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## Development of larvae, prepupae, pupae and adults of *Lucilia sericata* fed on animal tissue and organic food bio-wastes

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### Abstract

**Diptera:** Calliphoridae family *Lucilia sericata* is a bioagent in the fields of forensic entomology with its post-mortem minimum time interval (PMI) and cause of death predictions, behavioural ecology within the scope of differentiation in ecological diversity, and human and veterinary medicine with its larval debridement treatment effect. For researchers in these fields, the lack of a certain optimisation and standardisation of cultivation procedures under laboratory conditions poses a problem. In laboratory conditions, mostly animal tissue wastes were studied in the adult diet that complied with the protocol for *Lucilia sericata* colony continuity. In order to understand the physical and physiological effects, not only on ovarian development but also on reproductive potential, this study focuses on comparative determination of the effects on oviposition, larval, prepupal, pupal and adult development of adults on two groups of artificial diets consisting of Diet A (bovine brain, bovine spleen, fish and a mixture of all three foods) containing animal tissues and wastes and Diet B (sugar beet pulp, vegetable waste and household garbage, banana peel and a mixture of all three foods) containing food wastes. Mean value calculations and T-test results of Diet A and Diet B groups at the larval, prepupal and pupal stages showed that there was a significant difference between and within groups for nutrient substrates at each stage and this difference was statistically significant ( $p=0.001$ ).

**Keywords:** Calliphoridae, *Lucilia sericata*, artificial diet, biowaste, diet a/diet b, growth rate

### Introduction

Members of the Calliphoridae family are autogenous—the term autogenous means requiring protein to lay eggs and produce offspring (Carey 2003) [1]. *Lucilia sericata*, also known as the green bottle fly, is a common autogenous fly found in many parts of the world, and their ecology and biology are well characterized (Chandler *et al.* 2007) [2].

Postmortem interval (PMI) estimation is an essential task of forensic medicine and forensic biology, used by investigators in homicide cases to narrow down the list of suspects and clarify the circumstances of death. *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) is also a biological indicator for the estimation of the postmortem interval (PMI) and is, therefore, a necrophagous fly important in forensic medicine (Zurawski *et al.* 2009) [3].

Flies such as *Lucilia sericata* (Meigen) are typically the first organisms to reach a body after death, attracted to the cadaver by the odor produced during the early stages of decomposition. During the early stages of decomposition, larvae of such flies with growth rates follow predictable species-specific trajectories, the slopes of which depend mainly on several factors: These factors are temperature and larval age, which depends on the larval Diet and growth rate (Anderson 2000) [4]. Forensic entomologists construct a postmortem interval estimate based on the age of the larvae (Hall 2001) [5]. To enable the calculation of the age of the larvae, standard relationships between temperature and growth rate are used, and these are obtained by rearing larvae at generally constant temperatures, most commonly known in the laboratory where they feed on the liver of various mammalian species. Growth rates can be affected by daily fluctuations in variables such as food type and temperature

On the other hand, some species of fly larvae, such as *Lucilia sericata*, are also of particular importance in human medicine, where their feeding on necrotic tissues is used to heal slow-to-heal injuries that do not respond to conventional treatments, such as gangrenous ulcers or ulcers containing necrotic tissue (Cartier and Combemale 2008) [6].

Given the importance of *Lucilia sericata* in human and veterinary medicine, there is a need for more comprehensive information on its life cycle, life tables, and reproductive and population parameters, as this information could support the rearing of larvae as colonies under laboratory conditions to be used in larval therapy and also contribute to strategies for controlling the fly population in nature. According to recent studies, the tissues that *Lucilia sericata* larvae feed on significantly affect the growth rate (Tachibana and Numata 2001) [7]. The diet composition of larvae used in maggot treatment is usually animal tissue-based (Carey 2003) [1]. In larval rearing experiments, mammalian muscle (Smith 1986; Boatright and Tomberlin 2010) [8, 9], minced meat (Giao and Godoy 2007; Niederegger *et al.* 2010) [10, 11], mouse/rat carcasses (Tarone and Foran 2006) [12], fish or pigs (Ames and Turner 2003) [13] due to their easy availability and low cost. Previous studies have indicated that substrates can influence the entire life cycle, from egg production to the survival rate of synanthropic flies (Sukhaphanth *et al.* 1988) [14]. Despite diets containing this wide variety of food substrates, only limited information is available on the effect of the commonly used feeding medium on larval growth. A suitable larval diet can prepare a favorable condition for the growth of flies (Gobbi *et al.* 2013) [15]. The food consumed by *L. sericata* in laboratory conditions is critical as it affects biological development and population dynamics. Diets such as bovine or sheep liver, often used for rearing flies in the laboratory, produce offensive odors and contamination. Larval rearing in animal tissue may be undesirable due to the odor and lack of sterilization (Bambaradeniya 2017) [16]. Therefore, various artificial diets have been proposed and developed as alternatives for maintaining and rearing *L. sericata* (Tachibana and Numata 2001) [7].

In addition to having sufficient amounts of essential nutrients, artificial diets for flies must have appropriate nutritional stimuli to elicit a feeding response. Many nutrients, including sugars, some amino acids, lipids, and minerals, act as nutritional stimulants in the fly population dynamic effect (Chaudhury 2013) [17]. Studies on a chemically defined synthetic medium containing casein, yeast extract, cholesterol, inorganic salts, water, and agar are known to determine the nutritional requirements of fly larvae in terms of all essential nutrients such as amino acids, vitamins, minerals, fatty acids, nucleic acids, and carbohydrates (Gingrich 1964) [18]. In a study by Shefa (2013) [19], an artificial diet containing wheat germ, whole milk powder, dry yeast powder, cow blood, and eggs was used to feed the larvae.

Research has shown that different types of diets, such as dairy products, exhibit some advantages and disadvantages and need to be improved for the specific nutritional needs of some particular species. Thus, cultural continuity cannot be ensured (Chaudhury 2015) [20]. There are also studies in which an artificial dietary environment was created using various materials (poultry manure, pig feces, wheat bran, beer waste, fruit and vegetable peels, restaurant food waste.) consisting of decomposed plant, animal, and industrial organic wastes as a nutrient substrate for feeding and rearing larvae under laboratory conditions (Nyakeri *et al.* 2016) [21].

It has several consequences, such as increasing the world's population and improving food production and waste management systems (FAO 2011) [22]. Food waste of agricultural products represents a severe financial burden (FAO 2013) [23]. Organic waste management represents a

significant challenge, so innovations in food waste valorization technologies are crucial for the feasibility of sustainable waste management. Finding beneficial uses, developing new recycling systems, and reducing the number of losses and waste are helpful measures for both the ecological cycle in the living population and the environmental problems caused by waste (Saveyn and Eder 2014) [24].

The 1.3 billion tonnes of biowaste produced each year can be converted by some insects into insect biomass suitable for feeding animals. Effectively using insects can contribute to a sustainable circular economy (Veldkamp *et al.* 2012) [25]. Calliphoridae Dipterans and other decomposer families, such as Sarcophagidae, also play a vital role in the natural decomposition of organic matter and are favorable for mass rearing (Yang *et al.* 2014) [26]. Dipterans are one of the most common organisms found in decomposing organic matter. Some fly species offer great possibilities when setting up artificial waste decomposition systems, as they can be cultivated on different types of organic waste through the digestive processes of their larvae. Dipteran species have the advantages of relatively easy mass-rearing, high reproductive potential, and short life cycles (Putman 1983) [27]. *Hermetia illucens* (Linnaeus, 1758) (black soldier fly; BSF) and *Musca domestica* Linnaeus 1758 (housefly), which in their larval stages are commonly found in saprophagous compost heaps, carcasses, sewage, human and animal corpses and other decomposing organic matter, are the two main species with the potential to be utilized in the bioconversion of a range of different wastes in nutritious biomass (Parry *et al.* 2016) [28]. *H. illucens* has been found breeding in organic municipal waste, particularly in decomposing coffee grounds and banana peel waste (Diener *et al.* 2011) [29]. Despite the number of studies in which cultivation has been achieved on wastes containing high levels of meat or animal fat, plant wastes, manure, and a wide variety of organic wastes, not all waste products for bioconversion or other developed dietary media may be optimal for cultivation. The different types of organic waste that flies can degrade are highly variable in nutrient content. These differences lead to significant variations in larval body composition within the same species. The difficulty of rearing the animals under laboratory conditions should also be considered. Suitable larval Diet and optimum environmental conditions are crucial for successful mass rearing.

In this context, the main objective of the present study was to explain the effect of food type on larval growth and development in different variations of nutrient substrate media consisting of animal tissue-based and vegetable tissue-based nutrient substrate media comprised of organic food wastes in the cultivation of *Lucilia sericata* under laboratory conditions. The other aim is to investigate the possibilities of Dipteran *L. sericata* as an active substance in biowaste management systems and as a source for bioconversion, depending on consumption. Our main aim is to evaluate whether any differences that may arise in growth and development are due to the structure of the artificial dietary feeding environment or to the inherent structural properties of the tissue.

## Materials and Methods

This study was carried out between September 2022 and February 2023 in the Maggot Research and Development Laboratory of Ankara Yıldırım Beyazıt University, Çubuk

campus, within the scope of the Department of Traditional, Complementary and Integrative Medicine Practices.

### Stock maintenance

Adult *Lucilia sericata* (Greenbottle Blowfly) flies reared in the Maggot Research laboratory were used for the experiments. Cube-shaped cages of 40\*40\*40 cm were prepared for the experimental groups, and the environment was maintained at 25 °C  $\pm$  5 mean temperature, 60  $\pm$  5% relative humidity, and 12 h light and dark cycle photoperiodicity. A 10% sugar solution was prepared to provide a carbohydrate source, and a bottle with a cotton pad soaked in this solution was placed. The nutrient substrates to be used in the study were then introduced into the adult cages as a protein substitute necessary to initiate vitellogenesis.

### Collection of eggs for experimental cultures

An equal number of 300 *Lucilia sericata* pupae were placed in each cage for the research, and adults were expected to emerge. *Lucilia sericata* adults emerging from the pupae were fed from the bottle containing sugar solution and water in the cage for two days, and then the eight cages to be used for the two groups in the experiment were placed in small 150 mm diameter, 90 mm deep plastic bowls with equal grams of nutrient substrates, and the egg laying was monitored and the time was recorded. The eggs laid by the females in clusters were gently separated from the substrate with the help of a soft paintbrush and forceps. It is estimated that the eggs laid in the two experimental groups contained 100-5000 eggs as the minimum and maximum limit.

### Food substrates

### Composition and Preparation of the Diet

Two artificial diets consisting of eight food substrates were analyzed for their ability to support the larval development of *L. sericata* (Table 1). Diet A consisted of animal tissue-based bovine brain, bovine spleen, fish, and a mix-mix combination of these nutrients (bovine brain + bovine spleen + fish). At the same time, Diet B was prepared from vegetable organic food wastes such as sugar beet pulp waste, rotten vegetable kitchen waste, banana peel, and a mixed-mix combination of these waste nutrients (sugar beet pulp waste + kitchen waste + banana peel). The dry wastes in the Diet B were soaked with water once a day in order to create a bad odour incubation environment preference of necrophag *L. sericata*, which is one of the difficulties of growing in the laboratory, to ensure their ability to lay eggs in terms of moisture content in the feed and to consume food waste materials by breaking down depending on the composition of the feed. Food substrates in Diet-A were purchased from the same supplier on day 1, portioned and fresh frozen at -24 °C on the same day to ensure the same substrate quality for all experiments. Day and Wallman (2006) [30] mentioned in a study that larval growth on fresh substrate was comparable earlier.

On the day of the experiment, the substrate was taken out of the refrigerator to reach room temperature, decomposed, and putrefied, and the experimental process was continued this way. In total, 100 g of each type of animal waste food was weighed and transferred to small plastic bowls (diameter, 5 cm; height, 6.5 cm) weighing 25 g each. In the first and second diets, the combined groups obtained with the mixture to be formed were obtained by weighing the nutrient substrates specific to the diets in equal grams and passing them through the mixer to complete 100 grams.

**Table 1:** Diet A and Diet B Food substrates

Groups	DIET A	DIET B
Group I	Bovine brain	Sugar beet pulp
Group II	Bovine spleen	Vegetable waste and rubbish
Group III	Fish	Banana peel
Group IV	Mashed meat waste(brain, spleen, fish)	Mushed waste (sugar beet pulp, vegetable waste and rubbish, banana peel)

### Experimental setup

In two groups of 4 cages per Diet containing males and females of the species *Lucilia sericata*, the specified amount of food was given simultaneously for one day to allow females to oviposit and transition to the larval stage. Larvae were collected from each sample to standardize larval age for hatching and developing larvae; the time was 15 minutes. First, instar groups of 100 larvae were placed in 500 ml glass jars on dry wood chips. Mature larvae of the second and third stages were transferred to other 500 ml glass jars filled with a fixed number of dry wood chips. To prevent larvae from escaping, the mouths of the glass jars were covered with gauze, and a rubber band was placed over them. All the fauna were kept in the insectarium at an average temperature of 25 °C  $\pm$  5 °C., 60 $\pm$  5% relative humidity, and 12:12 LD photoperiod. Two replicate growth experiments were performed simultaneously by randomly collecting all larvae from the respective substrate from the rearing container each day. The collected larvae were killed by immersion in water for 30 seconds, dried with paper towels, and preserved in 80% ethanol (EtOH). Body lengths and weights of larvae at different stages on each respective substrate were measured. Only the data of the five most giant larvae from each collection were analyzed to ensure maximum consistency and

comparability between different substrates.

The larvae and developing pupae, which passed to different stages at different times, were separated with the help of a fine paintbrush. With the decrease in the mobility of the mobile fourth instar larvae that stopped feeding, the transition to the prepupa stage was followed by moistening the wood sawdust in the glass jar. Newly formed pupae were counted daily. Pupal lengths were measured, weighed, and then transferred to petri dishes (50 mm diameter, 10 mm deep). The emergence of adults was recorded daily. Pupae were killed by direct immersion in 80% ethanol (EtOH). Measurement, Data Processing, and Statistics The length of larvae and pupae was measured using a dissecting microscope and absolute digital calipers (Mitutoyo). Data were collected, and mean values were calculated with standard deviations. Statistical analyses were performed using t-independent samples t-test to compare independent observations between and within groups. The significance level was set as p=0.05 (95%). IBM SPSS Statistics 27 software was used for statistical analyses.

### Results

This study developed two diets with various nutrient substrates based on animal tissue and organic food waste. It

recorded and compared the growth and development parameters of *Lucilia sericata* flies reared on Diet A and Diet B.

### **Determination of *L. sericata* Life Cycle in different substrates**

#### **Egg Laying in Diet A Animal Tissues and Diet B Organic Food Waste**

For each egg retrieval medium experiment, there was a significant difference between the two dietary media in terms of egg laying time and favourability of the females when equal amounts of food were given to the control containers, and Diet A was faster and more efficient in laying eggs in animal tissues and organs. In Diet B food waste medium, the flies collected fewer eggs at a later time. There was no significant difference when the food substrates constituting Diet A and Diet B groups were compared with the substrates specific to their groups.

#### **Larval Development in Diet A Animal Tissues and Diet B Organic Food Waste**

The egg-laying and hatching time of flies feeding on bovine brain substrate was 22 hours. Most of the larvae had reached the second instar stage on the second day. With 30 mg of nutrient substrate supplemented daily to the larvae in the experimental groups, larvae that reached average size and those that did not develop were observed. Some larvae had pale white mouthparts, some had thinner mouthparts, and some had golden-colored mouthparts. According to the type of food substrate, the first larvae were seen in the bovine brain, the second larvae were seen in the bovine spleen on the second day, the third larvae were seen in fish on the third day, and the first stage and second stage larvae were seen at the end of the third day in the food group in which brain, spleen, and fish substrates were mixed into a slurry.

There was no significant difference between the larvae fed with animal tissues regarding length and weight ( $L_1$ - $L_2$ - $L_3$ ) developmental stages. When the five most giant larvae were selected and measured for size and weight, the average length of the larvae in the first larval stage ( $L_1$ ) and the last larval stage ( $L_3$ ) was 2.55-9.76 mm in bovine brain, 2.88-9.89 mm in bovine spleen and 0.13 mm longer than the larvae fed with bovine brain. In the fish substrate, the first-stage larvae were 2.43 mm long, and the third-stage larvae reached a length of 8.83 mm on average, while shorter larvae with a size of 2.38-7.60 mm were observed in the food medium with a mixture of these three substrates. The larvae fed with bovine brain and bovine spleen were 0.013-0.015 mg heavier in the second stage and 0.023-0.042 mg heavier in the third stage compared to the group fed with fish and chopped in the mix.

There were no significant inconsistent interactions between organs, organ structures, or animal species during the feeding period of larvae in the Diet A medium. Animal food source structure had no significant effect on larval crawling time. The difference was evident when compared to larvae fed on food waste in the Diet B environment. The egg-laying time and larval development of flies fed on Diet B medium, which was created by using various organic food wastes, were prolonged compared to Diet A. Larval lengths were shorter and lighter in terms of each stage. In larval development, a wide range of mortality was observed in each substrate from the first to the second stage.

According to the measurements of the larvae determined for data analysis in the larval stage as  $L_1$ - $L_2$ - $L_3$  stage, the longest

larvae were obtained in sugar beet pulp waste. On average, larvae with a length of 1.89 mm in the first stage could develop up to 5.12 mm in the third stage. Longer larvae than the other food waste-based nutrient substrate environments were obtained in the fourth group, mixed with sugar beet, vegetable rot, garbage waste, and banana peels, and the average larval period was 1.87- 4.36-5.16 mm. In the Diet B medium, the shortest larvae were observed and recorded in banana peels, which could develop up to 5.07 mm. This measurement result is consistent with other studies in the literature (Diener *et al.*, 2011) [29]. The longest larvae growing on vegetable and rubbish waste were, on average, 0.03 mm shorter than those growing on sugar beet pulp waste. Generally, the maximum length of larvae growing on food waste was equal to that of second-instar larvae feeding on animal tissue. Movement of the larvae during the crawling period was prolonged, raising the possibility that the food source's structure may affect the crawling period.

Since the larvae grown in the Diet B medium did not develop as much as those grown in the Diet-A medium, they could not be replicated more than the second replication, so the evaluations were made according to two replications. The larval weights of the larvae obtained were 0.046-0.091 mg in sugar beet pulp waste, 0.035-0.062 mg in kitchen waste, 0.024-0.042 mg in banana peels, 0.046-0.086 mg in a mixture of these substrates, which showed significant differences compared to the larval weight in Diet A medium. Low values were recorded in the species fed on different types of food in Diet B (Table 2). The result for *L. sericata* showed that the growth rate of flies fed on animal tissue organs showed a significant difference compared to those fed on food waste, and those cultured on food waste showed a considerable difference compared to those cultured on animal tissue, organ, or animal species, respectively.

#### **Prepupa and Pupa Development in Diet A Animal Tissues and Diet B Organic Food Waste**

In the larvae that stopped feeding after the third larval stage in Diet A, migration was observed on the fourth day in larvae reared on bovine brain and bovine spleen, on the fifth day in larvae reared on fish, and on the sixth day in the minced meat group in which all substrates were mixed. Of the prepupae that pupated on Diet A grown on animal tissues, the maximum prepupa length was 12.53 mm on average in larvae grown on bovine brain organs. In other food substrates, the length ranged between 10.83 and 11.45 mm. The first pupation was recorded on day eight on the bovine brain, day nine on the bovine spleen, day ten on fish, and day 11 on the mixed minced meat substrate. The maximum pupal length was 6.71 mm in those grown on bovine spleen organs, while the lengths of the pupae in the other food substrate media, which were not significantly different, ranged between 6.32 mm and 6.54 mm on average. The general appearance of the pupae was light brown or dark brown, and young pupae of white color were also present. The maximum pupal weight about length was 0.168 mg, obtained from those grown on a bovine spleen.

The migration time of larvae in the Diet B medium was longer than that in the Diet A medium on each food substrate. Prepupae were light brown, ranged from 6.11 mm to 7.78 mm long, and weighed from 0.055 to 0.103 mg minimum and maximum. According to the length and weight growth parameters, they were almost half the length and weight of those in Diet A medium. The largest and heaviest pupae were



obtained from those grown on sugar beet waste substrate with an average length of 5.41 mm and a weight of 0.086 mg parallel with larval development.

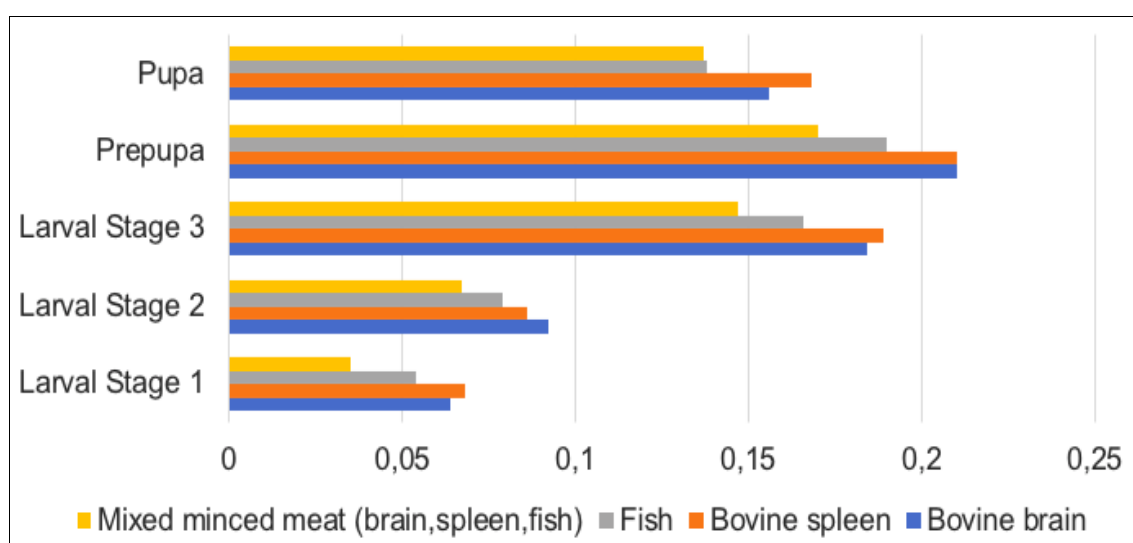
**Table 2:** Distribution and comparison of fly larval stage, prepupa, and pupa weights between Diet A and Diet B groups

Variable	Group				t- test	
	Diet A		Diet B			
	Mean	SS	Mean	SS	t- test	p
Larvae Stage 1	0,06	0,01	0,04	0,01	4,787	0,001*
Larvae Stage 2	0,08	0,01	0,04	0,01	12,100	0,001*
Larvae Stage 3	0,17	0,02	0,07	0,02	17,070	0,001*
Prepupa	0,20	0,02	0,08	0,02	20,900	0,001*
Pupa	0,15	0,01	0,06	0,02	16,110	0,001*

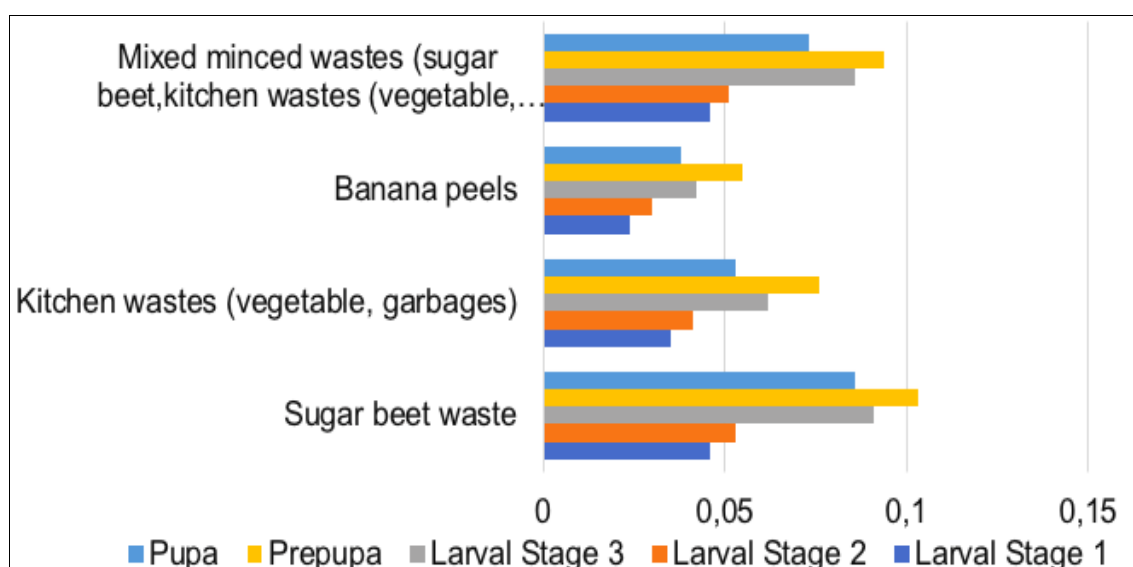
\* $p < 0,05$ ; t= independent samples t-test

According to the mean value calculations and T-test results of the Diet A group and Diet B group in the periodic larvae, prepupa, and pupa stages, it was observed that there was a significant difference between Diet A and Diet B at each

stage, and this difference was statistically significant ( $p=0.001$ ). It was determined that the weight levels of  $L_1$ ,  $L_2$ , and  $L_3$  larvae, prepupae, and pupae developing from larvae fed with Diet A were higher (Fig. 1a and Fig. 1b).



**Fig 1a:** Diet A Larvae stage ( $L_1$ ,  $L_2$ ,  $L_3$ ) Prepupae and pupae weight charge



**Fig 1b:** Diet B Larvae stage ( $L_1$ ,  $L_2$ ,  $L_3$ ) Prepupae and pupae weight charge

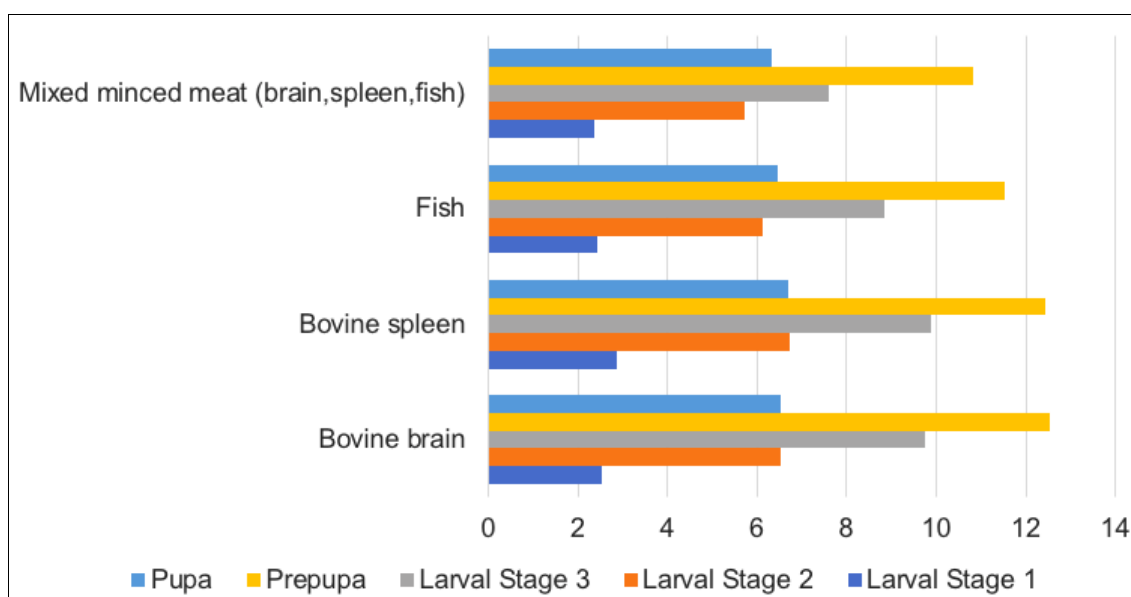
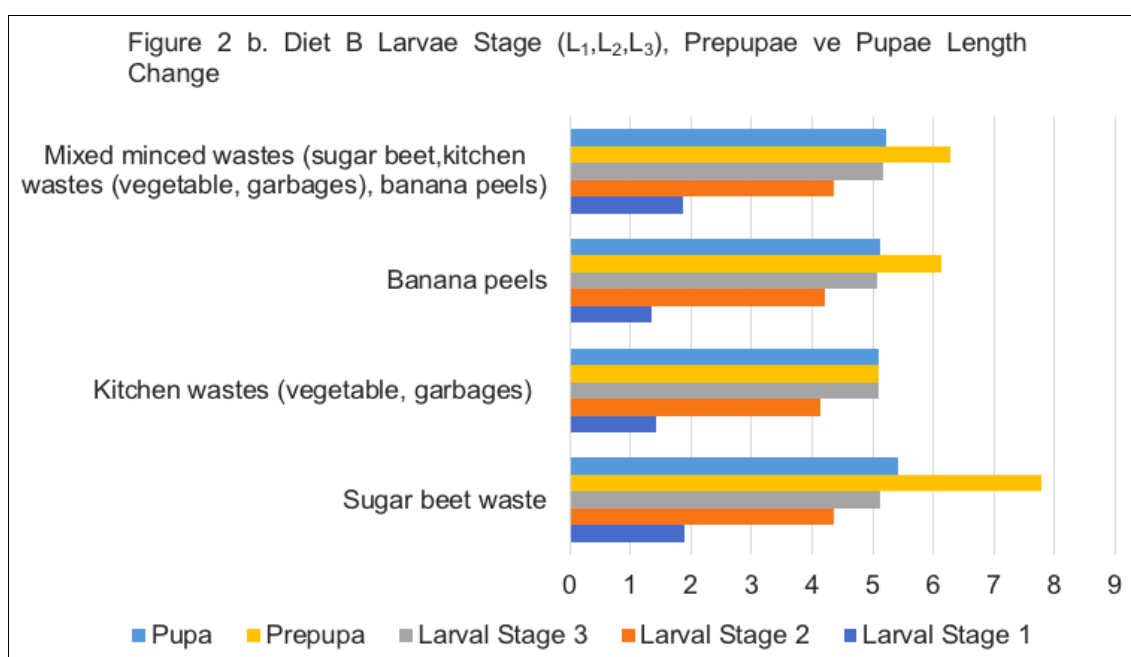
**Table 3:** Distribution and comparison of fly larval stage, prepupa, and pupa lengths between Diet A and Diet B groups

Variable	Group				t- test	
	DIET A		DIET B			
	Mean	SS	Mean	SS	t -test	p*
Larvae Stage 1	2,56	0,20	1,64	0,25	12,910	0,001*
Larvae Stage 2	6,28	0,39	4,25	0,09	22,400	0,001*
Larvae Stage 3	9,02	0,94	5,10	0,04	18,620	0,001*
Prepupa	11,83	0,72	6,54	0,74	22,910	0,001*
Pupa	6,51	0,15	5,18	0,15	27,870	0,001*

\* $p < 0,05$ ; t= independent samples t-test

According to the mean value calculations and T-test results of the Diet A group and Diet B group in the periodic larvae, prepupa, and pupa stages, there was a significant difference between Diet A and Diet B at each stage, and this difference

was statistically significant ( $p=0.001$ ) (Table 3). L<sub>1</sub>, L<sub>2</sub>, and L<sub>3</sub> larvae fed with Diet A, and prepupa and pupa length levels developing from larvae were higher (Fig. 2a and Fig. 2b).

**Fig 2a:** Diet A Larvae stage (L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>) Prepupae and pupae length charge**Fig 2b:** Diet A Larvae stage (L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>) Prepupae and pupae Length charge

**Table 4:** Comparison of fly larval stage, prepupa, and pupa weights in Diet A group within group food types

Variable	Category	Food Type		ANOVA		
		Mean	SS	F	p	Difference
Larvae Stage 1	Bovine Brain <sup>(1)</sup>	0,064	0,003	622,9	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	0,068	0,000			4<2
	Fish <sup>(3)</sup>	0,054	0,000			4<3
	Mixed Minced Meat <sup>(4)</sup>	0,035	0,001			
Larvae Stage 2	Bovine Brain <sup>(1)</sup>	0,092	0,001	326,6	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	0,086	0,000			4<2
	Fish <sup>(3)</sup>	0,079	0,001			4<3
	Mixed Minced Meat <sup>(4)</sup>	0,065	0,002			
Larvae Stage 3	Bovine Brain <sup>(1)</sup>	0,184	0,002	133,5	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	0,188	0,001			4<2
	Fish <sup>(3)</sup>	0,165	0,000			4<3
	Mixed Minced Meat <sup>(4)</sup>	0,146	0,001			
Prepupa	Bovine Brain <sup>(1)</sup>	0,212	0,011	48,74	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	0,210	0,000			4<2
	Fish <sup>(3)</sup>	0,194	0,000			4<3
	Mixed Minced Meat <sup>(4)</sup>	0,175	0,001			
Pupa	Bovine Brain <sup>(1)</sup>	0,156	0,001	233,5	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	0,168	0,000			4<2
	Fish <sup>(3)</sup>	0,138	0,000			4<3
	Mixed Minced Meat <sup>(4)</sup>	0,137	0,000			

\* $p < 0,05$ ; F=ANOVA Test; Difference =Tukey Test

In terms of the mean values of the weights of the first-stage larvae ( $L_1$ ) in the Diet A group consisting of different types of food, the weight of the first-stage larvae fed with bovine brain waste was 0.064 mg, those fed with bovine spleen waste was 0.068 mg, those fed with fish was 0.054 mg. The weight of those fed with a mixture of three food substrates of brain, spleen, and fish waste minced through a mixer was 0.035 mg. Since the mean value of the bovine spleen food type was the highest, it is seen that it is more effective in this category than the others. According to the ANOVA test, there was a significant difference between the categories ( $F = 622.9$ ,  $p < 0.001$ ). The mean weights of second instar larvae ( $L_2$ ) were 0.092 mg for those fed with bovine brain waste, 0.086 mg for those fed with bovine spleen waste, 0.079 mg for those fed with fish waste, and 0.065 mg for those fed with waste minced by mixing three food substrates of brain, spleen and fish waste. Since the mean value of the bovine brain waste food type was the highest, it is seen that it is more effective than the others in this category. According to the ANOVA test, there was a significant difference between the categories ( $F = 326.6$ ,  $p < 0.001$ ). The mean weights of third instar larvae ( $L_3$ ) were 0.184 mg for those fed with bovine brain waste, 0.188 mg for those fed with bovine spleen waste, 0.165 mg for those fed with fish waste, and 0.146 mg for those fed with waste minced by mixing three food substrates of brain, spleen and fish waste. Since the mean value of the bovine spleen waste food type was the highest, it is seen that it is more effective than the others in this category. According to the ANOVA test result, there is a significant difference between

the categories ( $F = 133.5$ ,  $p < 0.001$ ) (Table 4).

The average weight of the prepupae developing by feeding with bovine brain waste was 0.212 mg, the prepupae developing from bovine spleen waste were 0.210 mg, those fed with fish was 0.194 mg, and the weight of the prepupae developing by feeding with waste minced by passing the three nutrient substrates of bovine brain and spleen as well as fish waste through a mixer was 0.175 mg. Since the mean value of bovine brain waste food type is the highest, it is seen that it is more effective in this category than the others. According to the ANOVA test result, there is a significant difference between the categories ( $F = 48.74$ ,  $p < 0.001$ ) (Table 4).

The average weight of pupae developed by feeding with bovine brain waste was 0.156 mg, the average weight of pupae developed after feeding with bovine spleen waste was 0.168 mg, the average weight of pupae developed after feeding with fish waste was 0.138 mg, and the average weight of pupae developed by feeding with waste minced by passing three food substrates of bovine brain and spleen and fish waste through a mixer was 0.137 mg. Since the mean value of the bovine spleen food type was the highest, it is seen that it is more effective in this category than the others. According to the ANOVA test, there was a significant difference between the categories ( $F = 233.5$ ,  $p < 0.001$ ). These results indicate that different food types significantly affect growth and development in larval prepupal and pupal stages. However, which food type is effective in each category may vary (Table 4).

**Table 5:** Comparison of larval, prepupal, and pupal lengths of flies in Diet A group within food types

Variable	Category	Food Type		ANOVA		
		Ort.	SS	F	p*	Difference
Larvae Stage1	Bovine Brain <sup>(1)</sup>	2,55	0,02	297,6	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	2,88	0,03			4<2
	Fish <sup>(3)</sup>	2,43	0,03			4<3
	Mixed Minced Meat <sup>(4)</sup>	2,38	0,03			
Larvae Stage2	Bovine Brain <sup>(1)</sup>	6,52	0,03	130,3	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	6,74	0,04			4<2
	Fish <sup>(3)</sup>	6,12	0,03			4<3
	Mixed Minced Meat <sup>(4)</sup>	5,74	0,02			
Larvae Stage3	Bovine Brain <sup>(1)</sup>	9,76	0,02	604,9	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	9,89	0,04			4<2
	Fish <sup>(3)</sup>	8,83	0,03			4<3
	Mixed Minced Meat <sup>(4)</sup>	7,60	0,03			
Prepupa	Bovine Brain <sup>(1)</sup>	12,54	0,01	468,5	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	12,45	0,02			4<2
	Fish <sup>(3)</sup>	11,52	0,03			4<3
	Mixed Minced Meat <sup>(4)</sup>	10,83	0,04			
Pupa	Bovine Brain <sup>(1)</sup>	6,54	0,03	163	0,001*	4<1
	Bovine Spleen <sup>(2)</sup>	6,71	0,03			4<2
	Fish <sup>(3)</sup>	6,46	0,03			4<3
	Mixed Minced Meat <sup>(4)</sup>	6,32	0,03			

\* $p < 0,05$ ; F=ANOVA Test; Difference =Tukey Test

In the Diet A group, the mean length of the first stage larvae ( $L_1$ ) fed with bovine brain waste was 2.55 mm, while it was 2.88 mm for those fed with bovine spleen waste, 2.43 mm for those fed with fish, 2.38 mm for the first stage larvae fed with a mixture of three food substrates of bovine brain and spleen as well as fish waste. In the first stage larval ( $L_1$ ) length category, bovine spleen waste food type had the highest mean value (2.88 mm), followed by bovine brain waste (2.55 mm) and fish waste (2.43 mm). Those fed a mixture of three food substrates of bovine brain and spleen and fish waste minced in a mixer had the lowest mean value (2.38 mm).

According to the ANOVA test, there was a significant difference between the categories ( $F = 297.6$ ,  $p < 0.001$ ) (Table 5). In addition, according to the post-hoc test results, we observed that the fish waste food type was less than all other food types, and there was a significant difference. The mean lengths of second instar larvae ( $L_2$ ) were 6.52 mm for those fed with bovine brain waste, 6.74 mm for those fed with bovine spleen waste, 6.12 mm for those fed with fish, and 5.74 mm for those fed with a mixture of three food substrates of bovine brain and spleen and fish waste. In the second instar larval ( $L_2$ ) length category, bovine spleen waste food type had the highest mean value (6.74 mm), followed by bovine brain waste (6.52 mm) and fish waste (6.12 mm). The lowest mean value (5.74 mm) was for those fed a mixture of the three food substrates of bovine brain, spleen, and fish waste minced in a mixer. In addition, according to the post-hoc test results, it was observed that the fish waste food type was less than all other food types, and there was a significant difference. The mean lengths of second instar larvae ( $L_2$ ) were 6.52 mm for those fed with bovine brain waste, 6.74 mm for those fed with bovine spleen waste, 6.12 mm for those fed with fish, and 5.74 mm for those fed with a mixture of three food substrates of bovine brain and spleen and fish waste. In the second instar larval ( $L_2$ ) length category, bovine spleen waste food type had the highest mean value (6.74 mm), followed by bovine brain waste (6.52 mm) and fish waste (6.12 mm). The lowest mean value (5.74 mm) was for those fed a mixture of the three food substrates of bovine brain, spleen, and fish waste minced in a mixer.

According to the ANOVA test result, there is a significant difference between the categories ( $F = 130.3$ ,  $p < 0.001$ ). Also, according to the results of the post-hoc test, it was seen that the fish waste food type was less than all other food types, and there was a significant difference. In terms of the mean values of the lengths of third instar larvae ( $L_3$ ), it was determined that those fed with bovine brain waste reached 9.76 mm, those fed with bovine spleen waste reached 9.89 mm, those fed with fish waste reached 8.83 mm, and those fed with a mixture of bovine brain and bovine spleen as well as fish waste minced by passing three food substrates through a mixer reached 7.60 mm. In the third-period length category, bovine spleen waste food type had the highest mean value (9.89 mm), followed by those fed with bovine brain waste (9.76 mm) and fish waste (8.83 mm). The lowest mean value (7.60 mm) was found in those fed a mixture of the three food substrates of bovine brain and spleen and fish waste minced in a mixer (Table 5).

According to the ANOVA test result, there is a significant difference between the categories ( $F = 604.9$ ,  $p < 0.001$ ). In addition, according to the post-hoc test results, it was seen that the waste fish food type was less compared to all other food types, and there was a significant difference. The average length of prepupae fed with bovine brain waste was 12.54 mm, 12.45 mm, 11.52 mm, and 10.83 mm for those fed with bovine spleen waste, bovine brain and spleen, and fish waste, respectively. In the prepupal length category, bovine brain waste food type had the highest mean value (12.54 mm), followed by bovine spleen waste (12.45 mm) and fish waste (11.52 mm). Those fed a mixture of three food substrates of bovine brain and spleen and fish waste minced in a mixer had the lowest mean value (10.83 mm). According to the ANOVA test, there was a significant difference between the categories ( $F = 468.5$ ,  $p < 0.001$ ). In addition, according to the post-hoc test results, it was observed that fish food type was less than all other food types, and it was stated that there was a significant difference. The mean pupal length of the pupae fed with bovine brain waste was 6.54 mm, 6.71 mm for those fed with bovine spleen waste, 6.46 mm for those fed with fish, and 6.32 mm for those fed with a mixture of three food



substrates of bovine brain and spleen and fish waste. In the pupal length category, bovine spleen waste had the highest mean value (6.71 mm), followed by bovine brain waste (6.54 mm) and fish waste (6.46 mm). Those fed a mixture of three food substrates of bovine brain and spleen and fish waste minced in a mixer had the lowest mean value of 6.32 mm. According to the ANOVA test result, there is a significant difference between the categories ( $F = 163$ ,  $p < 0.001$ ) (Table 5). Also, according to the results of the post-hoc test, it was

seen that the fish waste food type was less compared to all other food types, and a significant difference was indicated. These assessments show the effect of different food types on weight and length variables at different larval stages and on prepupal and pupal weight and length variables. In particular, bovine brain and bovine spleen waste food types have the highest mean values in most cases and differ significantly according to the results of the ANOVA test.

**Table 6:** Comparison of fly larval stage, prepupa, and pupa weights between food types in Diet B group

Variable	Category	Food Type		ANOVA		
		Mean	SS	F	p*	Difference
Larvae Stage 1	Sugar Beet Waste <sup>(1)</sup>	0,05	0,00	733,4	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	0,04	0,00			3<2
	Banana Peels <sup>(3)</sup>	0,02	0,00			3<4
	Mixed Minced Wasted <sup>(4)</sup>	0,05	0,00			
Larvae Stage 2	Sugar Beet Waste <sup>(1)</sup>	0,05	0,00	209,7	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	0,04	0,00			3<2
	Banana Peels <sup>(3)</sup>	0,03	0,00			3<4
	Mixed Minced Wasted <sup>(4)</sup>	0,05	0,00			
Larvae Stage 3	Sugar Beet Waste <sup>(1)</sup>	0,09	0,00	440,7	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	0,06	0,00			3<2
	Banana Peels <sup>(3)</sup>	0,04	0,00			3<4
	Mixed Minced Wasted <sup>(4)</sup>	0,09	0,00			
Prepupa	Sugar Beet Waste <sup>(1)</sup>	0,10	0,00	250,8	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	0,08	0,00			3<2
	Banana Peels <sup>(3)</sup>	0,05	0,00			3<4
	Mixed Minced Wasted <sup>(4)</sup>	0,09	0,00			
Pupa	Sugar Beet Waste <sup>(1)</sup>	0,09	0,00	585,6	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	0,05	0,00			3<2
	Banana Peels <sup>(3)</sup>	0,04	0,00			3<4
	Mixed Minced Wasted <sup>(4)</sup>	0,08	0,00			

\* $p < 0,05$ ; F=ANOVA test; Difference=Tukey test

In the Diet B group, the average weight of the first-stage larvae ( $L_1$ ) was 0.05 mg for those fed with organic sugar beet waste, 0.04 mg for those fed with kitchen waste, 0.02 mg for banana peels, and 0.05 mg for those fed with a mixture of three food substrates of sugar beet kitchen waste and banana peels. Since the mean value of the sugar beet waste was the highest, it is seen that this food type is more effective than the others in this category. According to the result of the ANOVA test, there is a significant difference between the weights of the first instar larvae related to different types of plant food and organic plant waste food for this variable ( $F = 733.4$ ,  $p < 0.001$ ) (Table 6). The mean weights of second instar larvae ( $L_2$ ) were 0.05 mg for those fed with sugar beet waste, 0.04 mg for those fed with kitchen waste, 0.03 mg for those fed with banana peel waste, and 0.05 mg for those fed with a mixture of sugar beet kitchen waste and banana peel waste chopped by passing the three food substrates through a mixer. Since the mean value of sugar beet waste is the highest, it is seen that this food type is more effective in this category than the others.

According to the ANOVA test, there was a significant difference between the categories ( $F = 209.7$ ,  $p < 0.001$ ). The average weight of third instar larvae ( $L_3$ ) was 0.09 mg for those fed sugar beet waste, 0.06 mg for those fed kitchen waste, 0.04 mg for banana peel waste, and 0.09 mg for those fed a mixture of three food substrates of sugar beet kitchen waste and banana peel waste. Since the mean value of the sugar beet waste was the highest, it is seen that this food type

is more effective in this category than the others. According to the ANOVA test, there was a significant difference between the categories ( $F = 440.7$ ,  $p < 0.001$ ). The average weight of prepupae fed with sugar beet waste was 0.10 mg, prepupae fed with kitchen waste was 0.08 mg, those fed with banana peels was 0.05 mg, and prepupae fed with the combined mixture of sugar beet kitchen waste and banana peel waste three nutrients substrates chopped through a mixer was 0.09 mg. Since the mean value of the sugar beet waste substrate was the highest, this substrate was more effective than the others in this category. According to the ANOVA test, there was a significant difference between the categories ( $F = 250.8$ ,  $p < 0.001$ ) (Table 6).

Regarding pupal weights, the pupae fed with sugar beet waste were 0.09 mg, those fed with kitchen waste were 0.05 mg, and those fed with banana peels were 0.04 mg. The weight of the prepupae fed with the combined mixture of sugar beet kitchen waste and banana peel waste, three nutrient substrates chopped through a mixer, was 0.08 mg. Since the mean value of the sugar beet waste substrate was the highest, this substrate was more effective than the others in this category. According to the ANOVA test, there was a significant difference between the categories ( $F = 585.6$ ,  $p < 0.001$ ) (Table 6). The data shows that sugar beet waste is more effective than others in different larval, prepupal, and pupal stages. This may suggest that differences and diversity in food types play an important role in larval development, prepupa and pupa formation, and, thus, adult development.

**Table 7:** Comparison of larval, prepupal, and pupal lengths of flies in Diet B group within food types

Variable	Category	Food Type		ANOVA		
		Ort.	SS	F	p	Fark
Larvae Stage1	Sugar Beet Waste <sup>(1)</sup>	1,89	0,02	502,8	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	1,43	0,03			3<2
	Banana Peels <sup>(3)</sup>	1,36	0,01			3<4
	Mixed Minced Wasted <sup>(4)</sup>	1,86	0,04			
Larvae Stage2	Sugar Beet Waste <sup>(1)</sup>	4,35	0,02	112,8	0,001*	2<1
	Kitchen Wastes <sup>(2)</sup>	4,13	0,02			2<3
	Banana Peels <sup>(3)</sup>	4,20	0,02			2<4
	Mixed Minced Wasted <sup>(4)</sup>	4,33	0,03			
Larvae Stage3	Sugar Beet Waste <sup>(1)</sup>	5,12	0,02	12,95	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	5,09	0,02			3<2
	Banana Peels <sup>(3)</sup>	5,05	0,01			3<4
	Mixed Minced Wasted <sup>(4)</sup>	5,14	0,03			
Prepupa	Sugar Beet Waste <sup>(1)</sup>	7,78	0,03	748,1	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	6,11	0,03			3<2
	Banana Peels <sup>(3)</sup>	6,02	0,01			3<4
	Mixed Minced Wasted <sup>(4)</sup>	6,27	0,01			
Pupa	Sugar Beet Waste <sup>(1)</sup>	5,41	0,02	326	0,001*	3<1
	Kitchen Wastes <sup>(2)</sup>	5,08	0,03			3<2
	Banana Peels <sup>(3)</sup>	5,03	0,02			3<4
	Mixed Minced Wasted <sup>(4)</sup>	5,22	0,01			

\* $p < 0,05$ ; F=ANOVA test; Difference=Tukey test

In the Diet B group, the average length of the first larvae ( $L_1$ ) was 1.89 mm in those fed with organic sugar beet pulp waste, 1.43 mm in those fed with kitchen waste, 1.36 mm in banana peels, and 1.86 mm in those fed with a combined mixture of sugar beet kitchen waste and banana peels, chopped through a mixer of three food substrates. Since the mean value of the sugar beet waste was the highest, it is seen that this food type is more effective than the others in this category. According to the result of the ANOVA test, there is a significant difference between organic vegetable waste food for this variable in terms of the length of first instar larvae for different types of vegetable food ( $F = 502.8$ ,  $p < 0.001$ ) (Table 7). The mean length of the second instar larvae ( $L_2$ ) was 4.35 mm for those fed with sugar beet waste, 4.13 mm for those fed with kitchen waste, 4.20 mm for those fed with banana peel waste, and 4.33 mm for those fed with a mixture of sugar beet pulp, kitchen waste and banana peel waste substrate chopped through a mixer. Since the mean value of the sugar beet waste was the highest, it is seen that this food type is more effective in this category than the others. According to the ANOVA test, there was a significant difference between the categories ( $F = 112.8$ ,  $p < 0.001$ ) (Table 7). The average length of the third instar larvae ( $L_3$ ) was 5.12 mm for those fed sugar beet waste, 5.09 mm for those fed kitchen waste, 5.05 mm for banana peel waste, and 5.14 mm for those fed the combined mixture of sugar beet kitchen waste and banana peel waste, which was shredded by passing the three food substrates through a mixer. Since the mean value of the sugar beet waste was the highest, it is seen that this food type was more effective in this category than the others. According to the ANOVA test result, there is a significant difference between the categories ( $F = 12.95$ ,  $p < 0.001$ ) (Table 7). The mean length of the prepupae was 7.78 mm for those growing on sugar beet waste, 6.11 mm for those ever-increasing on rotten vegetable kitchen waste and rubbish, 6.02 mm for those ever-increasing on banana peels, and 6.27 mm for those growing on a combined mixture of sugar beet kitchen waste and banana peel waste chopped with a mixer of three nutrient substrates. Since the mean value of sugar beet waste is the highest, it is seen that this food type is more effective in this

category than the others. According to the ANOVA test result, there is a significant difference between the categories ( $F = 748.1$ ,  $p < 0.001$ ) (Table 7).

The pupal length of those developing with sugar beet waste was 5.41 mm, the average pupal length. In contrast, those developing by feeding on rotten vegetables, kitchen waste, and garbage were 5.08 mm, and those developing from banana peels were 5.03 mm. The pupal length was 5.22 mm in those developing with a combined mixture of three food substrates: sugar beet, kitchen waste, and banana peel waste. As in the previous stages of the life cycle, the average value of the sugar beet waste was the highest in pupal length development, and it was seen that this food type was more effective than the others in this category. According to the ANOVA test, there was a significant difference between the categories ( $F = 326$ ,  $p < 0.001$ ) (Table 7). As a result, the sugar beet waste seems more effective than others in different larval stages, pupal preparation, and pupal formation. This suggests that this species plays a vital role in the larval development of prepupa and pupa formation.

For data analyses, two replicate growth experiments were carried out on flies reared in different dietary substrates in which laboratory environmental conditions were kept constant. To determine the survival life table according to the number of larvae and pupae according to the growth stages, the flies grown in the Diet B medium were inadequate to obtain the second regeneration, perhaps because they could not adapt to the ambient conditions. For *Lucilia sericata* grown in Diet A and Diet B food groups, the life cycle table for the transition to the first larva, pupa, and adult individual according to the growth stages is shown (Table 8).

Of the flies reared on Diet A food substrate groups, two and more than two flies were reared on bovine brain, and  $\frac{3}{4}$  the survival rate was observed in those reared on bovine spleen. This was followed by those reared on bovine spleen, mixed and minced brain, spleen and fish food substrate, and finally fish food medium. Survival in Diet B nutrient substrate groups was 75% lower than in Diet A medium with single replication results. Among the food groups in the Diet B medium, the highest survival rate was observed in those

grown on sugar beet pulp waste, paralleling development and growth. Banana peel waste did not have the potential to fulfill

the pupation needs of the larvae (Fig. 3a and Fig. 3b).

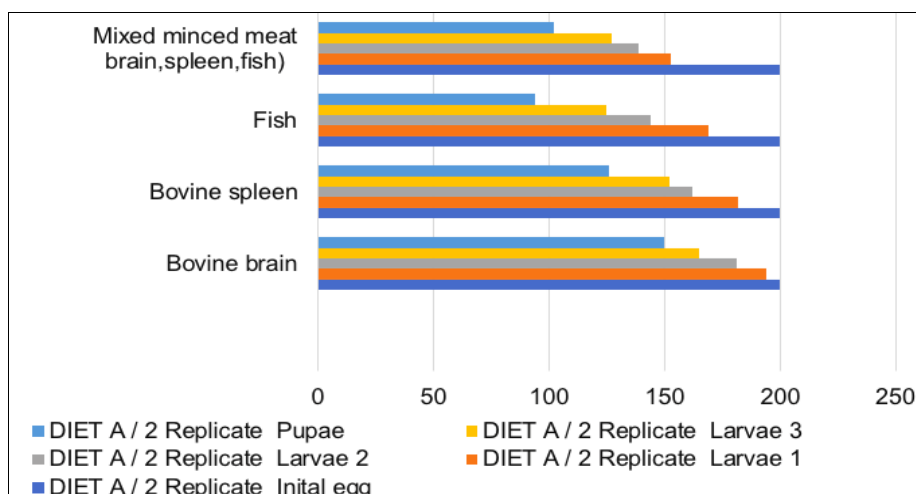
**Table 8:** Time of first larvae, pupae, and adult individuals in the life cycle of *Lucilia sericata* fed with different food types

DIET A	Food substrate type	First larva	First pupa	First adult
<i>Lucilia sericata</i>	Bovine brain	Day 2	Day 8	Day 13
	Bovine spleen	Day 2	Day 9	Day 14
	Fish	Day 3	Day 10	Day 15
	Mixed minced meat (brain,spleen,fish)	Day 3	Day 11	Day 16
DIET B	Food substrate type	First larva	First pupa	First adult
<i>Lucilia sericata</i>	Sugar beet pulp waste	Day 3	Day 10	Day 15
	Kitchen wastes (vegetable, garbages)	Day 4	Day 11	Day 16
	Banana peels	Day 4	Day 11	Day 18
	Mixed minced wastes (sugar beet, kitchen wastes (vegetable, garbages), banana peels)	Day 4	Day 11	Day 16

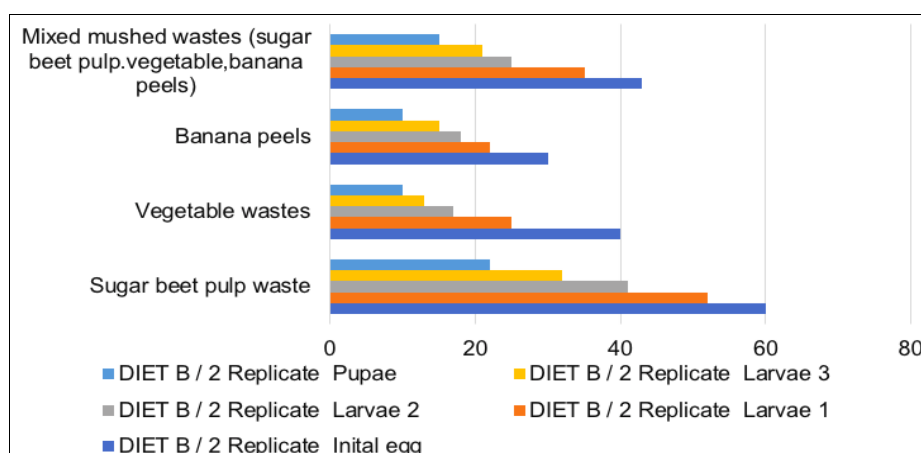
### Adult Development in Diet A Animal Tissues and Diet B Organic Food Waste

There was no significant difference between Diet A nutrient substrate groups in terms of time to pupal emergence. The first adult individual was seen on the 13th day in those cultured on bovine brain food substrate. In *Lucilia sericata* flies, which have a life cycle of 14-16 days, the first adult individuals were seen on the 14th day in those cultured on bovine spleen organ, on the 15th day in fish, and on the 16th day in the mixed and minced (brain, spleen, fish) meat group.

No different effects of animal species, organ type, or tissue structure on adult size were observed by qualitative observations. In Diet B medium, the first adult individuals were seen in those grown on sugar beet pulp waste on the 15th day, while adults were recorded on the 16th and 18th days in other food wastes. The size of adult individuals in the two artificial diet environments, Diet A and Diet B, significantly differed from qualitative observations. Individuals in the Diet B environment were smaller and had poor mobility compared to Diet A adults.



**Fig 3a:** Survival rates of eggs, larvae and pupae in Diet A medium after two replicates



**Fig 3b:** Survival rates of eggs, larvae and pupae in Diet B medium after two replicates

### Discussion

We measured how a larval diet of animal- and plant-derived waste food affects *L. sericata* fly species development,

survival, and body composition to optimize fly mass rearing using low-cost elements. Our observations and results conclude that *L. sericata* is a promising agent for digesting all

types of organic waste (manure, garbage, kitchen waste, or agricultural food waste). However, animal waste is a better agent than vegetable waste.

The study results showed that *L. sericata* fed on animal tissue-based and organic food waste substrates and that the larvae increased or decreased in length and weight differently. This increase or decrease in growth rate was attributed to the nutrients present in the substrates. The food's nutrient content contributes to the larvae's size by enabling the food species to feed on *L. sericata* (Clark *et al.* 2006) [31]. Regarding the growth rate of larvae in dipterous flies, the moisture content of food is a significant factor affecting feeding. Animal tissue-based food substrates positively affect *Lucilia*'s growth rate, except for making them larger (Chandler *et al.* 2007) [2]. This may be because *L. sericata* is a necrophagous fly species or because it can decompose decomposing meat tissues better. Therefore, *L. sericata* may be more able to mechanically break down animal tissue, organs, or animal-type food than organic food waste substrates. At the same time, this suggests that food substrates for cultivation should not be solid, liquid, moist, or humid. The lower weight and size of *L. sericata* grown on different organic waste substrates, which were more solid and had lower moisture content than animal tissues, confirmed our assessment. Hard tissues take longer to break down (Day and Wallman 2006) [30], and flies prefer soft, moist, delicate tissues rather than hard ones (Gullon and Cranston 2004) [32]. This explains why *L. sericata* developed on the brain gave significantly higher values for larval weight and length, pupal size, and adult development than those produced on other substrates, whether animal tissues, organs, or organic food waste.

White meat (brain) contains less connective tissue than red meat (meat and liver) (Robinson 2001) [33], so it is easier for flies to consume fiber-rich brain tissue for food. Variations in growth rates may be due to differences in the energy source of protein and high-fat content of food types and their preference by flies. Therefore, the main factors contributing to the observed differences in growth rate are tissue structure, the activity of proteolytic enzymes, and the amount of soluble protein. Still, other nutrients may also play a role. Females of the autogenous species *Lucilia sericata* require protein-sourced nutrients to initiate vitellogenesis (Hayes *et al.* 1999) [34]. For this purpose, the contribution of white and red meat and blood, the protein sources in Diet A, was probably more optimal than that in Diet B. This contribution helped the formation of embryos with better potential capabilities for adaptation and development (Browne 2001) [35]. The eggs obtained from these embryos were successful from the early stages of the biological cycle of the *L. sericata* fly until adult individuals were obtained. The protein nutrient sources in Diet A compared to Diet B support the lower mortality and rapid developmental adaptability of larvae hatched in the early stages of the life cycle. Davies and Hobson's theory (1935) [36] suggests that after nutrients, moisture is the most critical factor for survival in the early stages of the life cycle. Considering the composition of the two diets, the food substrates in Diet B may contribute to more significant water loss.

From a different perspective, using flies for bioconversion has significant benefits and commercial potential (Sánchez-Muros *et al.* 2014) [37]. Fly larvae can reduce and recycle plant waste (Tschirner and Simon 2015) [38]. Fly larvae are bioavailable tools for reducing and recycling plant waste. Fly larvae can effectively reduce various types of organic waste and produce

value-added products - protein and biofuels as raw materials in animal feed. The fly also has a significant 'green' role, especially in eliminating human biowaste and animal carcasses. Using flies such as *Hermetia illucens* and *Musca domestica*, large-scale microorganism-free (asepsis) control of animal feces (*H. illucens*) and urban rubbish (*M. domestica*) contaminated with pathogens harmful to humans has been carried out and has been successful (Sheppard *et al.* 2002; Hassan *et al.* 2016) [39,40]. Many Diptera larvae are particularly noteworthy in this field as they can thrive in various environments, reproduce rapidly, and are short-lived (Morales and Wolff 2010) [41]. Fly development in different types of waste can result in differences in growth rate and body composition.

In the study, which we set out with the aim of questioning the difference in nutrients in growth and development and determining the most suitable environment, the effect of decayed or putrefied animal tissue and organic food wastes on the growth and development of *L. sericata* larvae from the Diptera order, as in *Hermetia illucens* and *Musca domestica* larvae, and the effect of these wastes on growth and development, as well as the evaluation of these wastes, *L. sericata* larvae are stored in the body with high carbohydrate, protein, and lipid content, although the food source with animal tissue-based tissues and organs or animal carcass species such as fish leads to a decrease in body water content as a percentage of body mass. Therefore, in large-scale cultivation, the nutrient substrate environment of animal wastes is more nutritious than plant wastes. However, it should be emphasized that the relationship between the body water content of the larvae and the nutrient substrate is significant for a bioconversion plant. The body water content of larvae is an essential factor in growth and development, with lower body water content leading to lower growth rates and reduced mobility and feeding (Harrison *et al.* 2012) [42]. While various artificial diets have been described for insect larvae, adequate diets for adults have not been developed (Tachibana and Numata 2001) [7]. The maturation of ovaries in the pathogenic *Lucilia sericata* requires carbohydrates, mineral salts, and unknown low molecular weight materials, especially protein-dominated nutrients (Clift and McDonald 1976; Williams *et al.* 1979) [43, 44]. These elements affect the receptivity of females (Browne *et al.* 1980) [45] and odors, especially from animal tissue, influence the sexual activity of males (Shorey *et al.* 1969) [46]. Chemoreceptors on the ovipositor are the primary means females discriminate feeding conditions (Rice 1976) [47]. Abiotic factors such as temperature, humidity, food type and quantity, and food quality play multiple roles in the reproduction of *Lucilia*. The complexity created by these various factors may be one of the reasons why artificial diets have yet to be developed and defined in adults. Between the diets tested, Diet-A and Diet-B, the reproductive output and growth and development parameters of *L. sericata* showed significantly higher values with Diet-A, reflecting the optimal biological development of the life cycle during the five consecutive generations analyzed under controlled laboratory conditions, which made it possible to maintain the generation. In Diet B, reproductive output, population, and growth and development parameters were much lower than in Diet A. Each Diet had advantages and disadvantages in terms of preparation and utilization. Artificial diets such as Diet A, prepared with animal protein-based wastes, can be used for mass rearing and sustainability of fly larvae in applied studies. Deep analyses and extensive



laboratory-scale studies are needed to resolve the significant difficulties associated with artificial diets such as Diet B prepared with plant carbohydrate-dominated waste. Necrophagous fly larvae, such as saprophagous fly larvae, can also transform a wide range of organic wastes into valuable products. Sustainable waste management strategies can be developed by managing and recycling organic waste and producing new protein sources to increase its value.

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