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Original Article

Estimation of life expectancy and measurement of immature stages of *Lucilia sericata* fed on three kinds of diets

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Abstract

Background and Objectives: Finding the best diet is very important to rear *Lucilia sericata* larvae for therapeutic purposes and prepare standard curves in forensic entomology. The aim of this study was to find the best diet for larvae in maggot therapy. Furthermore, this study was conducted to obtain a vertical life table and measure the length and width of immature stages of *L. sericata* for forensic entomology.

Materials and Methods: Larvae of *L. sericata* (Karaj strain) were used to evaluate diets. The tests were carried out in three replicates of 100 eggs for each diet including chicken liver, blood agar, and fish food at the same time. Independent *t*-test, ANOVA, and Tukey's post-hoc tests were used to compare the mean length and width of larvae between different groups. A P-value of less than 0.05 was considered significance level.

Results: In contrast to the first and the second larval instars, there was a significant difference in the mean length and width of the third instar larvae ($P<0.001$). At the third instar larval stage, those larvae that had been fed on chicken liver were significantly larger than the others. There were also no significant differences between life expectancy, overall survivorship rate, and force of mortality of larval groups fed on three different diets.

Conclusions: Chicken liver is an advisable diet for nurturing larvae and plotting standard curves in forensic entomology. For rearing the first and the second larval instars in sterile conditions, blood agar and fish food can serve as suitable diets.

Keywords: Fly, *Lucilia sericata*, Life table, Larval diet, Forensic entomology, Maggot therapy

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Introduction

The members of family Calliphoridae are autogenous. This means that they need protein to lay eggs and produce progeny (1). A proper larval diet can prepare a suitable condition for the growth of flies (2). The composition of the diet of larvae that are used in maggot therapy is generally based on animal tissue (1). The size of the larvae can be changed by changing the diet which is important either in maggot therapy or in preparation of applicable and reliable standard database to estimate post mortem interval (3).

In previous studies, sheep liver (4) and beef (5) have been used as compatible and accessible media to nurture larvae of Calliphoridae family. The mean size and number of larvae grown with beef were higher compared to the other media and beef has been introduced as the most suitable substrate to nurture larvae. Due to the odor and lack of sterilization, rearing larvae on animal tissue can be undesirable (6). In 1988, Mandeville introduced dry cat food with CSMA (special food for fly larvae) as a suitable diet to rear *Lucillia sericata* (7); however, Sherman rejected it because of the impossibility of sterilizing this diet (8). A combination of liver and agar that could be sterilized by autoclave was used as a larval diet for *L. sericata* by Sherman (9). In a study conducted by Shefa in 2013, an artificial diet containing wheat bran, whole milk powder, cow blood, and egg was used to rear larvae (10). Other studies have shown that different kinds of diets such as milk products exhibit some advantages and disadvantages and may not provide certain nutritional needs for some specific species (1, 11). Larval diet is also important for its effect on life table parameters and life expectancy values of larvae. Today, the second instar larvae that are confined inside a bag called Biobag are used in maggot therapy (12). In this method, sterilization of larvae starts from the egg stage and sterile foods are used to grow larvae. Then, the second instar larvae are placed inside the bag to be used on wounds (13).

Despite various studies on the diet of fly larvae, there is no standard diet for rearing *L. sericata*. It seems that the effects of diets should be investigated separately and locally for each local strain. Long time rearing of this species in the laboratory can change the protein profile of saliva and hemolymph which are extremely important in activities of *L. sericata* in decomposition and wound healing measures. Experiences have shown that observable and morphological characteristics can be considered as evidence for usefulness of diets. The aim of this study was to examine the effects of chicken liver and carnivorous fish food, and blood agar on size of immature stage, life table, and life expectancy of *L. sericata* (Karaj strain).

Materials and Methods

This study was conducted from March 2017 to February 2018 at the Maggot Therapy Laboratory in the Department of Medical Entomology and Vector Control which was approved by the Ethics Committee of the Tehran University of Medical Sciences (TUMS), Tehran, Iran (IR.TUMS.REC.1395.1533)

Adult Breeding

Adult *L. sericata* which were raised at Maggot Therapy Laboratory were used in this study. They were grown in cages (45×45×45 cm) under 45% humidity, 24 ± 2°C, and 8-16 hour cycle of light and darkness with 5% sucrose solution. Chicken liver was used as a medium for laying eggs and growth of larvae.

Diet Preparation and Larval Measurement:

Three diets including chicken liver, carnivorous fish food, and blood agar were placed in dishes with 6×6×6 cm dimensions. The eggs that had been laid simultaneously with adults were washed with water. After separation of eggs from each other, 300 eggs were put on each diet at 26.5 ± 3°C (3 replicates of 100 eggs for each diet). In each test, counting the number of living larvae and recording their age stages were performed after hatching eggs until the emergence of adults. Moreover, 3 larvae were randomly selected from each dish to measure their length and width using binoculars (Olympus SZX12, Japan) and Labmed Pixel Pro Software (version 2.6.0.0).

Statistical Analysis

ANOVA and Turkey's post-hoc tests were used to analyze and compare the mean length and width of larvae at each growth stage. Independent *t*-test in SPSS version 18.0 was used to analyze and compare the mean length and width of larvae in each diet. A *P*-value of less than 0.05 was considered significance level.

Calculations of horizontal life table are shown in Tables 1 and 2 according to Henderson 2003 (14).

Results

The results of the comparison of the length and width of larvae at each stage of *L. sericata* (Karaj strain) are shown in Table 1. As seen, there was a significant difference in the length and width of the third instars of larvae grown with three different diets ($P<0.001$). Based on the results of Turkey's post-hoc test, the length of the third instar larvae fed on chicken liver was greater than that of the larvae fed on fish food and blood agar and accordingly, the width of the third age larvae which were fed on the chicken liver was greater. Only the larvae grown with blood agar and chicken liver became pupa and then adult. According to the result of the *t*-test, there was a significant difference in the mean length and width of pupae and wingspan between adult and mature flies ($P<0.001$). Therefore, the mean length and width of those which were fed on the chicken liver were significantly greater than those of flies grown with blood agar (Table 2).

The results regarding the number of larva and their growth stages and life table are shown in Tables 3 and 4,

Table 1. The Results of the Length and Width Measurements (millimeter) of Different Growth Stages of *L. sericata* in All Repetition of Diets

Diets	Number of initial eggs	Larval stage 1		Larval stage 2		Larval stage 3	
		Mean length	Mean width	Mean length	Mean width	Mean length	Mean width
Blood agar	100	2.46±0.48	0.44±0.10	5.62±1.13	0.96±0.24	10.28±1.69	2.17±1.64
Fish food	100	2.65±0.56	0.46±1.12	5.52±0.69	0.94±0.19	8.53±1.94	1.62±0.35
Chicken liver	100	2.59±0.68	0.49±0.09	6.29±0.86	1.03±0.18	11.78±2.49	2.32±0.44
P-value	-	0.55	0.55	0.56	0.2	< 0.001	< 0.001

P-value<0.05; data are presented as Mean±SD

Table 2. Analysis of the Results of the Length and Width Measurements (millimeter) of *L. sericata* Using *t*-test

Diet	pupa		Adult		wing	
	Mean length	Mean width	Mean length	Mean width	Mean length	Mean width
Blood agar	0.36±6.33	0.17±1.89	0.47±5	0.15±1.82	0.25±4.47	0.19±1.73
Chicken liver	0.34±7.62	0.22±3.16	0.50±7.92	0.33±3.35	0.36±7.41	0.19±3.04
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

P-value<0.05; data are presented as Mean±SD

Table 3. The Number of Living Larvae at Different Growth Stages of *L. sericata*

Diet	Replicate	Initial egg	Larval stage 1	Larval stage 2	Larval stage 3	Number of pupae	Number of adults
Blood agar	1	100	97	69	63	43	38
	2	100	91	74	56	31	26
	3	100	93	81	69	53	49
	Total	**300**	**281**	**244**	**188**	**127**	**113**
Fish food	1	100	82	47	40	0	0
	2	100	90	61	49	0	0
	3	100	88	47	59	0	0
	Total	**300**	**260**	**172**	**148**	**0**	**0**
Chicken liver	1	100	96	73	69	61	61
	2	100	97	90	85	76	75
	3	100	99	91	81	77	71
	Total	**300**	**292**	**254**	**235**	**214**	**207**

respectively. The highest survivorship rate of larval stages was observed when the larvae were fed on chicken liver. Despite the noticeable differences, the statistical analysis of data of survivorship rate, life expectancy, and force of mortality (K_X) of *L. sericata* using ANOVA test showed no significant difference among fly groups of three different diets. The survivorship curve of *L. sericata* that was prepared based on life expectancy did not show any significant difference among the flies grown with three different diets. Carnivorous fish food did not have the potential to meet the needs of larvae to become pupa (Figure 1).

Discussion

The effect of diets on survivorship rate, life expectancy (15), and force of mortality (K_x) of *L. sericata* has been proved (16). However, our study did not show any significant difference between the growth stages of flies of the three diets. Overall, survivorship rate and life expectancy of larvae that had eaten chicken liver were high. The trend in life expectancy in the three diets was similar (Table 4 and Figure 1). Additionally, none of the larvae that had eaten fish food survived to the pupa stage and no progeny was obtained.

Different studies have shown that chicken liver is a suitable diet for growing larvae of the family Calliphoridae. However, this diet has some disadvantages such as keeping, having a bad smell, sterilizing, and creating sludge that causes the larvae to die (16, 17). In this study, the measurement of different stages of *L. sericata* grown on three different diets and statistical analysis showed that the mean length and width of the third instar larvae, pupa, and adult fed on the chicken liver were significantly greater compared with blood agar (Tables 1 and 2). In addition, the chicken liver was appropriate for the growth of *L. sericata*; however, the ideal diet for mass production in maggot therapy should be sterile and odorless. Therefore, fish food and blood agar can be used instead of the chicken liver.

One of the most important and practical methods of determining the post mortem interval in forensic entomology is to measure the length of immature stages of flies that were found on corpses. This information is based on the plotting of isomegalen and isomorphen diagrams. The isomegalen diagram is based on the length of larvae at different temperatures. But, isomorphen diagram is based on the length of different immature stages, including larval stage one to larval stage three and prepupa (13). According to behavioral and biological changes of different strains of flies, isomegalen and isomorphen diagrams should not be used internationally. They should be prepared locally or regionally for different strains of flies in each area using suitable diets. Obviously, different diets can have different effects on the body size of immature stages of flies (18). Therefore, the plotting of standard diagrams should be carried out in standard conditions in the laboratory and the most suitable diet should be used for this purpose. The results of the current study showed that chicken

r is the best diet to prepare isomegallen and isomorphen diagrams (Tables 1, 3, and 4).

Blood agar enriched with some other ingredients showed promosing results in a previous study (16). The compounds added to blood agar include albumin, vitamin B, and some other proteins that are essential elements for larval growth of Calliphoridae family (15, 19). Blood agar that was used in this study was pure and contained no additional materials. The results of the current study showed that both blood agar and fish food are suitable for larval growth until the second instar stage due to their nonsignificant differences. Therefore, these two diets can be used to produce Biobags

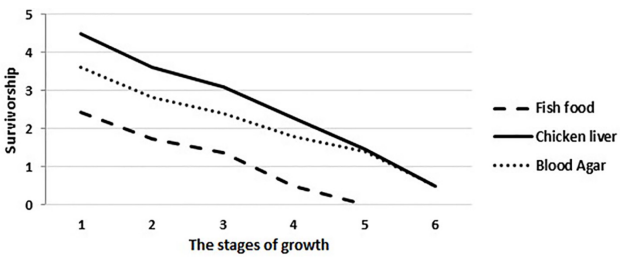

Figure 1. Survivorship Curve of *L. sericata* Based on life Expectancy

Table 4. The Life Table of *L. sericata*

X	Diet	n_x	ad_x	l_x	d_x	q_x	K_x	T_x	L_x	e_x
Egg	Blood agar	300	19	1	0.063	0.063	0.028	3.61	0.968	3.61
	Fish food	300	40	1	0.133	0.133	0.062	2.433	0.933	2.433
	Chicken liver	300	8	1	0.026	0.026	0.0117	4.506	0.986	4.506
Larval stage 1	Blood agar	281	57	0.936	0.19	0.202	0.098	2.644	0.841	2.82
	Fish food	260	38	0.866	0.293	0.338	0.179	1.5	0.72	1.73
	Chicken liver	292	38	0.973	0.126	0.13	0.06	3.52	0.91	3.616
Larval stage 2	Blood agar	224	36	0.746	0.12	0.16	0.07609	1.8	0.686	2.41
	Fish food	172	24	0.573	0.08	0.139	0.065	0.78	0.533	1.36
	Chicken liver	254	19	0.846	0.063	0.074	0.033	2.61	0.815	3.082
Larval stage 3	Blood agar	188	61	0.626	0.203	0.324	0.17	1.113	0.525	1.776
	Fish food	148	148	0.493	0.493	1	2.17	0.246	0.246	0.5
	Chicken liver	253	21	0.783	0.07	0.089	0.04	1.795	0.748	2.291
Pupas	Blood agar	127	14	0.423	0.046	0.11	0.05	0.588	0.04	1.389
	Chicken liver	214	7	0.713	0.023	0.032	0.014	1.046	0.701	1.467
Adults	Blood agar	113	113	0.376	0.376	1	0.053	0.188	0.188	0.5
	Chicken liver	207	207	0.69	0.69	1	2.35	0.345	0.345	0.5

***x*:** different stages, n_x**:** initial number, ad_x**:** number of dead, l_x**:** the ratio of live individuals, d_x**:** the ratio of dead individuals, q_x**:** mortality rate, K_x**:** mortality pressure, T_x**:** the number of days that larvae have survived in each stage and subsequent stages, L_x**:** the number of days that larvae have survived in each growth stage, and e^x**:** life expectancy at the beginning of each stage.

for maggot therapy. Moreover, the larvae fed on fish food and blood agar were too weak to reach pupa and adult stages, which can be beneficial for the Biobag producers. In this condition, the larvae which are used on the patient's ulcer in a Biobag are unable to become adult; therefore, their use can prevent the spread of flies in the environment. However, according to international guidelines, larvae should be considered potentially contaminated and destroyed after being used in the maggot therapy process (20).

The duration of the larval stage of the flies fed on a fish diet was much longer than the duration of the larval stage of those fed on blood agar and chicken liver, and the mean length and width of larvae were lower in fish food. Larvae grown with fish food diet did not become pupae. Fish food may not have some of the essential components for the development of pupa. Protein is the main component of fish food. In addition to protein, vitamin B and albumin are necessary for the growth of larvae of family Calliphoridae (15, 19). Vitamin B and albumin may be essential elements for larvae to reach the pupal stage.

Conclusion

In maggot therapy, it is important to keep larvae until the second larval instar in sterile conditions. Therefore, to develop the first larval instar to the second larval instar, blood agar and fish food could serve as suitable diets. Additionally, the results of this study showed that chicken liver is an advisable diet to nurture larvae and prepare standard biological diagrams and life table in forensic entomology. It is also recommended that standard diagrams be prepared for different strains of flies in each area in standard laboratory conditions.

Conflict of Interests

Authors declare no conflicts of interests.

Ethical Approval

This study has been approved in ethical committee of School of Public Health of Tehran University of Medical Sciences by the code of IR.TUMS.SPH.REC.1395.1533.

Authors Contributions

KA designed and was responsible for scientific support. OD conducted the trial and prepared the manuscript. JR supported technically. SMT supported clinically. SA and AR edited and helped in arrangement of the text. ARF helped for analyzing the data.

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References

1. Carey JR. *Longevity: the biology and demography of life span*. New Jersey: Princeton University Press; 2003. (pp. 157-176).
2. Gobbi P, Martinez-Sanchez A, Rojo S. The effects of larval diet on adult life-history traits of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae). Eur J Entomol. 2013;110(3):461-468.
3. Lecheta MC, Moura MO. Estimating the Age of Forensically Useful Blowfly, *Sarconesia chlorogaster* (Diptera: Calliphoridae), Using Larval Length and Weight. J Med

Entomol. 2019;56(4):915-20.
4. Day DM, Wallman JF. Influence of substrate tissue type on larval growth in *Calliphora augur* and *Lucilia cuprina* (Diptera: Calliphoridae). J Forensic Sci. 2006;51(3):657-63.
5. Anderson GS. Minimum and maximum development rates of some forensically important Calliphoridae (Diptera). J Forensic Sci. 2000;45(4):824-32.
6. Bambaradeniya YTB, Karunaratne WIP, Tomberlin JK, Goonerathne I, Kotakadeniya RB. Temperature and tissue type impact development of *Lucilia cuprina* (Diptera: Calliphoridae) in Sri Lanka. J Med Entomol. 2017;55(2):285-91.
7. Mandeville JD. Rearing *Phaenicia sericata* (Diptera: Calliphoridae) on dry cat food with CSMA. J Med Entomol. 1988;25(3):197-8.
8. Sherman RA, TRAN JMT. A simple, sterile food source for rearing the larvae of *Lucilia sericata* (Diptera: Calliphoridae). Med Vet Entomol. 1995;9(4):393-8.
9. Hobson R. Studies on the Nutrition of Blow-Fly Larvae: II. Role of the Intestinal Flora in Digestion. J Exp Biol. 1932;9(2):128-38.
10. Shefa K, Hossain M, Islam M, Islam A, Saifullah A. An artificial larval diet for blowfly, *Lucilia cuprina* (Diptera Calliphoridae). J Entomol Zool Stud. 2013:99-102.
11. Chaudhury M, Chen H, Sagel A, Skoda S. Effects of new dietary ingredients used in artificial diet for screwworm larvae (Diptera: Calliphoridae). J Econ Entomol. 2015;108(3):1429-34.
12. Čičková H, Čambal M, Kozánek M, Takáč P. Growth and survival of bagged *Lucilia sericata* maggots in wounds of patients undergoing maggot debridement therapy. Evid Based Complement Alternat Med. 2013;2013. doi. org/10.1155/2013/192149.
13. Limsopatham K, Khamnoi P, Sukontason KL, Boonyawan D, Chaiwong T, Sukontason K. Sterilization of blow fly eggs, *Chrysomya megacephala* and *Lucilia cuprina*,(Diptera: Calliphoridae) for maggot debridement therapy application. Parasitol Res. 2017;116(5):1581-9.
14. Henderson PA. *Practical methods in ecology*. Oxford: Blackwell Publiching; 2003. (pp. 108-115).
15. Zheng L, Crippen TL, Dabney A, Gordy A, Tomberlin JK. Evaluation of Sterilized Artificial Diets for Mass Rearing the *Lucilia sericata* (Diptera: Calliphoridae). J Med Entomol. 2017;54(5):1122-8.
16. Rueda LC, Ortega LG, Segura NA, Acero VM, Bello F. *Lucilia sericata* strain from Colombia: Experimental colonization, life tables and evaluation of two artifcial diets of the blowfy *Lucilia sericata* (Meigen)(Diptera: Calliphoridae), Bogotá, Colombia Strain. Biol Res. 2010;43(2):197-203.
17. Archer MS, Jones SD, Wallman JF. Delayed reception of live blowfly (*Calliphora vicina* and *Chrysomya rufifacies*) larval samples: implications for minimum postmortem interval estimates. Forensic Sci Res. 2018;3(1):27-39.
18. Robinson L, Bryson D, Bulling MT, Sparks N, Wellard K. Post-feeding activity of *Lucilia sericata* (Diptera: Calliphoridae) on common domestic indoor surfaces and its effect on development. Forensic Sci Int. 2018;286:177-84.
19. Kadner C. The vitamin requirements of *Phaenicia sericata* (Meigen) larvae (Diptera: Calliphoridae). Wasmann J Biol. 1951;9:129-36.
20. The All Wales Guidance for the Use of Larval Debridement Therapy (LDT). Publisher: London: Wounds UK; 2013. (Accessed online at: https://www.wounds-uk.com/ and http://www.welshwoundnetwork.org/download_file/95/228/).