.

In the Study 2: "Effect of individual components on growth and development of larvae", we tested the effect of individual components of the AD on larval growth and development. The obtained results were presented in Table 5, Table S2 and Fig. 3.

Based on the results, we can conclude that the lengths of 3rd instar larvae across food groups significantly differ among the food deficiencies ($P < 0.001$) in generation G_0. The mean larval length feeding on food lacking in omega-acids (-Ω) and the complete diet have the highest values, while deficiency of powdered milk (-milk) resulted in the shortest lengths of the maggots. Wald's statistic ($\chi^2 = 148.51$) confirms strong differences among food groups. G_1 also demonstrates significant differences ($P = 0.0067$), but the variation was smaller compared to G_0. Pupa length for G_0 showed significant differences across different food deficiencies ($P < 0.001$). Food (-Ω) produced the largest pupae, while food (-milk) produced the smallest. The Wald statistic ($\chi^2 = 184.8$) supports meaningful variation in the pupal size due to food composition. In G_1, pupae size is still affected by food composition significantly ($P < 0.001$). Complete food shows the largest pupae and food (-milk) the smallest. Food (-milk) consistently results in the smallest larvae and pupa sizes across generations, indicating it may be nutritionally deficient (Brust & Fraenkel, 1955). The results indicate that the larvae's diet should provide them with adequate amounts of lipids, proteins and carbohydrates, which not only allow for proper develop-

Table 5. Comparison of larvae growth against diets with deficiencies of ingredients.

	Parameters	A+milk+prot+sup II					
		-vit	-milk	-chl	-prot	-Ω	complete
G_0	3rd instar larvae length (mm)	11.10 ± 1.71	9.11 ± 1.25	12.04 ± 0.97	10.34 ± 1.38	12.49 ± 1.64	12.75 ± 1.52
	Total larvae (30 larvae) weight (g)	1.44	0.60	1.76	0.97	1.78	1.65
	Pupa length (mm)	7.70 ± 0.46	7.02 ± 0.47	7.93 ± 0.43	7.42 ± 0.40	8.02 ± 0.39	7.88 ± 0.38
	Pupa weight (mg)	0.959	0.697	1.120	0.814	1.120	1.100
	Pupation rate (%, ± SE)	23.81 ± 5.93	59.29 ± 2.36	68.98 ± 1.00	60.93 ± 11.69	51.58 ± 3.02	58.25 ± 12.75
	Eclosion rate (% ± SE)	39.54 ± 9.97	42.95 ± 3.19	46.97 ± 0.94	48.28 ± 0.33	46.42 ± 1.10	50.10 ± 8.64
G_1	Number of eggs laid (g)	–	34.93±2.05	122.90 ± 21.38	32.63 ± 5.35	70.27 ± 28.19	75.43 ± 42.45
	3rd instar larvae length (mm)	–	10.12 ± 1.78	11.27 ± 0.87	7.85 ± 1.94	10.27 ± 2.57	11.60 ± 1.05
	Total larvae weight (g)	–	1.20	2.32	1.50	1.39	1.04
	Pupae length (mm)	–	6.58 ± 0.49	7.97 ± 0.45	7.85 ± 0.27	7.78 ± 0.42	8.10 ± 0.34
	Pupation rate (%, ± SE)	–	31.00 ± 7.19	36.5 ± 1.62	39.13[a]	43.19 ± 0.87	41.13 ± 1.73[a]
	Eclosion rate (% ± SE)	–	30.69 ± 15.37	37.04 ± 10.31	40.00[a]	42.15 ± 3.06	49.00 ± 1.00

[a] Combined replicates.

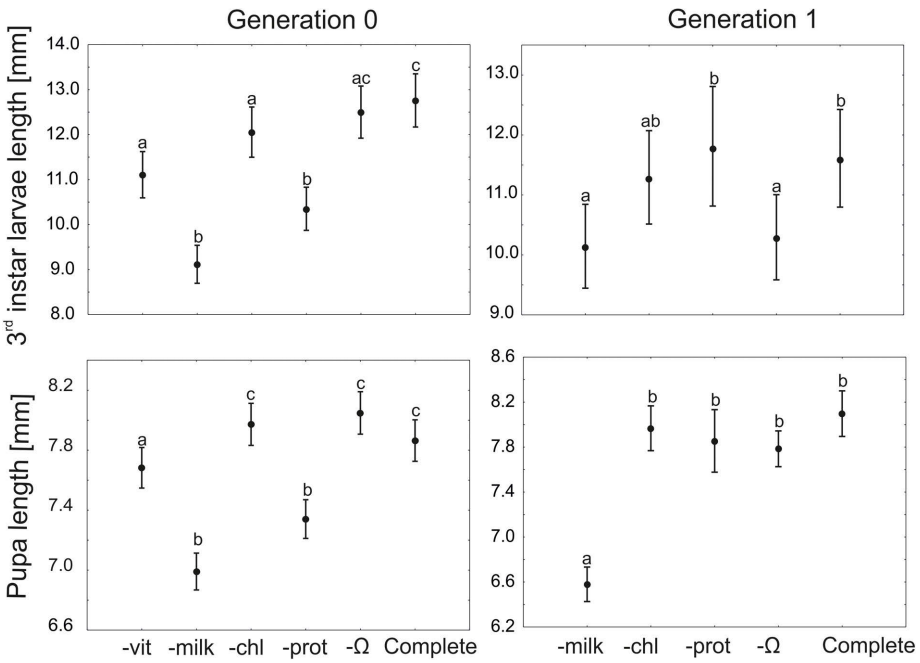


Fig. 3. The mean confidence interval (+CI) of the third instar larvae and pupa length in relation to different food deficits in the first and second generations. The letters indicate differences (Tukey HSD test result) among various diets.

ment, but also provide an adequate reservoir of substances necessary during the pupation (Beenakkers et al., 1985).

Although larger sizes of larvae and pupa from G_0 with food deficit in vitamins (-vit), the mature females failed to lay eggs for the next generation. The results obtained may indicate that the B vitamin content of the milk is sufficient for the growth of the larvae, but not for the proper development of the flies (Hobson, 1933).

The effect of each dietary component on fly development in comparison to control is shown in a statistical summary in Fig. 4, the original data with description of the calculation shown in Table S3.

In our study (Study 3) we also investigated the bioactivity of secretions from larvae fed on our AD and on control liver. The activity was determined using the radial diffusion assay from both secretions. Secretion from AD-fed larvae showed a zone of inhibition against *E. coli* ATCC 25922

(A)

Parameters	Generation	-vit	-milk	-chl	-prot	-Ω
3rd larvae length	G_0					
Pupa length	G_0					
Pupae weight (mg)	G_0					
Eclosion failure	G_0					
Adult flies survival	G_0					
Total dead flies	G_0					
Female egg-laying capacity	G_1					
3rd larvae length	G_1					
Larvae survivorship until day 4	G_1					
Pupa length	G_1					
Eclosion failure	G_1					
Adult flies survival	G_1					
Total dead flies	G_1					

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

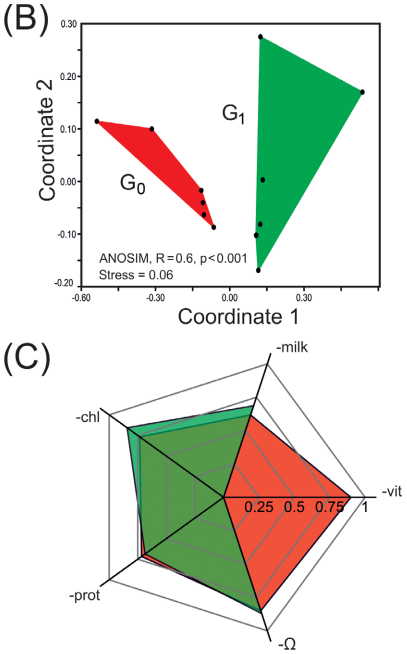


Fig. 4. A – Heat map of the influence of deficit ingredients with respect to control composition on *Ls* development across generations (G_0 and G_1). The darkest red shade indicate that lack of ingredients had higher negative impact on larvae development. Grey colour means lack of data. B – A nonmetric multidimensional scaling analysis, showing that different factors (food ingredients) are responsible for G_0 and G_1 development. C – SIMPER analysis showing that different factors (food deficiencies) are responsible for G_0 and G_1 development.

in concentrations ranging from 7 to 56 mg/mL. Activity against *P. aeruginosa* ATCC 27853 was only observed as loss of yellow-green pigment (pyoverdine) from both liver and AD secretion, as shown in Fig. S2 and Fig. S3. Notably, no activity was observed against Gram-positive bacteria from any secretion on diffusion assay.

DISCUSSION

Meat-free diets based on ingredients such as eggs, milk, fish and yeast are increasingly being developed for *Ls* larval development (Stadler & Takáč, 2022), but information on macronutrient composition, that is, % of protein, lipid, or carbohydrate, is still insufficiently documented. Knowledge of the exact composition of larval food is particularly important when comparing and verifying results obtained by different laboratories, or when experiments must be performed under controlled conditions. (Shefa et al., 2013; Sherman & My-Tien Tran, 1995). We have indicated that dried cow milk, protein powder, and supplements such as multivitamins, cholesterol, and omega acids are suitable for rearing *Ls* larvae efficiently. Larvae fed on the developed diet exhibited growth comparable to those fed on the control diet of autoclaved puréed beef liver with agar (Zheng et al., 2017; Dehghan et al., 2020).

The ingredients present in our food have similar nutritional values in comparison to fresh beef liver (which is often used as a larval food), as shown in Table 4. The proposed composition is formulated, reproducible, and independent of factors such as the quality of animal tissues, the risk of animal pathogen transmission, and rearing conditions, which may influence the liver composition (Enser et al., 1998). The downside of the liver is that it produces a strong malodour upon decomposition, and the presence of probable toxins present in the liver may inhibit larval growth (Hernández-Herrero et al., 1999; Clark et al., 2006; Kharbouche et al., 2008). Additionally, liver is prone to microbial contamination, making it less suitable for applications where sterility is critical – such as medical maggot therapy and experimental investigations; due to the risk of transmitting pathogenic bacteria to the patient's body (Sherman & My-Tien Tran, 1995; Zhang et al., 2009). Autoclaving meat helps reduce contamination; however, this process can lead to protein coagulation, potentially reducing its bioavailability to larvae (Fine & Alexander, 1934).

We also evaluated the effects of individual nutrients as dietary deficiencies on larval growth dynamics and development. Among the tested components, vitamin deficiency produced the most severe adverse outcomes, followed by deficiencies in milk, protein, cholesterol, and omega-3 acids (Table 5). These findings are consistent with previous studies emphasising the essential roles of lipids, cholesterol, proteins, and vitamins in insect development (Brust & Fraenkel, 1955; Beenakkers et al., 1985; Barragan-Fonseca et al., 2018). Vitamin deficiency has the most pronounced negative impact on metabolic processes, energy production, and cellular function of maggots (Hobson, 1933; Brust & Fraenkel, 1955; Tachibana & Numata, 2001), and studies utilising dried yeast, a rich source of B vitamins, have supported the influence of B vitamins on larval growth and reproductive success. Larvae reared on this diet exhibit significantly enhanced development compared to those maintained on a diet deficient in B vitamins (Tachibana & Numata, 2001). Our findings also confirm that B vitamins are essential for the development and reproduction of *Ls* larvae because larvae reared on food without vitamins were significantly smaller than those fed on the liver diet. Additionally, the mortality rate was the highest among all presented diets (Table 5), and the fecundity of adult females was greatly limited in the next generation.

Another food ingredient that had an essential effect on larval growth was whole milk powder, which was selected as the primary ingredient in the formulated diet because of its high fat and protein content, particularly albumin and sterols, which are critical for larval growth and development (Shefa et al., 2013; Polat et al., 2024). Milk-deficient diets resulted in significantly smaller third-instar larvae and pupae, along with reduced pupal weight. These findings are in agreement with observations reported by Barragan-Fonseca et al. (2018). Although milk contains many components that are essential for the development of larvae, it is not possible to use it as a complete food (Zheng et al., 2017). The powdered milk used in our experiments contained small amounts of B vitamins (0.005%) and cholesterol (0.1%). Both parameters are crucial for the proper development of larvae (Brust & Fraenkel, 1955; Tachibana & Numata, 2001). In our study, we found that the amount of cholesterol provided in the milk powder was sufficient to increase larval growth close to that of the control, but not sufficient to ensure adequate health and fitness in adult flies. In addition, the amount of protein provided as a component of the milk used in preparing the food was found to be insufficient. The diet containing only milk together with supplements had a negative effect on larval development; the larvae and third instar pupae were significantly smaller, losing their weight (Daniels et al., 1991). The lack of protein also affects the fecundity of flies. Wall et al. (2002) showed that an adequate supply of protein at the larval stage affects the ability of adult females to lay eggs. In our case, we supplemented the protein content using protein powder made from concentrated cow's milk to match the amounts typical of animal tissues (Zarkadas et al., 1996). Replacing a part of the milk in the developed feed formula with a high-protein product not only improved the nutritional value of the feed, which had a positive effect on the condition of the larvae, but also improved the artificial food consistency (photos shared in the repository DOI:10.5281/zenodo.15302601). An adequate amount of moisture is also crucial to avoid drowning of larvae in excessively liquid food and desiccation of eggs or dry food with poor moisture. Moisture is one of the most critical factors for the survival of *Ls* in the early stages of the life cycle (Davies & Hobson, 1935). The negative effect of liquid food on the development of *Ls* flies was confirmed by Byrd (2009), who noted that chicken liver is an unsuitable growth medium for larvae because it easily liquefies as it decomposes. Extremely high humidity of the substrate is

also unsuitable, as it increases the time of larval development (Kökdener & Şahin Yurtgan, 2022). The posterior spiracles at the posterior end of the larvae enable the larvae to regulate oxygen exchange in a semi-liquid environment (Keilin, 1994; Magni et al., 2021).

To enhance the solid consistency of the food, we added agar. Sherman & My-Tien Tran (1995) applied a similar solution to ensure the optimal consistency of the liver during autoclaving. The fluid consistency of agar after autoclaving also allows the preparation of diets containing nutrients or ingredients essential for larval development that are sensitive to the autoclaving process (McCollum & Davis, 1915; Yeung et al., 2006). These ingredients only need to be added to the agar before it solidifies.

In our procedure, the diet was prepared in a laminar airflow chamber using autoclaved distilled water with agar, with brief microwave heating to maintain sterility. Prepared this way, the food remains sterile and nutritionally available to larvae, which might otherwise be lost during heat sterilisation (McCollum & Davis, 1915). Such readily usable food can be stored for extended periods at 4°C while maintaining sterility for a month. This is a substantial advantage, especially when continuous mass production of larvae is required.

In summary, the proposed meat-free sterile diet offers a compelling option for both healthcare providers and researchers. It is nutritionally complete, pathogen-free, and can be prepared easily (within 1–2 h) and stored, making it useful in medical wound debridement therapy and controlled experimental settings. Additionally, the ingredients necessary for its preparation are readily available, inexpensive (the cost of feeding 100 larvae from the 1st instar to the pupa is 0.6 USD), and compliant with food-grade or dietary supplement standards. Moreover, in our research, larvae maintained on this diet retained the capability to produce biologically active secretions. Which leads us to believe that the developed food may find future application for the rearing and maintenance of fly larvae lines in health and research facilities or supply units.

ACKNOWLEDGEMENTS. This research was funded in whole by National Science Centre, Poland (Grant number 2021/43/D/NZ7/03119). For Open Access, the author has applied a CC-BY public copyright licence to any Author Accepted Manuscript (AAM) version arising from this submission.

STATEMENTS AND DECLARATIONS. The authors have no conflicts of interest to declare. I. Kubasik: performing the experiment, methodology, validation, investigation, resources, writing – original draft, writing – review and editing. A. Michalczuk: performing the experiment, methodology, validation, investigation, resources, writing – original draft, writing – review and editing. J.K. Jandoo: conceptualization, performing the experiment, data analysis, methodology, validation, investigation, resources, writing – original draft, writing – review and editing. T. Skalski: methodology, statistical analysis, writing – original draft, writing – review and editing resources. A. Góra: conceptualization, visualisation, writing – review and editing. K. Papaj: conceptualisation, methodology, visualisation, project administration, writing – original draft, supervision.

REFERENCES

Baer W.S. 1931: The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). — *J. Bone Jt. Surg. Am.* **13**: 438–475.

Barragan-Fonseca K.B., Dicke M. & van Loon J.J.A. 2018: Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). — *Entomol. Exp. Appl.* **166**: 761–770.

Beenakkers A.M.T., Van der Horst D.J. & Van Marrewijk W.J.A. 1985: Insect lipids and lipoproteins, and their role in physiological processes. — *Prog. Lipid Res.* **24**: 19–67.

Borkataki S., Katoch R. & Goswami P. 2019: Developmental time variation of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) colonization in two bacteriological culture media vs red meat of sheep. — *J. Entomol. Zool. Stud.* **7**: 1222–1225.

Brust M. & Fraenkel G. 1955: The nutritional requirements of the larvae of a blowfly, *Phormia regina* (Meig.). — *Physiol. Zool.* **28**: 186–204.

Byrd J.H. 2009: Laboratory rearing of forensic insects. In Byrd J.H. & Castner J.L. (eds): *Forensic Entomology: The Utility of Arthropods in Legal Investigations.* CRC Press, Boca Raton, pp. 121–143.

Clark K., Evans L. & Wall R. 2006: Growth rates of the blowfly, *Lucilia sericata*, on different body tissues. — *Forensic Sci. Int.* **156**: 145–149.

Daniels S., Simkiss K. & Smith R.H. 1991: A simple larval diet for population studies on the blowfly *Lucilia sericata* (Diptera: Calliphoridae). — *Med. Vet. Entomol.* **5**: 283–292.

Davies W.M. & Hobson R.P. 1935: The relationship of humidity to blowfly attack. — *Ann. Appl. Biol.* **22**: 279–293.

Dehghan O., Tabaie S.M., Rafinejad J., Azarmi S., Froooshani A.R., Rafinejad A. & Akbarzadeh K. 2020: Estimation of life expectancy and measurement of immature stages of *Lucilia sericata* fed on three kinds of diets. — *J. Shahrekord Univ. Med. Sci*. **22**: 121–125.

Enser M., Hallett K., Hewitt B., Fursey G.A.J. & Wood J.D. 1996: Fatty acid content and composition of English beef, lamb and pork at retail. — *Meat Sci*. **42**: 443–456.

Enser M., Hallett K.G., Hewett B., Fursey G.A.J., Wood J.D. & Harrington G. 1998: The polyunsaturated fatty acid composition of beef and lamb liver. — *Meat Sci.* **49**: 321–327.

Fine A. & Alexander H. 1934: Maggot therapy: technique and clinical application. — *J. Bone Jt. Surg.* **16**: 572–582.

Georgescu M., Marinas O., Popa M., Stan T., Lazar V., Bertesteanu S.V. & Chifiriuc M.C. 2016: Natural compounds for wound healing. In Fonseca C. (ed.): *Worldwide Wound Healing – Innovation in Natural and Conventional Methods*. InTech, London, pp. 61–89.

Grassberger M. & Reiter C. 2001: Effect of temperature on *Lucilia sericata* (Diptera: Calliphoridae) development with special reference to the isomegalen- and isomorphen-diagram. — *Forensic Sci. Int.* **120**: 32–36.

Hammer Ø., Harper D.A.T. & Ryan P.D. 2001: PAST: Paleontological statistics software package for education and data analysis. — *Palaeontol. Electron.* **4**(1): 4, 9 pp.

Hernández-Herrero M.M., Roig-Sagués A.X., López-Sabater E.I., Rodríguez-Jerez J.J. & Mora-Ventura M.T. 1999: Influence of storage temperature on the quality of beef liver; pH as a reliable indicator of beef liver spoilage. — *J. Sci. Food Agric*. **79**: 2035–2039.

Hobson R.P. 1933: Growth of blow-fly larvae on blood and serum: Response of aseptic larvae to vitamin B. — *Biochem. J.* **27**: 1900–1909.

Hobson R.P 1935: On a fat-soluble growth factor required by blow-fly larvae: Identity of the growth factor with cholesterol. — *Biochem. J.* **29**: 2023–2026.

Kadner C.G. & LaFleur F.M. 1951: The vitamin requirements of *Phaenicia sericata* (Meigen) larvae (Diptera: Calliphoridae). — *Wasmann J. Biol.* **9**: 129–136.

Keilin D. 1944: Respiratory systems and respiratory adaptations in larvae and pupae of Diptera. — *Parasitology* **36**: 1–66.

Kharbouche H., Augsburger M., Cherix D., Sporkert F., Giroud C., Wyss C. & Mangin P. 2008: Codeine accumulation and elimination in larvae, pupae, and imago of the blowfly *Lucilia sericata* and effects on its development. — *Int. J. Leg. Med.* **122**: 205–211.

Khatun R., Mostafa G., Haque E., Ahmad S. & Akter T. 2018: Formulation and evaluation of the efficacy of an artificial diet for two forensically important flies. — *Asian Australas. J. Biosci. Biotechnol.* **3**: 86–92.

Kökdener M. & Şahin Yurtgan M. 2022: The effect of soil type and moisture level on the development of *Lucilia sericata* (Diptera: Calliphoridae). — *J. Med. Entomol.* **59**: 508–513.

Liu Y.-F., Ni P.-W., Huang Y. & Xie T. 2022: Therapeutic strategies for chronic wound infection. — *Chinese J. Traumatol.* **25**: 11–16.

Magni P.A., Senigaglia V., Robinson S.C. & Dadour I.R. 2021: The effect of submersion in different types of water on the survival and eclosion of blow-fly intra-puparial forms (Diptera: Calliphoridae). — *Forensic Sci. Int.* **319**: 110663, 11 pp.

McCollum E.V. & Davis M. 1915: The cause of the loss of nutritive efficiency of heated milk. — *J. Biol. Chem.* **23**: 247–254.

Michelbacher A.E., Hoskins W.M. & Herms W.B. 1932: The nutrition of flesh fly larvae, *Lucilia sericata* (Meig.). — *J. Exp. Zool.* **64**: 109–131.

O'Hara H.R.J. 1997: Fitting generalized linear models to retrospectively sampled clusters with categorical responses. — *Can. J. Stat.* **25**: 159–174.

Pascacio-Villafán C. & Cohen A.C. 2023: How rearing systems for various species of flies benefit humanity. — *Insects* **14**: 553, 29 pp.

Pritchard D.I., Čeřovský V., Nigam Y., Pickles S.F., Cazander G., Nibbering P.H., Bültemann A. & Jung W. 2016: TIME management by medicinal larvae. — *Int. Wound J.* **13**: 475–484.

Rueda L.C., Ortega L.G., Segura N.A., Acero V.M. & Bello F. 2010: *Lucilia sericata* strain from Colombia: Experimental colonization, life tables and evaluation of two artificial diets of the blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae), Bogotá, Colombia strain. — *Biol. Res.* **43**: 197–203.

Sawicki R.M. & Holbrook D.V. 1961: The rearing, handling and biology of house flies (*Musca domestica L.*) for assay of insecticides by the application of measured drops. — *Pyrethrum Post* **6**: 3–18.

Schultz G.S, Barillo D.J., Mozingo D.W. & Chin G.A. 2004: Wound bed preparation and a brief history of TIME. — *Int. Wound. J.* **1**: 19–32.

Shefa K., Hossain M.M., Islam M.H., Islam A.T.M.F. & Saifullah A.S.M. 2013: An artificial larval diet for blowfly, *Lucilia cuprina* (Diptera: Calliphoridae). — *J. Entomol. Zool. Stud.* **1**: 99–102.

Sherman R.A. & My-Tien Tran J.M. 1995: A simple, sterile food source for rearing the larvae of *Lucilia sericata* (Diptera: Calliphoridae). — *Med. Vet. Entomol.* **9**: 393–398.

Stadler F. & Takáč P. 2022: Medicinal maggot production. In Stadler F. (ed.) *A Complete Guide to Maggot Therapy: Clinical Practice, Therapeutic Principles, Production, Distribution, and Ethics*. Open Book Publishers, Cambridge (UK), pp. 289–330.

Tachibana S.I. & Numata H. 2001: An artificial diet for blow fly larvae, *Lucilia sericata* (Meigen) (Diptera: Calliphoridae). — *Appl. Entomol. Zool.* **36**: 521–523.

USDA 2015: *Composition of Foods Raw, Processed, Prepared USDA National Nutrient Database for Standard Reference, Release 28. Documentation and User Guide.* Agricultural Research Service, Nutrient Data Laboratory, URL: http://www.ars.usda.gov/ba/bhnrc/ndl (last accessed Apr. 2025).

Wall R., Wearmouth V.J. & Smith K.E. 2002: Reproductive allocation by the blow fly *Lucilia sericata* in response to protein limitation. — *Physiol. Entomol.* **27**: 261–266.

Williams P. 2007: Key nutrients delivered by red meat in the diet. — *Nut. Diet.* **64**: S113–S119.

Wolff H. & Hansson C. 2005: Rearing larvae of *Lucilia sericata* for chronic ulcer treatment – an improved method. — *Acta Derm. Venereol.* **85**: 126–131.

Yeung C.Y., Lee H.C., Lin S.P., Yang Y.C., Huang F.Y. & Chuang C.K. 2006: Negative effect of heat sterilization on the free amino acid concentrations in infant formula. — *Eur. J. Clin. Nutr.* **60**: 136–141.

Zarkadas G.C., Karatzas C.D. & Zarkadas C.G. 1996: Assessing the myofibrillar and connective tissue protein contents and protein quality of beef tripe. — *J. Agric. Food Chem.* **44**: 2563–2572.

Zhang B., Numata H., Mitsui H. & Goto S.G. 2009: A simple, heat-sterilizable artificial diet excluding animal-derived ingredients for adult blowfly, *Lucilia sericata*. — *Med. Vet. Entomol.* **23**: 443–447.

Zheng L., Crippen T.L., Dabney A., Gordy A. & Tomberlin J.K. 2017: Evaluation of sterilized artificial diets for mass rearing the *Lucilia sericata* (Diptera: Calliphoridae). — *J. Med. Entomol.* **54**: 1122–1128.

Received May 13, 2025; revised and accepted October 29, 2025
Published online December 19, 2025

Table S1. Results of generalised linear modelling of maggot's length variation in relation to quality of artificial diet.

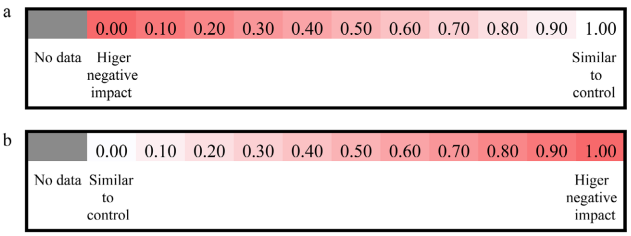
	Variables	df	Wald stat.	*P*
First day	Intercept	1	2305.92	< 0.001
	Artificial diet	5	692.99	< 0.001
Second day	Intercept	1	19071.72	< 0.001
	Artificial diet	5	603.04	< 0.001
Third day	Intercept	1	18744.68	< 0.001
	Artificial diet	5	2011.97	< 0.001

Table S2. Generalised linear modeling results of third instar larvae and pupae length analysis through significance comparison in G_0 and G_1 generations.

	Dependent variable	Independent variable	SS	df	MS	F	*P*	Wald stat.	Significance
G_0	3rd instar larvae length	Intercept	22997.76	1	22997.76	11183.2	< 0.001	62730.13	Significant
		Artificial diet	294.81	5	58.96	28.67	< 0.001	148.51	Significant
	Pupae length	Intercept	13338.39	1	13338.39	73316.22	< 0.001	307923.2	Significant
		Artificial diet	31.92	5	6.38	35.09	< 0.001	184.8	Significant
G_1	3rd instar larvae length	Intercept	16402.82	1	16402.82	5194.923	< 0.001	20885.22	Significant
		Artificial diet	62.6	4	15.65	4.956	< 0.001	14.2	Significant
	Pupae length	Intercept	5291.379	1	5291.379	28743.47	< 0.001	114354.9	Significant
		Artificial diet	33.181	4	8.295	45.06	< 0.001	193.3	Significant

Table S3. Heatmap calculation. Normalised data to control data value in percentage was divided by maximum value in each parameter. The darkest red shade indicates lack of ingredients that had higher negative impact on larvae development. Grey colour means lack of data. Shades of red were achieved using MS Excel conditional colour scale formatting. The numeric scale is from value 0 to 1 with 0.1 minor scale. Scale[a] describes situations where the highest value is desirable and close to the control value. Scale[b] describes a situation where the high ratio is an undesirable situation and differs from the control value.

Parameters	Generation	-vit	-milk	-chl	-prot	-Ω
3rd larvae length[a]	G_0	0.89	0.73	0.96	0.83	1.00
Pupa length[a]	G_0	0.95	0.90	1.00	0.92	1.00
Pupae weight (mg)[a]	G_0	0.86	0.62	1.00	0.73	1.00
Eclosion failure[b]	G_0	1.00	0.24	-	0.41	0.63
Adult flies survival[a]	G_0	0.56	0.94	1.00	0.88	0.88
Total dead flies[b]	G_0	1.00	0.43	0.43	0.57	0.57
Female egg-laying capacity[a]	G_1	–	0.28	1.00	0.26	0.57
3rd larvae length[a]	G_1	–	0.86	0.96	1.00	0.87
Larvae survivorship until day 4[b]	G_1	–	0.96	0.81	0.56	1.00
Pupa length[a]	G_1	–	0.83	1.00	0.99	0.98
Eclosion failure[b]	G_1	–	0.75	0.62	1.00	0.77
Adult flies survival[a]	G_1	–	1.00	0.94	0.88	0.81
Total dead flies[b]	G_1	–	0.00	0.57	0.00	1.00



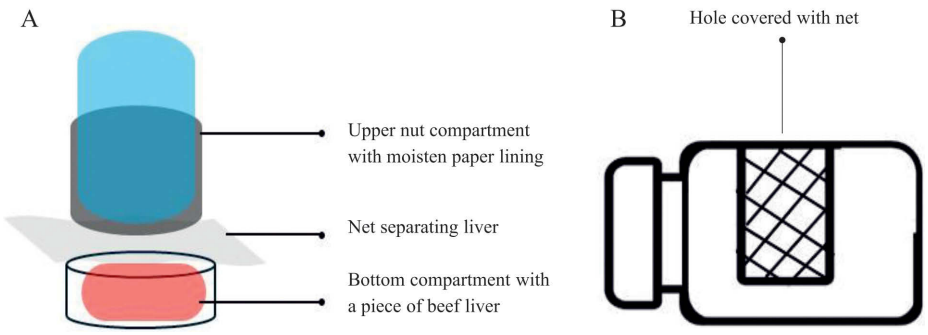
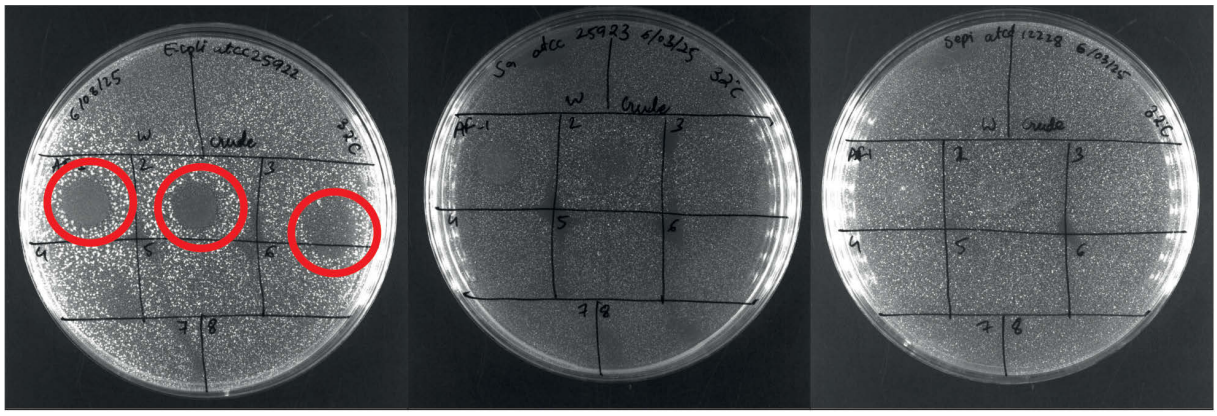
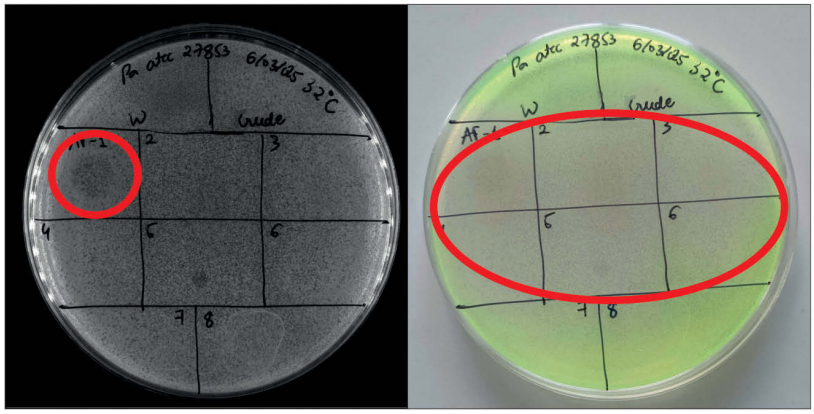


Fig. S1. A – Illustration of a trap used for oviposition of eggs. B – Illustration of the bottle used for larval growth in the study 2.

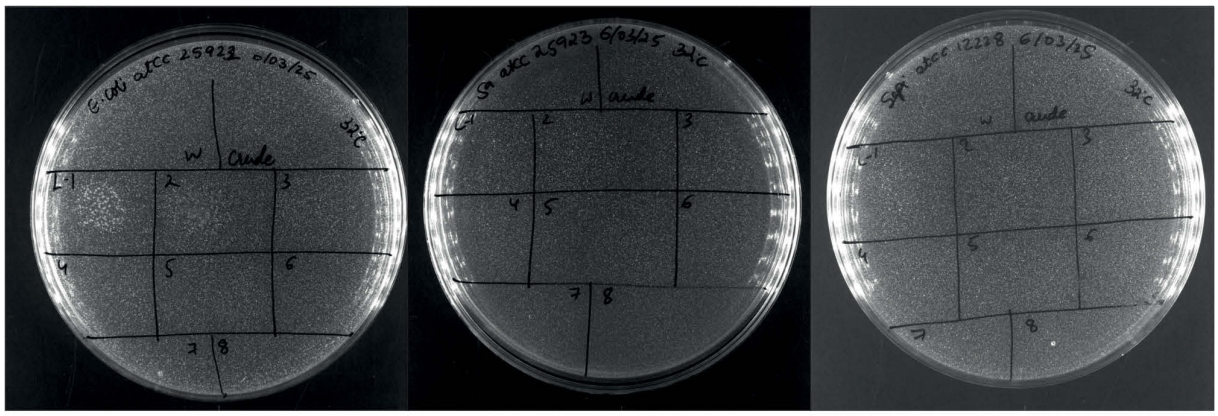


Escherichia coli ATCC 25922 *Staphylococcus aureus ATCC 25923* *Staphylococcus epidermidis ATCC 12228*

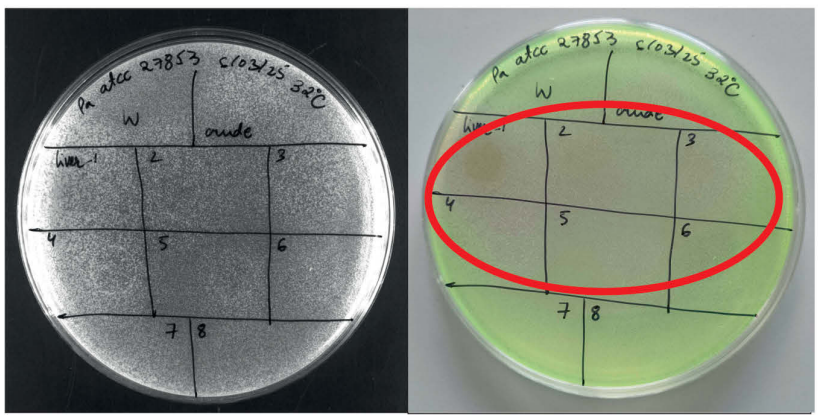


Pseudomonas aeruginosa ATCC 27853 *Pseudomonas aeruginosa ATCC 27853*

Fig. S2. Antimicrobial activity of secretions from *L. sericata* larvae reared on the artificial diet against Gram-negative and Gram-positive reference bacteria. Representative radial diffusion test plates showing zones of inhibition against *E. coli* growth. Against *P. aeruginosa,* a zone with loss of pigment was observed. Numbers on the plate indicate a twofold decreasing concentration of secretion from 56.0 to 0.3 mg/mL, until the 8th dilutions. Pictures were taken using the Syngene G:BOX high-resolution camera. The red circle shows the zone of inhibition of bacterial growth, and the red oval shows the area where the bacteria did not produce pigment.



Escherichia coli ATCC 25922 *Staphylococcus aureus ATCC 25923* *Staphylococcus epidermidis ATCC 12228*



Pseudomonas aeruginosa ATCC 27853 *Pseudomonas aeruginosa ATCC 27853*

Fig. S3. Antimicrobial activity of secretions from *L. sericata* fly larvae reared on liver against Gram-negative and Gram-positive bacteria. Representative radial diffusion plates demonstrate that secretions collected from larvae reared on the liver show no antimicrobial activity against the tested microorganisms; however, only loss of pigment when tested against *P.aeruginosa* was observed. Numbers on the plate indicate a twofold decreasing concentration of secretion from 56.0 to 0.3 mg/mL, until the 8th dilution. Pictures were taken using the Syngene G:BOX high-resolution camera. The red oval shows the area where the bacteria did not produce pigment.