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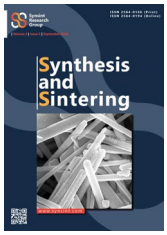

Research article

Evaluation of the contribution of media derived from various animal livers on the production of Lucilia sericata

Erdal Polat [a,b,*], **Zahra Bahararjmand** [c,d,*], **Kübra Tugtekin** [e], **Merve Cil** [f], **Emre Deymenci** [g], **Serhat Sirekbasan** [h]

[a] *Department of Medical Microbiology, İstanbul University-Cerrahpaşa School of Medicine, İstanbul, Turkey*
[b] *Traditional and Complementary Medicine Application and Research Center, İstanbul University-Cerrahpaşa, İstanbul, Turkey*
[c] *Department of Biophysics, İstanbul University-Cerrahpaşa School of Medicine, Istanbul, Turkey*
[d] *Department of Biophysics, İstanbul Nisantasi University School of Medicine Istanbul, Turkey*
[e] *Department of Medical Biology, İstanbul University-Cerrahpaşa School of Medicine, İstanbul, Turkey*
[f] *Institute of Forensic Sciences and Legal Medicine, Istanbul University-Cerrahpasa, Istanbul, Turkey*
[g] *Dogus University, School of Vocational, Department of Pathology Laboratory Techniques, Istanbul, Turkey*
[h] *Department of Medical Laboratory Techniques, Şabanözü Vocational School, Çankırı Karatekin University, Çankırı, Turkey*

A B S T R A C T

The effects of liver from different animals and agar- media on the production of Lucilia sericata (Meigen 1826) larvae were investigated to determine the best medium for producing larvae for wound therapy. The research was conducted in two phases. The best liver for generating L. sericata larvae was determined in the first phase, using media with beef, porcine, lamb, and chicken livers gelled with agar. In the first phase of the research, it was established that chicken liver was acceptable since the number of flies emerging from puparia was the highest at 80.75%. The preparation and content of the best medium for developing L. sericata larvae were determined in the second phase using chicken liver, raw, cooked, agar, and agar + salt. The number of flies emerging from puparia on the medium with chicken liver + salt + agar was 95.7% in the second phase, followed by 95% of flies coming out of the pupa in the medium prepared with chicken liver and agar. Finally, as the number of flies developing in these two mediums was not significantly different, we believe that the chicken liver and agar medium are most suitable for developing larvae.

KEYWORDS

Lucilia sericata
Maggot
Different mediums
Medium comparison

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1. Introduction

The species Lucilia sericata (Meigen 1826), commonly known as the "green bottle fly," is characterized by its metallic green color and has significant roles in both medical and forensic fields [1–7]. In medical entomology, the larvae of L. sericata have been utilized in Maggot Debridement Therapy (MDT) due to their ability to selectively degrade necrotic tissue [8, 9]. MDT, which involves applying live larvae to non-healing wounds, dates back to ancient times but saw a decline in use with the discovery of antibiotics in the mid-20th century. However, the rise of antibiotic-resistant pathogens has renewed interest in MDT, particularly in cases where conventional antibiotic treatments fail to resolve chronic or infected wounds [3, 10, 11].

L. sericata larvae produce proteolytic enzymes that break down necrotic tissue while leaving healthy tissue intact, making them highly effective in wound cleaning [11–13]. Additionally, their secretions

* Corresponding authors. *E-mail address:* erdalp@iuc.edu.tr (E. Polat), zahra.bahararjmand@nisantasi.edu.tr (Z. Bahararjmand)
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have been shown to disrupt bacterial biofilms, which are often resistant to antibiotics, further enhancing the therapeutic potential of MDT [8, 9]. This dual action of removing dead tissue and reducing bacterial infection has made MDT a valuable alternative treatment in modern medicine, especially in the management of diabetic ulcers, pressure sores, and post-surgical wounds [2, 11, 14].

One of the most significant challenges in the widespread adoption of MDT is the need for consistent and high-quality larval production. The nutritional substrate used to rear L. sericata larvae plays a critical role in their development, size, and overall efficacy. While various substrates have been tested, including different types of animal liver, there remains a gap in the literature regarding the optimal media for large-scale larval production. This gap is particularly relevant given the increasing demand for sterile larvae in wound care treatments, which are now used in over 20 countries [3, 14–17].

In addition to its medical applications, L. sericata is a key species in forensic entomology. The life cycle of the fly, particularly its predictable developmental stages, is used to estimate the post-mortem interval (PMI) in forensic investigations. Forensic entomologists rely on the detailed knowledge of L. sericata development under various environmental conditions to accurately determine the time since death in legal cases. Given the significance of L. sericata in both MDT and forensic applications, understanding the factors that influence its growth and development is critical [7, 18–22].

Several studies have examined the dietary requirements of L. sericata larvae to optimize their growth. Early research by Tenquist [23] demonstrated that a combination of water, sugar, and milk powder significantly reduced the time required for colony formation. More recent studies have explored the use of different animal tissues as larval substrates, with the liver being one of the most commonly tested due to its high nutrient content [24]. However, there is still no consensus on which type of liver—lamb, bovine, porcine, or chicken—provides the best results in terms of larval yield, size, and adult fly emergence.

The selection of an appropriate medium is also crucial for ensuring the reproducibility and efficiency of larval production. Different studies have tested various media, including raw and cooked liver, as well as media supplemented with agar to improve consistency and prevent spoilage [17]. The variability in results across studies highlights the need for further research to standardize larval production methods. A standardized medium would not only enhance the quality of larvae for MDT but also improve the reliability of forensic investigations involving L. sericata.

This study aims to address these gaps by systematically evaluating the effects of different animal livers and media compositions on the development of L. sericata larvae. In the first phase, we compared the growth of larvae on media prepared with lamb, bovine, porcine, and chicken liver to identify the best-performing substrate. The second phase of the study focused on optimizing the chicken liver medium identified as the most effective in the first phase by testing different preparations, including raw, cooked, agar-based, and salt-enriched media. The goal of this research is to provide empirical evidence on which liver-based media yield the highest quality larvae for use in both medical and forensic applications.

The significance of this research extends beyond the immediate findings related to larval development. By optimizing the production of L. sericata larvae, this study has the potential to impact both medical and forensic fields. In the medical field, improved larval production could lead to more consistent and effective MDT treatments, ultimately benefiting patients with chronic wounds that are resistant to conventional therapies. In the forensic field, understanding the factors that influence larval growth could lead to more accurate PMI estimations, thus strengthening the reliability of forensic evidence in legal cases.

Despite the growing body of research on L. sericata larval production, there remains a critical need for standardized protocols that can be easily replicated in laboratory settings. This study contributes to this effort by providing a detailed comparison of liver-based media and offering practical recommendations for optimizing larval production. The findings of this research will not only fill a gap in the existing literature but also provide a foundation for future studies aimed at improving the use of L. sericata in both medical and forensic contexts.

L. sericata plays a vital role in MDT and forensic entomology, and optimizing its larval production is essential for enhancing its applications in both fields. This study seeks to identify the most effective liver-based media for larval growth and development, to improve the quality and consistency of larvae produced for medical and forensic use. By addressing the nutritional needs of L. sericata larvae and refining the media used for their production, we hope to contribute to the ongoing efforts to improve MDT and forensic practices.

2. Materials and methods

2.1. Study design

Livers for the investigation were sourced from a butcher. Initially, cages were set up, and colonies were established. The livers were then processed for use in the media. The second phase was structured based on observations and data collected during the study. The effects of the liver-based media, prepared in various ways, were analyzed. Results were monitored and processed using Microsoft Excel.

2.2. Creating the cages

Eight cages, each measuring 30×30×30 cm (27,000 cm³), were constructed for the study and lined with fine tulle to allow proper ventilation for the adult flies and to facilitate observation of their movements. Water and sugar were provided in plastic containers inside the cages to feed the fly colonies (Fig. 1a). Each cage was labeled according to the liver type used (chicken, bovine, porcine, and lamb). The cages were maintained under controlled conditions in a laboratory set at 24–26 °C and 40% humidity, with a 24-hour light cycle. Observations were conducted for seven weeks after the flies emerged from the pupae until 90% or more had died (Fig. 1b–c).

2.3. Preparation of media

Phase I

The media were prepared separately using chicken, lamb, bovine, and porcine livers. Bacteriological agar (20 g) was added to 650 ml of distilled water and heated until fully dissolved. Then, 500 g of blended liver was incorporated to create a homogenous mixture. The media were divided into 250 ml portions, placed in autoclave-safe bottles, and sterilized at 121 °C for 15 minutes. After sterilization, the media were stored in a refrigerator at +4 °C until use.

Phase II

The second phase involved media prepared with chicken liver, which yielded the best results during the preliminary phase of the study.

Fig. 1. a) Adult flies cage, b) pupa, c) adult fly, d) egg, e) III stages larvae, and f) stigma.

Cooked medium: The cooked medium was prepared using 500 g of chicken liver. Initially, the liver was blended to achieve a homogenous mixture. The blended liver was then subjected to cooking in a water bath at 100 °C for 30 minutes to ensure proper denaturation of proteins and to reduce microbial contamination. After the cooking process, the liver was allowed to cool down to room temperature.

Once cooled, the liver was mixed with bacteriological agar dissolved in distilled water. The agar was heated until it completely melted, ensuring the mixture was homogenous. After combining the cooked liver and melted agar, the medium was divided into 250 ml portions, placed into autoclave-safe bottles, and sterilized at 121 °C for 15 minutes in an autoclave to ensure complete sterility.

The sterilized medium was then stored at +4 °C until use. Before each experiment, the medium was thawed and 25–30 g portions were placed onto 90 mm sterile petri dishes inside a sterile cabinet to avoid contamination.

Agar agar medium: Bacteriological agar agar (20 g) was dissolved in distilled water (650 ml) and heated. Then, 500 g of blended chicken liver was added and stirred to achieve a homogeneous mixture. The prepared medium was divided into 500 ml autoclave bottles, with 250 ml per bottle. The bottles were autoclaved at 121 °C for 15 minutes and stored in a refrigerator at +4 °C until use.

Raw medium: Chicken liver (500 g) was blended and stored in a freezer at -20 °C until needed. Livers used for feeding adults were preserved in a deep freezer set at -20 °C. For use, 25–30 g of the liver was placed onto 90 mm petri dishes within a sterile cabinet.

2.4. Obtaining eggs

During the study, 25–30 g of chicken, lamb, bovine, and porcine livers were placed in cages containing 1000–1100 L. sericata flies for 4 hours before being removed and used with the eggs (Fig. 1d). In each petri dish containing medium made from different animal livers and chicken livers, 100 eggs were introduced. The petri dishes were then placed in identical 25×16×11 cm boxes and incubated for 72 hours in a 30 °C incubator (Nüve EN 400®). After confirming with a microscope that the larvae were in the 3rd stage (Fig. 1e & 1f), 10 larvae from each experimental group were killed in boiling water. Their weights (measured with a Denver instrument® TP 214) and lengths (measured with a Horex®) were recorded. The data sets were then tabulated. The larvae were placed in identical 25×16×11 cm transparent boxes filled with sawdust to complete the post-feeding stages and the pupal stage. The heights and weights of the pupae, which developed after an average of 72 hours, were tabulated. Pupae were collected from the sawdust and placed in laboratory cages with 24–26 °C and 40% humidity to allow adult flies to emerge. The emerging adult flies were counted and recorded. To feed the flies and monitor the next generation, 25 g of liver was placed in the cages once a week and removed with the eggs after 4 hours. A suitable medium was provided to feed the hatching larvae, and equal 25×16×11 cm transparent boxes were used for this purpose.

Each research group was inspected three times, and adult fly cages were monitored for seven weeks or until 90% of the flies died. All analyses were repeated three times to ensure statistical reliability and to reduce the impact of potential anomalies. The average, standard deviation and relative standard deviation of each experimental group were calculated accordingly.

3. Results and discussion

The number of larvae hatched from the eggs (Table 1), the length and weight of the larvae and pupae (Table 1), and the number of adults

Table 1. Evolutionary stages, population ratios, mean values, and standard deviation data of L. sericata on media prepared with various animal livers (average ± standard deviation (SD)).

	Lamb (n=100)		**Bovine (n=100)**		**Porcine (n=100)**		**Chicken (n=100)**		**Probability value (P value)**	
Hatched eggs (mean)	80.33		75.33		78.67		**81.67***			
Mean ± standard deviation (SD)	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**
Larvae (mm/g)	**12.42**±1.14	0.33±0.08	11.89±1.77	0.30±0.13	11.83±1.86	**0.36**±0.09	11.04±2.09	0.29±0.2	0.139	0.364
Pupae (mm/g)	**6.92**±2.23	0.27±0.09	6.33±1.83	0.24±0.08	6.58±1.74	**0.28**±0.09	6.28±1.81	0.20±0.09	0.736	0.068
	Total	**Rate**	**Total**	**Rate**	**Total**	**Rate**	**Total**	**Rate**		
Adults coming out the pupae (%) (mean)	666.88	78.35%	1051.36	78.23%	360.19	78.45%	**1347.31**	80.75%	0.002	

* The highest values are indicated in bold.

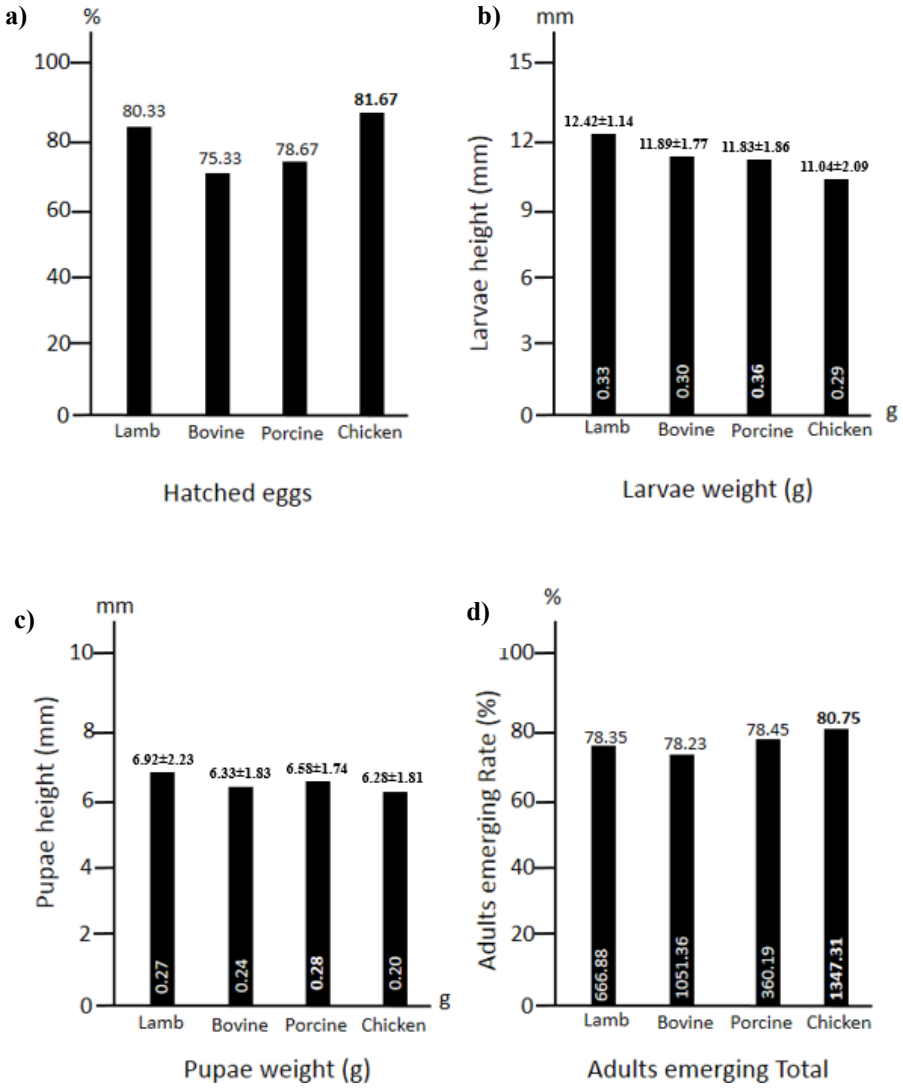

Fig. 2. First phase, a) average proportions of larvae hatching from eggs planted in the medium as 100 pieces each, b) average length/weight ratios of larvae seen to have reached the 3rd stage, c) average length/weight ratios of the pupal stage, and d) adult fly ratio/number average values emerging from the pupa.

emerging from the pupae (Table 1) were demonstrated in the medium created with lamb, bovine, porcine, and chicken liver in the first phase of the study. When compared to other mediums, chicken liver had the highest success rate (81.67%) in raising larvae that had just emerged from the egg (Fig. 2a). While the larva fed with the medium prepared with lamb liver was measured as the largest at 12.42 mm (Fig. 2b) and the length of the pupae at 6.92 mm (Fig. 2c), the weight of the larvae fed with the medium prepared with pig liver was 0.36 g (Fig. 2b) and the weight of the pupae were determined as the heaviest with 0.28 g (Fig. 2c). In comparison with other data, it was decided to use chicken liver in the second phase of the study because the number of adult flies that came out of the pupa fed with the medium made with chicken liver was 1347.31 and the rate was 80.75%. (Fig. 2d). The media were created in the second phase of the investigation by adding chicken liver and raw, cooked salt+agar agar, and agar agar. The results gained from the study on various media are presented in the tables, including the number of larvae hatched from the egg (Table 2), the length and weight of the larvae and pupae (Table 2), and the number of adults emerging from the pupae (Table 2). When compared to other mediums, chicken liver + salt+ agar agar had the highest success rate (78.7%) in raising larvae that had just emerged from the egg (Fig. 3a). Larvae fed with autoclaved chicken liver medium measured 13.39 mm and 0.40 g in weight (Fig. 3b), with pupae measuring 6.91 mm and weighing 0.30 g (Fig. 3c). Even though the adult flies from the pupa fed with the media with chicken liver + salt + agar agar had the greatest rate of coming out (95.7%), we saw in research as the adult flies from the pupa fed with the media with chicken liver + agar agar had a rate of 95% (Fig. 3d).

L. sericata is used not only in the treatment of larvae debridement but also in forensic entomology to determine the time, the identity of the corpse, and the location of death [3, 7, 19, 24]. L. sericata larvae, of which there have been interdisciplinary studies in many subjects, must be produced sterile to be used in MDT therapy [3, 11, 13]. Genç [25] classified the nutritional needs of oviparous insects in her review, which also showed the connection between nutrition and egg

Table 2. Evolutionary stages, population ratios, mean values, and standard deviation data of L. sericata in media made using various procedures and substances derived from the chicken liver (average ± SD).

Chicken	Cooked (n=100)		Raw (n=100)		w/salt (n=100)		w/agar (n=100)		P value	
Hatched eggs (mean)	60.0		60.0		**78.7***		64.7			
	Height (mm)	**Weight (g)**	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**	**Height (mm)**	**Weight (g)**
Larvae (mm/g)	**13.39**±0.91	**0.40**±0.12	12.47±1.6	0.30±0.1	12.23±1.33	0.32±0.1	11.96±1.57	0.30±0.11	0.027	0.047
Pupae (mm/g)	**6.91**±0.79	**0.30**±0.13	6.74±0.62	0.29±0.14	6.39±0.89	0.24±0.13	6.24±1.07	0.24±0.13	0.091	0.416
	Total	**Rate**	**Total**	**Rate**	**Total**	**Rate**	**Total**	**Rate**		
Adults coming out the pupae (%) (mean)	1010.4	91.20%	653.9	94.60%	1249.6	**95.70%**	**1416.1**	95.0%	0.187	

* The highest values are indicated in bold.

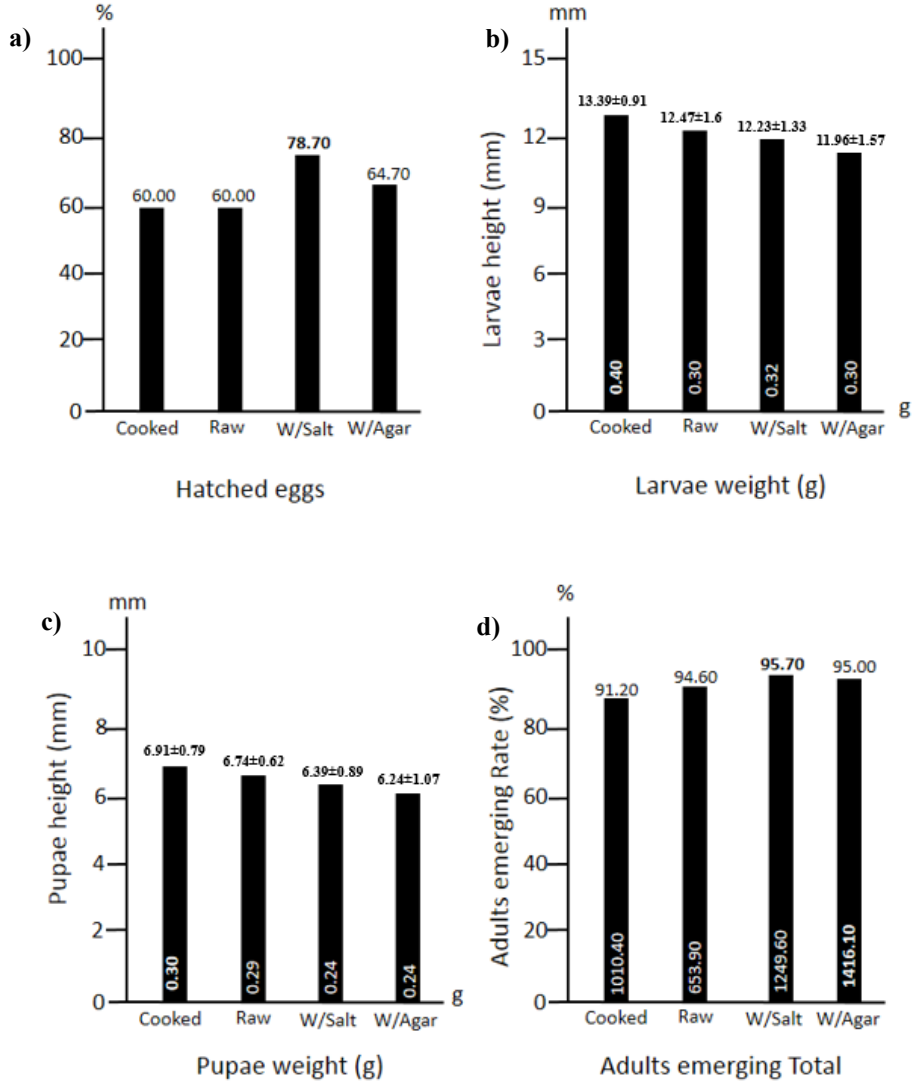

Fig. 3. Second phase, a) average proportions of larvae hatching from eggs planted in the medium as 100 pieces each, b) average length/weight ratios ratios of larvae seen to have reached the 3rd stage, c) average length/weight ratios of the pupal stage, and d) adult fly ratio/number average values emerging from the pupa.

Table 3. Content of lamb, chicken, bovine, and porcine livers per 100 g*.

References	Unit	Lamb	Chicken	Bovine	Porcine
Water	g	71.37	76.46	70.81	71.06
Energy	kcal	139.00	119.00	135.00	134.00
Protein	g	20.38	16.92	20.36	21.39
Total lipid (fat)	g	5.02	4.83	3.63	3.65
Calcium (Ca)	mg	7.00	8.00	5.00	9.00
Magnesium (Mg)	mg	19.00	19.00	18.00	18.00
Potassium (K)	mg	313.00	230.00	313.00	273.00
Sodium (Na)	mg	70.00	71.00	69.00	87.00
Cholesterol	mg	371.00	345.00	275.00	301.00
Tryptophan	g	0.236	0.176	0.263	0.301
Threonine	g	0.882	0.725	0.869	0.91
Isoleucine	g	0.878	0.813	0.967	0.85
Methionine	g	0.442	0.432	0.543	0.53
Histidine	g	0.479	0.507	0.629	0.582

*The information comes from the United States Department of Agriculture's Food Data Center. (Date of access: 10.04.2022) Genç (2006) work was used to create this.

production (Table 3). Various researches on the diet, lifestyle, and life duration of L. sericata have been undertaken. L. sericata dietary habits and developmental phases have been studied since 1971. According to research, some observe that feeding flies with a mixture of water, sugar, and milk powder in a microenvironment of 27 °C and 55–60% humidity reduces the time necessary for them to build a colony by half [23]. Sherman and Tran [26] grew L. sericata larvae on a sterile medium made from bovine liver. Tachibana and Numata [27] investigated the growth and life span of larvae, pupae, and adults in various media containing beef liver, milk, wheat, cellulose powder, dry yeast, and propionic acid. Clark et al. [24] examined the feeding and developmental phases of L. sericata in media made from pig and cow, heart, lungs, and liver. In a research using the blowfly Calliphora stygia (Fabricius 1782) (Diptera: Calliphoridae), Ujvari et al. [28] studied the effects of the low and high-fat medium on larval growth, sex distribution, and life duration. It was observed that the low-fat diet group survived longer than the high-fat diet group. When the process from egg to adult is taken into consideration in the study by Rueda et al. [29] on the development of L. sericata with two different artificial diets, the transformation rate is over 90% in larvae given diet 1 (agar, ram blood, powdered liver, sodium acid phosphate, potassium acid phosphate, sodium chloride, glucose, and distilled water) and roughly 40% in those fed diets 2 (blood flour, egg flour, powdered milk, agar, and water) were detected. Rabêlo et al. [30] studied the diet, behavior, larval stages and length, weight, mortality, sex, and life duration of Chrysomya megacephala (Fabricius, 1794) and Chrysomya putoria (Wiedemann, 1830) (Diptera: Calliphoridae) larvae in a medium made from ground beef, sardines, tripe, and chicken eggs. Similarly, El-Moaty and Kheirallah [31] studied the nutrition, behavior, larval phases, and length, weight, and life span of L. sericata larvae on medium containing cow's liver, brain, heart, lung, kidney, intestine, and minced meat. C. megacephala development was observed in a medium containing four distinct tissues (meat, liver, fats, and mixed) by El Hadi Mohamed et al. [32]. When the length ratios of the 3rd stage larvae were compared, it was determined that the larvae and pupae fed with mixed food were the longest (15.23–11.17 mm) and the ones fed with fat were the shortest (10.93–7.7 mm). Noblesse et al. [33] investigated the development of L. sericata and Phormia regina (Meigen, 1826) (Diptera: Calliphoridae) flies on a minced beef medium with varying amounts of fat (10%, 20%, or 27%). As a result, the rise in fat ratios had a detrimental effect on P. regina, yet it did not cause a difference in sex ratios in L. sericata. The medium was employed uncooked in many research exploring the life cycle of flies and larval development.

Our investigation occurred in 30×30×30 cm cages at 24–26 °C, 40% humidity, and 24-hour lighting in an insectarium. The size of the cages not only allowed adult flies to wander freely, but also allowed for observation. L. sericata larvae and pupae fed on a medium containing lamb liver were found to be greater compared to other mediums in the initial investigation on four different animal liver media (Fig. 2b & 2c). The weight of the larvae fed with the porcine liver medium was greater (Fig. 2b). It was found that the medium made with chicken liver produced the greatest number of adult flies (Fig. 2d).

Several nations employ sterile L. sericata larvae grown in a laboratory setting for MDT. 1st and 2nd stage larvae are utilized in the therapy as 6–10 pieces of 1 cm^2 depending on the wound size. The size or weight of the larvae is not considered for medical uses. As a result, in the second phase of the investigation, it was chosen to utilize chicken liver, from which most adult flies were obtained. Only chicken liver was employed as a medium in the second phase of the research, and the number of L. sericata larvae, pupae, and adults was observed to be near to the one in other mediums produced with chicken liver+agar agar (Fig. 3b–d). The medium containing chicken liver and agar agar and salt produced the highest growth. When L. sericata larvae are used MDT, it would be difficult to collect them from cooked and raw mediums. Thus, we believe that chicken liver+agar agar and chicken liver+agar agar+salt will be the most suitable media.

4. Conclusions

Based on the study, it was decided that the chicken medium was the most efficient. Although lamb and bovine mediums produced efficient results, the high data of chicken mediums is noticeable. We believe that the use of chicken liver as a medium is the most appropriate when it is evaluated financially from an economic perspective in laboratories and health institutions that are intensively studied. When preparing chicken medium, it is more practical to use agar agar medium rather than cooked medium during therapies. Larval losses occurred in the study because raw and cooked medium (especially raw) liquefied more than expected from agar agar-free medium prepared in different sets. Those certain losses were excluded from the data.

CRediT authorship contribution statement

Erdal Polat: Validation, Data Curation, Visualization, Writing – original draft.
Zahra Bahararjmand: Methodology, Writing – review & editing.
Kübra Tugtekin: Methodology, Investigation, Writing – review & editing.
Merve Cil: Formal analysis, Resources, Software, Writing – review & editing.
Emre Deymenci: Conceptualization, Methodology, Investigation, Writing – review & editing.
Serhat Sirekbasan: Project administration, Supervision, Writing – review & editing.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

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