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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Developing *Lucilia sericata* Larva on the Natural Liver or Liver Agar

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Abstract: Under laboratory conditions, food type is an important factor for *Lucilia sericata* fly colony production and regeneration. Females generally prefer animal tissues or organs with high protein and moisture content for laying their eggs. However, contamination can occur when these preferred foods become rotten and putrid. In order to ensure mass rearing control and sterility in fly colony production, to evaluate structural differences in the food source and to have an idea about the extent to which the growth rate will change in the life cycle stages, raw liver and sterile tissue-based liver agar food sources in autoclaved plant tissue culture dishes were used. For larval stage (L1, L2, L3) development monitoring, a subgroup was formed for each larval stage, so that the experiments were continued for two generations with 3 sub-study groups for each food type and 6 study groups in total. The growth rate of larvae reared on a sterile 1:1 mixture of pureed liver and 3% Bacto agar was equivalent or higher than that of larvae reared on raw liver, resulting in larger adults. At the same time, the sterile liver agar rearing medium showed the advantage of no contamination and no offensive odour. There was a significant difference ($p<0.05$) between first instar larval length, first instar larval weight, second instar larval weight, third instar larval length, third instar larval weight, pupal length and adult length measurements of *L. sericata* larvae fed with liver and liver agar. However, there was no significant difference between the second instar larval length measurements of *L. sericata* larvae fed with liver-agar and liver ($p>0,05$).

Keywords: Food media, Growth and development, *Lucilia sericata*, Mass-rearing.

Doğal Karaciğer veya Karaciğer Agarı Üzerinde Gelişen *Lucilia sericata* Larvası

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Öz: Laboratuvar koşullarında *Lucilia sericata* sinek koloni üretimi ve rejenerasyonu için besin türü önemli bir etkendir. Dişilerin yumurtalarını bırakmasında besin tercihleri genellikle protein oranı ve nem içeriği yüksek hayvan doku veya organlardır. Ancak tercih ettikleri bu besinlerin çürümeye ve kokuşmaya yüz tutmasıyla kontaminasyon gelişebilir. Sinek koloni üretiminde kitlesel yetiştirme kontrol ve steriliteyi sağlamak, besin kaynağındaki yapısal farklılıkları değerlendirmek ve yaşam döngü evrelerindeki büyüme oranının ne ölçüde değişeceği hakkında fikir sahibi olmak için çiğ ciğer ve otoklavlanmış bitki doku kültürü kapları içinde steril doku bazlı ciğer agar besin kaynakları kullanıldı. Larval dönem (L1, L2, L3) gelişim takibi için her bir larva dönemi için birer alt grup oluşturulmuş dolayısıyla her besin çeşidi için 3 alt çalışma grup totalde 6 çalışma grup ile denemeler iki nesil sürdürülmüştür. Püre haline getirilmiş ciğer ile %3 Bacto agarın steril 1:1 karışımı üzerinde yetiştirilen larvaların büyüme hızı çiğ karaciğer üzerinde yetiştirilen larvalarınkine eşdeğer veya daha yüksekti böylelikle daha büyük yetişkinler elde edilmiştir. Aynı zamanda steril ciğer agar yetiştirme ortamı kontaminasyon gelişmeme ve rahatsız edici koku olmadan yetiştirme ortam avantajı sergilemiştir. *L. sericata* larvalarından ciğer ve ciğer agar ile beslenen birinci dönem larva boy, birinci dönem larva ağırlık, ikinci dönem larva ağırlık, üçüncü dönem larva boy, üçüncü dönem larva ağırlık, pupaya geçiş dönemindeki boy ve ergin dönemindeki boy ölçümleri arasında anlamlı fark elde edilmiştir ($p<0,05$). Ancak ciğer-agar ve ciğer ile beslenen *L. sericata* larvalarının ikinci dönem larva boy ölçümleri arasında fark yoktur ($p>0,05$).

Anahtar kelimeler: Besin ortamı, Büyüme ve gelişme, Kitlesel yetiştirme, *Lucilia sericata*..

INTRODUCTION

The necrophagous larvae of Calliphoridae family Diptera flies live by feeding on carrion, dead tissue, rubbish or rotten food. After the larval stage in their life cycle, they leave the feeding substrate and move to a cool and shady place for the next stage of their development, the pupal stage (Gomes et al., 2006). Abiotic and biotic factors affect these flies' development and development time during the life cycle stages. Egg, larval stage (1st stage L1, 2nd stage L2, and 3rd stage L3), prepupa, pupa, and adult stage refer to the life cycle of a fly in fly morphology. In their life cycle, periodic larvae and adults constitute the mobile developmental stage, prepupae the semi-mobile stage, and eggs and pupae the immobile stage (Bomphrey et al. 2009). At each stage transition within the larval stage, larvae shed their cuticle, and this continues until the final outer cuticle shrinks. It hardens in this way until the pupal stage (Gunn, 2009). The transition to developmental stages in biological life cycles requires a developmental threshold in size and weight. For this reason, adequate nutrition of larvae during the larval stage is essential (Shaaya & Levenbook, 1982). Pupation time is related to food competition according to larval density. In insufficient food substrate, larvae pupate in a short time. They seek a cool and dark environment for pupation. This stage after feeding indicates the larvae's behavioral movement (Gomes et al. 2006). The metamorphosis period is completed in the puparium until the adult fly emerges (Mohr, 2012).

Diptera: Calliphoridae larvae development rate and duration are related to abiotic factors such as temperature, ambient relative humidity, and photoperiod cycle in laboratory growing conditions, as well as biotic factors such as food (Benbow et al., 2015). Food diversity is important for the fertility of male and female individuals in reproduction and development, especially if the structure and chemical properties of the food are suitable for these organisms. It has been reported that the moisture content of the food substrate significantly affects the growth of the green bottle fly *L. sericata*, with a developmental difference of up to 7.4 days (Tarone & Foran, 2006). *L. sericata* larvae feed on dead, necrotic tissue or decayed food for 3-10 days, depending on environmental conditions and food substrate quality. Scientists have repeatedly observed that feeding on soft food with high protein content affects larval development time rich diet affects larval development time (Clark et al., 2006; El-Moaty & Kheirallah, 2013). It has been reported that a larva consumes 0.3 g of tissue per day. Recent studies reported that 200 larvae (maggots) could consume 15 g of necrotic tissue or tissue fluid in one day (Wollina et al., 2000). However, it has been reported that very rapid larval development may also cause permanent later-stage effects, such as reduced immunity and reduced adult longevity (Cotter et al., 2004). This maximum larval feeding rate is explained by the larvae's powerful pharyngeal pumps, which pass it through a filter that concentrates it five times. They can digest up to half their body weight in just five minutes. Thus, a larva can increase its body size 100 times in a few days (Wollina et al., 2000).

It is medically important to obtain sterile larvae in the application of a biological ‘dressing’ larval treatment to reduce debridement, bacteria and bad odour in chronic wounds. For this reason, it is aimed to produce a colony of sterile adults by maintaining sterility from eggs taken from fly colonies grown in the laboratory to larval development, from larvae to pupae and adult. In this direction, liver and liver agar food types were used and the development of each stage in the life cycle of *L. sericata* was examined comparatively.

MATERIAL AND METHOD

Fly rearing: *L. sericata* pupae obtained from the Biotherapy Laboratory of Istanbul Cerrahpaşa University were reared as adults within 7-10 days in specially prepared 45x45x45 cm wire cages covered with tulle. The adult individuals were initially fed with 20% sugar water solution (sucrose was used as sugar) in 250 ml Erlenmeyers. The flasks were sealed with cotton wool so that one end extended to the bottom of the flask, one end remained outside, and water was provided as ad libitum. The flies were maintained at room temperature (22 ± 3◦C), 50% R.H. humidity, and 12:12 (L:D) photoperiod to ensure colony continuity. Chicken hearts were used as a protein source and oviposition medium as food substrate to provide a sufficient number of adults of the species for the targeted studies.

Pre-Experiment Preparations and Design: In the transition of the obtained *L. sericata* eggs to the next stages in their biological life cycle, the protocol was followed, and a sufficient number of adults from the species were provided for the experimental plan and targeted studies. The number of flies in a cage was kept between 500 and 1000 for the targeted studies. The eggs of the females that laid eggs within the specified time (within 2-4 hours) in the cages where chicken hearts were used for laying eggs were taken from the cages immediately after the end of laying. In the second stage, a sterile liver-agar medium was prepared using bovine liver as a nutrient substrate for experimental analyses. Egg packets of each female were placed in falcon tubes containing 9-10 ml of distilled water to separate and count the eggs. The eggs were shaken manually for 1-2 minutes until the separation occurred. The eggs separated from each other were filtered through sterile filter paper, and 100 eggs were counted separately for each group and transferred to the

media. The media containing the eggs were incubated at 25-30 °C overnight. For larval stage (L1, L2, L3) development monitoring, a subgroup was formed for each larval stage, so that the experiments were continued for two generations with 3 sub-study groups for each food type and 6 study groups in total.

Experimental Procedures:

Preparation of Liver and Liver-agar Medium: In preparation of liver and sterile liver-agar medium, chicken livers were washed thoroughly with distilled water and then cleaned from fat and veins and passed through distilled water again. The liver was shredded to gel consistency with a kitchen rondo and 45 grams each for liver-agar medium and 50 grams each for liver medium were taken into heat-resistant 15 cm diameter sterile petri dishes. To prepare 5% agar solution to be used in liver agar medium preparation; 5 grams of dehydrated agar powder was weighed and distilled water was added until 100 millilitres was added. The agar powder was stirred until the agar powder dissolved and kept in agarotoclave at 121ºC temperature and 1 atmosphere pressure for 15 minutes. When the agar solution cooled down to 45-50 degrees, 5 ml of the agar solution was added to the petri dishes containing 45 grams of liver for liver-agar medium and mixed thoroughly and homogenous distribution was ensured. For each food type, 30 petri dishes were used, therefore 10 petri dishes were used in each subgroup. Petri dishes with liver and liver-agar were sterilised in an autoclave at 121ºC temperature and 1 atmosphere pressure for 15 minutes and then covered with sterile petri covers and allowed to cool.

To determine the average length and weight of a larva at the end of days 0-2, days 2-4, and days 4-7 following the first opening of the eggs, 10 larvae of similar size were randomly selected from each experimental study group. These larvae were rinsed in distilled water and dried with blotting paper, then their weights were measured with a precision balance, and lengths were measured with calipers. For each food type, the sums of the size and weight data of 30 larvae (since there were three groups) were taken and divided by 30, and the final data obtained was recorded as the length-weight value of a larva. Traveling larvae generally require a dry, cool, shaded environment during the transition from the prepupal stage to the pupal stage. Glass jars (15x10x25cm) were used to provide this environment, and sawdust was placed inside. During metamorphosis, a hole was made in the lid of the container or glass jar, and this part was covered with gauze to allow the air necessary for the larvae's life to enter and prevent the larvae from getting out of the container. Following the incubation period of 3-5 days, Petri dishes containing different media containing the larvae, whose mobility decreased after the maximum feeding stage 3, were placed in the jar on the sawdust. The larvae were expected to move away from the nutrient medium into the sawdust, and the petri dishes were removed after they left the medium. After the incubation period, the pupae were removed from the sawdust, placed in another plastic container, and placed in a new fly cage. For pupal length measurement, ten normal-sized 2-3 days dark-colored 10 pupae of each food species were measured with calipers, and the sum of the length values was calculated and divided by 30 and recorded as the average length value. Adult lengths were calculated in the same way as pupal lengths. The data obtained as a result of mathematical calculations were compared.

Statistical Analysis: IBM SPSS Statistics 24.0 package program was used in the statistical analysis of the data obtained in the study. Firstly, the descriptive statistics' minimum, maximum, skewness, and kurtosis values according to the variables were included. Skewness and kurtosis values are statistics that give information about the normality of the variables, and when they are between ±1.5, the distribution is normal (Tabachnick & Fidell 2013). Another important assumption for parametric methods in statistical analyses is that the number of data should be sufficient in the group (N>30), and when the number of data is not enough, nonparametric methods should be used even if the scores are normally distributed (Pallant & Tennant 2007). Accordingly, the dependent group's t-test method was used to compare the weights and lengths of larvae after feeding them different foods. The comparison was made at $p<0.05$ significance level for statistical analyses.

RESULTS

Since the ambient temperature and humidity will increase during the feeding process of *L. sericata* larvae depending on their activity (Early & Goff, 1986), the laboratory ambient temperature was fixed at 30°C and 50% humidity. Since *L. sericata* flies consume all their energy reserves within a few days and die when they do not have access to energy-rich food in the form of lipids or carbohydrates, the initial feeding of adults in our study was provided with table sugar and water. A moist and high-protein meal was fed to mature the ovaries of females and produce viable eggs. Sterile liver and sterile liver-agar medium were used to identify growth and developmental differences and deviations of *L. sericata* larvae about food sources at constant temperature and humidity. The opening times of the eggs of *L. sericata* larvae left to feed on different food sources, the transition times to the next stage depending on the larvae's development, length-weight measurements, pupal entry times, pupal lengths, pupal exit times, and adult lengths were determined at (0-2), (2-4), and (4-7) day intervals after the larvae hatched. These values were recorded in the table below (Table 1).

Table 1. Growth and development times of *Lucilia sericata* in different food sources.

Lucilia sericata	Liver	Sterile liver-agar
Egg laying time	9-11 hour	7-9 hour
Time of entry into the second larval stage	27-31 hour	23-30 hour
Time of entry into the third larval stage	45-49 hour	41-46 hour
End of prepupa, the first entry into pupa	10th day	7th day
First exit from the pupa	14th day	12th day

Table 2. Length-weight averages of Lucilia sericata about food type (± means represent standard deviation).

L. sericata	Liver medium		Sterile liver - agar medium	
	Length (mm)	Weight (gr)	Length (mm)	Weight (gr)
(0-2) day L_1	4.5±0.097	0.011±0.001	4.8±0.079	0.012±0.001
(2-4) day L_2	8.8±0.126	0.024±0.001	8.9±0.084	0.025±0.001
(4-7) day L_3	12±0.092	0.060±0.001	12.3±0.103	0.062±0.002
(7-9) day Pupa	7.2±0.03	---	7.6±0.019	---
(10-30) day Adult	7.72±0.013	---	7.9±0.023	---

In this section, the findings examined and analyzed within the scope of the research are given (Table 2). Descriptive statistics of *L. sericata* larvae (L1, L2, L3), pupae, and adults according to their developmental characteristics in liver and sterile liver-agar food type are given in Table 3.

Table 3. Descriptive Statistics Table for *Lucilia sericata* Larvae (L1, L2, L3), Pupae and Adult Developmental Characteristics in Food Type.

Food type	Measurements	Min.	Max.	Skewness	Kurtosis
Liver	**Larva I (L_1) length**	4.340	4.660	0	-1.175
	Larva I (L_1) weight	0.010	0.013	0	-1.219
	Larva II (L_2) length	8.760	9.040	0	-1.127
	Larva II (L_2) weight	0.022	0.026	0	-1.175
	Larva III(L_3) length	11.850	12.150	0	-1.269
	LarvaIII (L_3) weight	0.058	0.062	0	-1.193
	Pupa length	7.150	7.250	0	-1.197
	Adult length	7.700	7.740	0	-0.943
Sterile liver - agar	**Larva I (L_1) length**	4.670	4.930	0	-1.227
	Larva I (L_1) weight	0.011	0.013	0	-1.183
	Larva II (L_2) length	8.760	9.040	0	-1.127
	Larva II (L_2) weight	0.023	0.027	0	-1.205
	Larva III(L_3) length	12.130	12.470	0	-1.182
	Larva III(L_3) weight	0.059	0.065	0	-1.197
	Pupa length	7.570	7.630	0	-1.174
	Adult length	7.860	7.940	-0.044	-1.163

The length and weight measurements of *L. sericata* larvae fed with liver and liver-agar food and the smallest and largest values of pupal and adult length periods are given according to the periods. The length and weight of pupae and adults generally increased in both food types depending on the larvae's development. The skewness and kurtosis values show that the larval period measurements fed with both food types are normally distributed since they are between ±1.5 (Table 3). A dependent group t-test was used to determine whether there was a difference between the length and weight measurements of *L. sericata* larvae fed with liver and liver-agar food and the length measurements in pupal and adult stages. The scores were normally distributed, and there were two different measurements for each larva, liver and liver-agar.

A significant difference was obtained between the first larval length, first larval weight, second larval weight, third larval length, third larval weight, third larval weight, pupal length, and adult length measurements of *L. sericata* larvae fed with liver and liver-agar ($p<0.05$). *L. sericata* larvae fed with liver-agar were higher than those fed with liver and for *L. sericata* larvae, feeding with liver-agar increased the measurements compared to feeding with liver. However, there was no difference between the larval length measurements of *L. sericata* larvae fed with liver-agar and liver ($p>0.05$) (Table 4).

Table 4. Dependent Groups T-Test between Length and Weight Measurements of *Lucilia sericata* Larvae (L1, L2, L3), Pupae, and Adults fed with Liver and Sterile Liver-agar Food.

Group	Liver	Sterile liver – agar	t	p
	Mean±SD)	(Mean±SD)		
Larvae Ⅰ (L_1) length	4.5±0.097	4.8±0.079	-13.67	**.000***
Larvae Ⅰ (L_1) weight	0.011±0.001	0.012±0.001	-2.902	**0.007***
Larvae Ⅱ (L_2) length	8.8±0.126	8.9±0.084	0	**1**
Larvae Ⅱ (L_2) weight	0.024±0.001	0.025±0.001	-2.083	**0.046***
Larvae Ⅲ(L_3) length	12±0.092	12.3±0.103	-12.379	**.000***
Larvae Ⅲ(L_3) weight	0.060±0.001	0.062±0.002	-5.025	**.000***
Pupa length	7.2±0.03	7.6±0.019	-62.26	**.000***
Adult length	7.72±0.013	7.9±0.023	-36.547	**.000***

Figure 1. *Lucilia sericata* larvae reared on different food media (a) larvae fed with liver, (b) larvae fed with liver-agar.

Figure 2. *Lucilia sericata* larvae reared on different food media and at 96 hours (a) larvae fed with liver-agar, (b) larvae fed with liver.

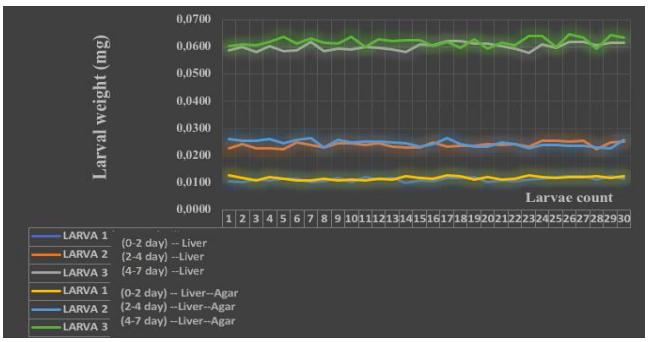

Figure 3. Comparison of larval weights of *Lucilia sericata* larvae according to the stages (L1, L2, L3) in liver and liver-agar medium.

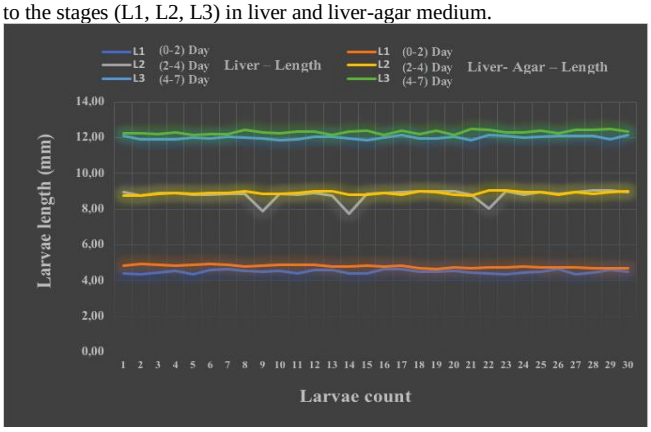

Figure 4. Comparison of the lengths of *Lucilia sericata* larvae according to the stages (L1, L2, L3) in liver and sterile liver-agar medium.

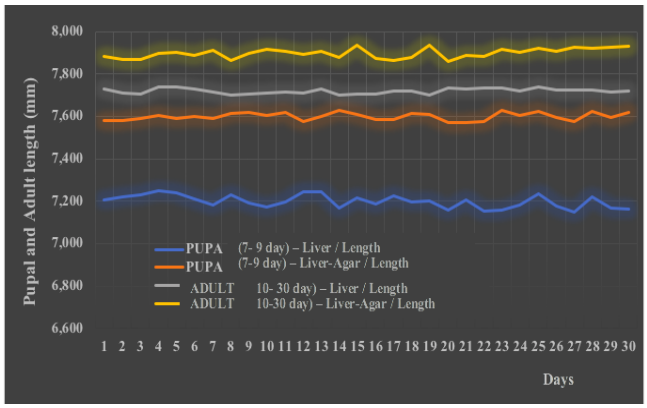

Figure 5. Comparison of pupa and adult lengths in liver and sterile liver-agar medium.

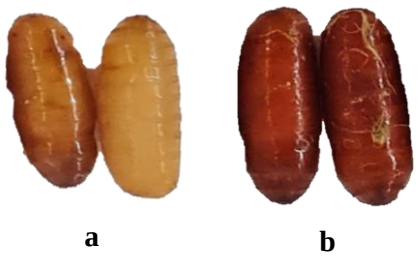

Figure 6. *Lucilia sericata* pupae (a) pupa developing from liver-fed larvae, (b) pupa developing from liver-agar-fed larvae

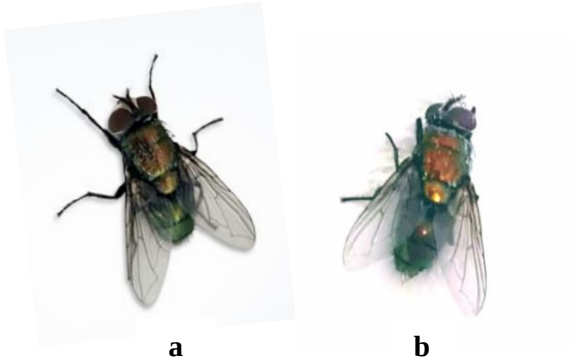

Figure 7. *Lucilia sericata* adult (a) adult developing from liver-fed larvae, (b) adult developing from liver-agar-fed larvae.

DISCUSSION AND CONCLUSION

It is the subject of the study that supplementation of agar medium to the feeding with liver, which is the known nutrient substrate in the procedure in the cultivation of *L. sericata*, may have an effect on oviposition, development during the larval feeding period, developmental time variability, and the developmental rate and time of pupae developing from larvae and adults developing from pupae after feeding. Our results confirm our hypothesis, although less than the results of developmental variability with different nutrient substrates. Based on our observations and the data we obtained, if conditions affect development during the larval period, it exhibits results that accelerate or slow down the development and process after feeding. For example, the larval density in the maximum feeding stage causes food competition to affect the metamorphosis completed in the puparium (Faris et al., 2020). In most flies, the final adult size is directly proportional to the rate of development (Nijhout et al., 2014). However, individual species-specific weight may vary depending on environmental factors (e.g., bacterial accumulation, food limitation) (Komo & Charabidze, 2021). The rapid increase of microorganisms through the decomposition process of saprophytes deteriorates food quality and adversely affects larval development (Benbow et al., 2019). According to some scientists, even though Calliphorid flies are known as carrion flies, they prefer fresh rather than decaying food (Burkepile et al., 2006). In our study, we observed that the rate of mass larval feeding on fresh liver was at the maximum level, but as the food decayed, the larvae avoided and slowed down feeding. These observations showed the social behavior of the larvae. The food consumed by *L. sericata* under laboratory conditions is a key factor in biological development and population dynamics (Sherman & My-Tien Tran, 1995). When larvae feed, larval activity increases, and thus, the temperature of the food medium increases (Early & Goff, 1986). For this reason, the ambient temperature and humidity in the laboratory were not kept too high and were kept constant at 30ºC and 50% humidity. When the hatching times of the eggs of *L. sericata* were placed in different media, the time intervals of the transition of the living larvae to the next stage, the length and weight of the larvae according to the 24th, 48th and 96th hour periods of hatching, the time of first entry into the pupa, pupal length, pupal exit time and adult length were evaluated, no extreme variability was observed in the hatching time of the eggs in both food types. Byrd and Castner, (2001) emphasized that environmental conditions and humidity are the most important factors for egg laying, survival, and hatching. One of the results of our study is the observation that the opening times of the eggs laid on both food types were similar in both species. The similarity in the hatching times of the eggs explains that the humidity ratios

of the liver and liver-agar medium are similar, and the environmental conditions are suitable. Our findings are based on Byrd and Castner (2001). According to Greenberg and Kunich, (2002) larval maturity depends on feeding during the larval period, and the minimum larval weight for maturity is the weight of a few adult flies. In our study, when the level of increase in the weight of the larvae in L1 (0-2), L2 (2-4), and L3 (4-7) day intervals of the larvae were evaluated, it was seen that it was over 100% in both food types. *L. sericata* larval period (L1, L2, L3) weight measurements were significantly different ($p<0.05$) as a result of dependent groups T Test comparison analysis. Our weight gain results in different food types, which indicates that food type is an important factor for growth and development (Figure 1 and Figure 3), as shown by Greenberg and Kunich, (2002). According to Firoozfar et al., (2011), nutritional differences significantly affect growth and development in *L. sericata* larvae, and food type plays an important role in development. Ronald and Sherman (1995) examined the development of sterile *L. sericata* larvae reared on liver puree and sterile liver-agar. They reported that 79% (±26) of larvae reared on liver puree were viable, and 92% (±8) were viable on sterile liver-agar medium. There were no significant differences in the weights of larvae and pupae reared on the two nutrient media. According to the results obtained from our study, the percentage of larvae fed on liver agar medium was higher than the percentage of larvae fed on liver agar medium. A significant difference was obtained between the first instar larval length, first instar larval weight, second instar larval weight, third instar larval length, third instar larval weight, pupal length, and adult length measurements of *L. sericata* larvae fed with liver and liver-agar ($p<0.05$). Accordingly, the mean of the first instar larval length (Figure 4), first instar larval weight, (Figure 3), second instar larval weight (Figure 3), third instar larval length (Figure 4), third instar larval weight (Figure 3), pupal length and adult length (Figure 5) measurements of *L. sericata* larvae fed with liver-agar were higher than those fed with liver. Our findings are in agreement with Ronald and Sherman, (1995) but not in agreement with ($p>0,05$) in the sense that there was no difference between the second instar larval length measurements of *L. sericata* larvae fed with liver-agar and liver ($p>0.05$). The difference in larval second instar length measurements is due to the competition between the larvae fed at maximum speed.

Greenberg and Kunich, (2002) reported that a newly hatched Calliphorid larva, which was 0.1 milligram, was 0.0840 grams at the end of the fifth day and could increase its weight up to 800 times in 5 days with feeding. According to the findings obtained from our study, in the first period (0–2) days in *L. sericata*, those fed with liver weighed 0.011±0.001 g, and those fed with liver-agar weighed 0.012±0.001 g. In the third period (4–7) days, those fed with liver weighed 0.060±0.001 g, and those fed with liver-agar weighed 0.062±0.002 g. According to the findings obtained from our study, it was determined that the larvae can increase up to 600-650 times in 5 days, and the high rate of weight gain of the larvae is parallel with the literature data.

Hassan, (2008) and Firoozfar et al., (2011) reported that different food substrates significantly affected larval development and duration in *L. sericata* larvae. According to the findings obtained from *L. sericata* larvae reared on different nutrient substrates, it was determined that the larval viability of larvae reared on liver and liver-agar were similar to each other, and larval weights of *L. sericata* larvae reared on liver-agar were only 3.42% (±0.69) higher than those reared on liver-agar (Figure 2 and Table 2). Our findings on viability rates and weight gain are by Hassan, (2008) and Firoozfar et al., (2011).

Çakan et al., (2009) investigated the development of *L. sericata* larvae at 24ºC, 27ºC, 30ºC, 35ºC, and 50% humidity and obtained the longest larvae at 27ºC. According to our findings, the maximum length of larvae was obtained at 30ºC temperature and 50% humidity when the working environment was kept constant. Çoban, (2009) reported that the eggs of *L. sericata* opened in 8-11 hours, completed their larval stages in 80-110 hours, and the adult emerged from the pupa in 5-8 days at 27ºC-35ºC temperature and 45-65% humidity range. According to the findings of our study, it was determined that the eggs of L. sericata fed with liver-agar opened in 8-11 hours, completed their larval period between 96-168 hours, entered the pupa on the 6th day, and the adult emerged from the pupa on the 12th day. While our findings are compatible with the findings of Çoban, (2009) regarding egg opening time, they are not compatible with the completion of the larval period and the late emergence of the adult from the pupa. We think this difference is because the temperature and humidity were constant in our study, while the temperature and humidity were not constant in Çoban's (2009) study. Barnes and Gennard (2013) reared sterile *L. sericata* eggs on the non-sterile liver and sterile horse blood-agar medium, and the growth and density rates of larvae reared on blood medium were lower than those reared on the liver. Compared with our study, it was found that the larvae reared on liver and sterile liver-agar medium were better in size than those reared on liver-agar medium. However, there were no significant variations or developmental deviations.

For our findings that pupal development took place at a later time in sterile liver-agar medium compared to sterile liver medium, we think that the completion of metamorphosis in the puparium takes place at a later time, and this may be caused by the delay in the secretion of ecdysone hormone which terminates larval development. The pupae developing from the larvae whose larval development lasted longer in the sterile liver-agar medium

were morphologically darker and larger than those developing from the liver medium. In contrast, those in the liver medium were shorter and lighter in color (Figure 6). The size of the adult individuals developing from the pupae was close to each other in both food types, but the difference in the wing morphotypes of the adults observed on liver-agar medium was remarkable. Wing morphology is analysed in detail using geometric morphometric methods. However, in our study, the left wings of adults from both nutrient media were removed and the longitudinal and transverse vein length was measured to the nearest 0.1 mm under a dissecting miroscope and it was observed that the wing size of adults from liver agar medium was larger (Figure 7). In view of the extant literature (Espra et al. 2015), it can be hypothesised that this phenomenon may be attributable to disparities in the growth and development of larvae, as well as the reflection of genetic and environmental conditions.

In conclusion one of the main factors explaining the biological development and population changes of *L. sericata* and the reasons for these changes is the type and amount of food consumed in cultivation under laboratory conditions. Natural diets such as chicken liver or bovine liver, which have high reproductive potential, are often used in aquaculture. However, nowadays, various artificial diets have been developed as an option for maintaining and cultivating *L. sericata*. Although nutrient substrates such as chicken liver or beef liver, which are high in protein, have been used for rearing flies in the laboratory (Sherman & My-Tien Tran, 1995), the decomposition of these nutrient substrates can cause offensive odors due to off-gassing and contamination over time (Tachibana & Numata, 2001). However, the proposed artificial diets must be standardized to meet species-specific nutritional needs. The study we targeted was a comparative analysis of the development and duration of flies on a commonly used natural diet, sterile liver, which has a high moisture content in its nutrient content and a high tendency of female flies to lay eggs, and on an artificial diet, sterile liver-agar medium, which contains both a natural diet and an artificial diet to prevent contamination. The results of this natural diet-integrated artificial diet study in terms of developmental speed and time, hatching from eggs, moving to the next level earlier in each larval stage, completing the pupal adult formation three days earlier on average, and at the same time, larval, pupal and adult larvae in terms of weight and size are more developed compared to the natural diet. Our findings conclude that sterile liver agar media are more suitable for mass production and medical larvae supply without contamination in a short time.

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