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**Smari Hadjer-Kounouz**
Laboratory of Biosystématic and Ecology of Arthropods – Faculty SNV – University Mentouri Constantine - Constantine - Algeria 25000

**Louadi Kamel**
Laboratory of Biosystématic and Ecology of Arthropods – Faculty SNV – University Mentouri Constantine - Constantine - Algeria 25000

**Correspondence**
**Smari Hadjer-Kounouz**
Laboratory of Biosystématic and Ecology of Arthropods – Faculty SNV – University Mentouri Constantine - Constantine - Algeria 25000

# Development of *Calliphora vicina* (Robineau-Desvoid) (Diptera: Calliphoridae) under different biotic and abiotic conditions

**Smari Hadjer-Kounouz and Louadi Kamel**

**Abstract**
This study was conducted to evaluate the effect of the nature of the nutrient substrate on the weight and metric growth and the development time of necrophagous fly larvae *Calliphora vicina* (Robineau-Desvoid) (Diptera: Calliphoridae) used in forensic entomology. Eggs of the insect harvested from an animal corpse are deposited on the camel meat, goat meat and the fish for their breeding under constant temperatures of 20 and 30 °C. The weight and length as well as the longevity of the larval stages are compared by the scheffe post-hoc test. The results obtained indicate that the larvae fed on different substrates cannot complete their development until the emergence of adults at 30 °C. Larvae raised on fish are affected compared with those fed on camel and goat meats; they increase in weight and length slowly. Sometimes, they produce pupae that are significantly smaller and lighter than other types of substrates. Longevity differs a little between larval stages under the two temperatures and between the three nutrient substrates.

**Keywords:** Larval development, nutrient substrate, weight, length, longevity, *Calliphora vicina*

## Introduction
*C. vicina* (Diptera: Calliphoridae), commonly referred to as "meat flies", has a limited deployment to areas where summer temperatures do not exceed 30 °C for long periods **[1-2]**. Usually, this insect species is active only during winter in subtropical regions **[3-4]**. However, these authors found that temperature fluctuations tend to slow down the development time. Synchronization of temperature cycle is also important because the growth rate may change as the larvae pass through various stages of development [2]. Calliphoridae life cycle includes an egg stage, three larval stages, prepupa, a pupa and the adult stage. The larva feeds only during the three juvenile stages [5]. Different species of blowflies grow at different speeds and several works, including [1-6-8], have been devoted to the development of certain species of medico-legal importance including *C. vicina* [2]. A range of tissues and organs are available as food sources on a corpse. Necrophagous larvae colonize especially the soft tissue attack it first before consuming it faster than others [9]. The liver of beef [10-15], of porc [16, 17], and lamb [18, 19], have been used to as food substrates. Muscle tissues of beef [4], pork [11, 20], and fish [21] have been used used also. Other authors, such as [22] and **[20]** have promoted an artificial diet in larval growth studies of the species *Phaenicia sericata*. This diet consists of dry cat food [9]. It is also important to know on which corpse tissue the larvae arise. In laboratory, weight and metric growth models are the basis of the PMI estimate. The substrates are selected based on criteria of convenience and low odor **[20]**. The liver is most often used because it is frequently available and relatively cheaper. Estimation of larvae age by body weight was attempted by [23]. The aim of this study is to examine the development of *C. vicina* (Robineau-Desvoid) on camel and goat meat and on fish tissues at temperatures of 20 and 30 °C in order to assess the average weight, length and longevity of each larval stage.

## Material and methods
### Preparation of the experiment
To carry out the experiments, a species of necrophagous fly "*C. vicina*" (Robineau-Desvoid) (Diptera, Calliphoridae) was used. This fly is a Diptera Brachycera whose larvae are strictly necrophagous [24]. Many species associated with corpses are punctually associated with these ecosystems. Adult females of this Diptera are constantly searching for the protein intake necessary for vitellogenesis (maturation of eggs) [25]. The protocol followed in our work is

based on the effects of various biotic and abiotic factors on the larval development of this necrophagous fly including growth in weight, length and longevity. The species studied "*C. vicina*" was chosen because of its high frequency on the corpse compared to other necrophages in winter period of the year. Study was conducted over four months: from December 2014 to March 2015.

**Field Sampling, food choice and Larvae breeding**

Eggs are collected from animal corpse during the fly active period [13] and within 30 min of oviposition [26]. Eggs are collected on a 12.5 kg puppy corpse (exactly from the muzzle) which was handed over to us by the hygiene department of the city of Constantine after picking it up. The latter is stored in an iron cage of 2m X 2m X 2m made up of a wire mesh of small netting to facilitate only the entry of the insects and to protect the corpse of possible scavengers and to allow its conservation during the experiment [27]. It has been reported that flies can detect carcasses inside buildings and enter habitats for egg laying [28].This cage was placed at an isolated place near the research laboratory located in Chaabat Ersas. As soon as the corpse was placed, observations began to make our egg sampling. Females lay their eggs on wounds or natural orifices of the corpse [29-30]. These are collected one hour after deposition of the puppy using a needle mounted onto a mandrel or flexible metal clips. The eggs are placed in boxes of petri dishes and transported to the laboratory. It is possible that the organ on which the larvae feed can significantly alter the growth rate [31].Moreover, not all tissues have the same nutritional value for larvae [9]. The selected nutrient substrates are camel meat, goat meat and fish. Meat and fresh fish are bought at the same time; each meat is divided into three portions of 40 g each for three repetitions. 30 eggs are then placed on each substrate in a petri dish without a cap to simply serve as a support. The petri dishes are then placed in plastic cages of 25cm long, 15 cm wide and 17cm of high. The cages are provided with a tulle cover to allow the larvae breathing. They are then introduced into two climatic chambers (ovens) set at 20 °C and 30 °C.

**Measurement of length and weight of larval body**

The necrophagous Diptera are holometabolous insects. Their development cycle is composed of four stages: egg, larva, pupa and adult [32]. Observations of the maggots development are made daily for stages 2, 3, prepupae and pupae. The different stages are weighed with a precision balance and are measured with a graph paper. To make these measurements a dozen of meat maggots were recovered and cleaned on paper towels. The maggots are then paralyzed by the cold by introducing them for a few minutes in a refrigerator in order to be able to manipulate them. Regarding the final stage "pupe", the measurement of the length is done with a calliper due to the roundness of its ends. Measurements are made every day on the feeding and post-feeding stages. The post-feeding phase can be identified by the ability of larvae to actively move away from the food source and the way they contract in a barrel shape when touched [2]. In fact, the number of individuals taken is 10 larvae. It is less than the initial number placed in each rearing cage because some larvae sink deep into the meat, which makes their extraction very difficult. 10 newly hatched larvae are collected randomly every 24 hours [26]. The same manipulation is performed for all applications. The picked and cleaned maggots are placed in glass petri dishes to be weighted. Manipulated maggots are put back into the original meat to continue growing. The time taken for the recovery of the substrates larvae is limited to 30 minutes in order to respect the larvae's age. We manipulated the larvae starting from stage 2 because stage 1 is very fragile and light.

**Specimens identification and statistical Study**

Adults are more easily identified by their physical characteristics, and even larvae of some species can generally be distinguished. Keys have been used for the identification of adult larvae [33-35]. The distinction between larval stages is made using a binocular lens. The development timeline is based on the observation of posterior respiratory spiracles. The statistical study was carried out using the SPSS statistics 17.0 software. ANOVA (variance analysis) allowed us to compare the growth stages of the different larvae from the three substrates under the two temperatures. Weight and length were determined using mean and standard deviation (mean ± gap) (Arkin and Colton cited by [26]. The results are confirmed by 3 replications.

**Results**

**1. Development time of *C. vicina***

In this study, *C. vicina* was raised in two temperatures (20 and 30 °C) and fed with fish, camel meat and goat meat.

**1.1. Longevity at 20 °C**

*C. vicina* Larvae develop from the initial stage to the final stage to give adults, after emergence of pupae, at a temperature of 20 °C. 24 hours after egg laying on the substrates, stage 1 maggots are born. After 48 hours, all the larvae reach stage 2 on the three substrates. At 72 hours, the maggots develop to stage 3. This stage lasts 3 days for maggots fed on camel meat. However, for maggots fed with goat meat and fish, stage 3 lasts about 4 days. The high camel meat maggots start leaving the substrate after 144 hours and turn to become pre-pupae. The pupae are stabilized and become pupae after 216 hours. After 480 hours the emergence of the first adults is observed. Those raised from goat meat and fish begin to leave their substrates after 168 hours. At 240 hours, all the pre-pupae become pupae and the emergence begins at 504 hours.

**1.2. Longevity at 30 °C**

After two days, stage 1 larvae reach stage 2 on the three substrates. Maggots fed of camel meat and fish grow in stage 3 in 2 days. However, those fed of goat meat become in stages 3 in 3 days. When the larvae finish feeding, they start to migrate away from the substrates, and after 96 hours, maggots fed of camel meat and fish become pre-pupae, while those fed of goat meat become so at 120 hours. The pupal stage is reached after 144 hours for those who are fed of camel meat and fish, and 168 hours for those fed of goat meat. Under this temperature there is no emergence of adults. All pupae are desiccated and damaged.

**2. Measurement of mean weight of *C. vicina***

**2.2. At 20 °C Temperature**

Table 1 and figure 1 show the mean weights during the development period of this species at 20 °C. The weight of stage 1 larvae is negligible. On the other hand, a slight increase in weight was observed after 48 hours. The number of individuals remains unchanged (n = 30) due to the absence of mortality at this temperature. The analysis results of the data variance indicate that the highest values of the mean weight of maggots fed of camel and goat meat are observed at

120 hours (110.90 ± 9.93) mg and (99. 85 ± 20.10) mg. However, for fish-fed maggots, the highest value was observed at 144 hours (75.55 ± 12.50 mg). The weight of fish-fed maggots is low throughout the development period compared to maggots fed of both types of meat. The Scheffe's post-hoc test indicates a significant difference at 72, 96, 120, 168 and 192 hours between the three types of food (camel meat, goat meat and fish) (Scheffe's test p = 0.000). This significant difference recorded during the development period at 20 °C denotes that the type of food affects maggots' weight. However, at 48 hours, there was only a significant difference between the goat group and the other two groups (camel meat and fish) (p = 0.000), while there was no significant difference between the camel group and the fish group (p = 0.998). At 144, 216, 240 and 312 hours, significant differences were constantly detected between the fish group with both camel and goat meat (p = 0.000). Though, there was no significant difference between the camel group and the goat one (p = 0.186), (p = 0.249), (p = 0.243) and (p = 0.300) respectively.

**Table 1**: Average weight of *C. vicina* maggots fed of camel and goat meat and fish during the development period at 20 °C. (*: P≤0.05 significant; **: 0.05 <*p* <0.01 highly significant; ***: 0.001 <*p* <0.0001 very highly significant)

| Age (hour : h) | Type of food | Number of individuals (n) | weight (mg) Mean/ gap | F |
|---|---|---|---|---|
| 48 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (1.52±1.42) mg<br>(3.08±1.39) mg<br>(1.5±0.51) mg | 17.454*** |
| 72 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (38.12±5.75) mg<br>(20.63±4.35) mg<br>(9.09±2.80) mg | 320.405*** |
| 96 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (93.71±14.07) mg<br>(71.76±13.74) mg<br>(42.97±5.91) mg | 138.043*** |
| 120 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (110.90±9.93) mg<br>(99.85±20.10) mg<br>(65.53±17.60) mg | 62.008*** |
| 144 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (102.40±8.68) mg<br>(96.60±14.63) mg<br>(75.55±12.30) mg | 40.714*** |
| 168 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (83.27±6.28) mg<br>(91.67±10.01) mg<br>(70.45±8.38) mg | 48.924*** |
| 192 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (75.70±6.51) mg<br>(86.96±9.77) mg<br>(60.01±8.18) mg | 80.449*** |
| 216 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (68.60±14.05) mg<br>(73.33±10.91) mg<br>(54.88±6.30) mg | 23.206*** |
| 240 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (64.91±17.14) mg<br>(70.09±6.88) mg<br>(48.72±8.78) mg | 26.734*** |
| 312 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (63.68±16.61) mg<br>(68.16±7.48) mg<br>(46.14±6.18) mg | 32.901*** |

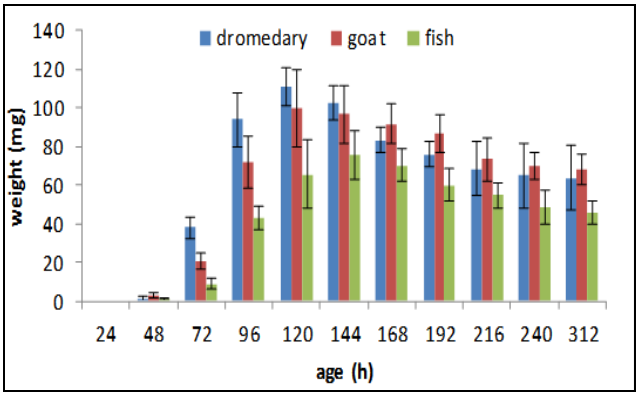


**Fig 1:** Mean weight of *C. vicina* maggots fed of goat meat and fish during the development period at 20 °C.

### 2.3. At 30 °C Temperature

Table 2 and figure 2 show the mean weight measurements during the development period of *C. vicina* fed by the three types of substrates at 30 °C. The egg hatching gives first-stage maggot. The weight of the first-stage larvae, being very low, was neglected. We began to observe an increase in weight after around 48 hours. The number of individuals is not identical for the three groups (n ≠ 30) because of larval mortality in this temperature. The results of the data variance analysis indicate that the highest values of the mean weight of maggots fed by the three types of foods, camel meat, goat meat and fish, are observed at 96 hours respectively (71.94 ± 15, 17) mg, (65.62 ± 13.41) mg and (38.39 ± 9.82) mg. The weight of maggots fed of fish is low throughout the development period compared to maggots fed by both types of meat. Under this temperature, the *C. vicina* does not develop optimally on the three examined substrates. The difference between the three groups is significant (p = 0.000). At 48h and 72h, the Scheffe's post-hoc test indicates a significant difference between the goat group and the other two groups (camel and fish) (p = 0.000) but no differences between Camel group and fish group is noticed at 48 h (p = 0.585) and 72 h (p = 0.674). At 96 h, 120 h and 144 h there was a significant difference between the fish group and the two other groups (camel and goat) (p = 0.000). On the other hand, no differences were noticed between the two types of meat at 96 h (p = 0.254), 120 h (p = 0.470) and 144 h (p =

0.798), respectively. At 192 h, a significant difference is noticed between the camel group and the other two groups (goat and fish) (p = 0.000). However, there was no difference between goat and fish groups (p = 0.072). At 168 h, 216 h and 240 h a significant difference was noticed between the three types of food (p = 0.000).

**Table 2:** Mean weights of *C. vicina* maggots fed of camel and goat meat and fish during the development period at 30 °C. (*: P≤0.05 significant; **: 0.05 <*p*<0.01 highly significant; ***: 0.001 <*p*<0.0001 very highly significant)

| Age (hour : h) | Type of food | Number of individuals (n) | weight (mg) Mean/ gap | F |
|---|---|---|---|---|
| 48 | Dromedary<br>Goat<br>Fish | 28<br>27<br>18 | (2.56±1.76) mg<br>(5.80±2.97) mg<br>(3.36±2.78) mg | 11.930*** |
| 72 | Dromedary<br>Goat<br>Fish | 27<br>25<br>18 | (37.57±14.08) mg<br>(24.83±5.24) mg<br>(34.74±9.36) mg | 10.322*** |
| 96 | Dromedary<br>Goat<br>Fish | 27<br>25<br>13 | (71.94±15.17) mg<br>(65.62±13.41) mg<br>(38.39±9.82) mg | 27.581*** |
| 120 | Dromedary<br>Goat<br>Fish | 24<br>24<br>10 | (55.22±15.91) mg<br>(60.12±13.73) mg<br>(30.22±4.79) mg | 17.301*** |
| 144 | Dromedary<br>Goat<br>Fish | 23<br>24<br>9 | (54.25±13.31) mg<br>(56.40±10.04) mg<br>(24.24±4.16) mg | 30.669*** |
| 168 | Dromedary<br>Goat<br>Fish | 18<br>24<br>5 | (56.20±8.37) mg<br>(43.38±8.02) mg<br>(18.14±2.75) mg | 48.137*** |
| 192 | Dromedary<br>Goat<br>Fish | 18<br>24<br>5 | (53.40±8.56) mg<br>(23.28±10.59) mg<br>(12.36±3.79) mg | 67.142*** |
| 216 | Dromedary<br>Goat<br>Fish | 18<br>24<br>5 | (50.54±8.62) mg<br>(23.75±10.19) mg<br>(10.54±2.28) mg | 60.461*** |
| 240 | Dromedary<br>Goat<br>Fish | 18<br>24<br>5 | (51.61±8.10) mg<br>(23.04±10.60) mg<br>(10.04±1.91) mg | 66.555*** |

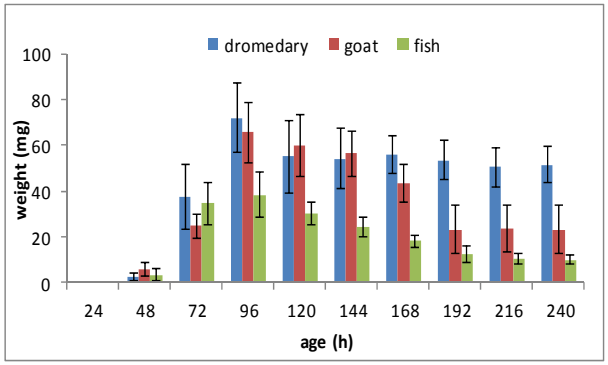


**Fig 2:** Mean weights of *C. vicina* maggots fed on camel and goat meat and fish during the development period at 30 °C.

## 3. Larvae Metric growth

### 3.1. At 20 °C temperature

Table 3 shows the average lengths during the development period of the *C. vicina* farmed on the three types of foods at 20 °C. The number of individuals is constant (n = 30). According to table 3 and figure 3 representing the lengths of larvae at T° = 20 °C, the highest average length value is observed at the camel meat at 96 h (15.05 ± 0.62 mm). On the other hand, the highest values in larval groups fed with goat meat and fish were obtained at 120 h (14.51 ± 1.13 mm) and 144 h (13.15 ± 0.84 mm) respectively. The length of fish-fed maggots is small compared to those fed of camel and goat meat throughout the development period. Once the eggs are hatched on all substrates, the larvae of the second and third feeding stages grow rapidly in size. They increase significantly in larval length (*p*<0.05). However, the length of the third larval stage decreases significantly once the maggots enter post-feeding stage (*p*<0.05). The Scheffe post hoc test carried out between pairs of substrates showed that a significant difference is noticed between the mean length values of the maggots fed by the three substrates during development (Scheffe test: p = 0.000). However, at 48 h, 120 h and 216 h, the test showed no significant difference between the meat only and are respectively at (p = 0.242), (p = 0.363) and (p = 0.086). However, a significant difference exists between the fish group and the two other meat-fed groups (p = 0.000).

**Table 3:** Average lengths of *C. vicina* larva during the development period at 20 ° C. (*: P≤0.05 significant; **: 0.05 <p <0.01 highly significant; ***: 0.001 <p <0.0001 very highly significant).

| Age (hour : h) | Type of food | Number of individuals (n) | Lengths (mm) Mean/ gap | F |
|---|---|---|---|---|
| 48 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (4.81±0.56) mm<br>(4.58±0.65) mm<br>(3.43±0.31) mm | 58.069*** |
| 72 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (11.73±0.98) mm<br>(9.53±0.96) mm<br>(6.90±0.56) mm | 238.688*** |

| | | | | |
|---|---|---|---|---|
| 96 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (15.05±0.62) mm<br>(13.26±0.98) mm<br>(11.61±1.08) mm | 105.585*** |
| 120 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (14.86±0.55) mm<br>(14.51±1.13) mm<br>(13.11±1.04) mm | 28.675*** |
| 144 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (14.63±0.45) mm<br>(13.85±0.65) mm<br>(13.15±0.84) mm | 36.741*** |
| 168 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (14.11±0.92) mm<br>(13.38±0.98) mm<br>(12.03±1.04) mm | 34.433*** |
| 192 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (9.28±0.59) mm<br>(13.20±0.79) mm<br>(11.86±0.93) mm | 191.271*** |
| 216 | Dromedary<br>Goat<br>Fish | 30<br>30<br>30 | (9.28±0.59) mm<br>(9.03±0.29) mm<br>(8.03±0.34) mm | 66.621*** |

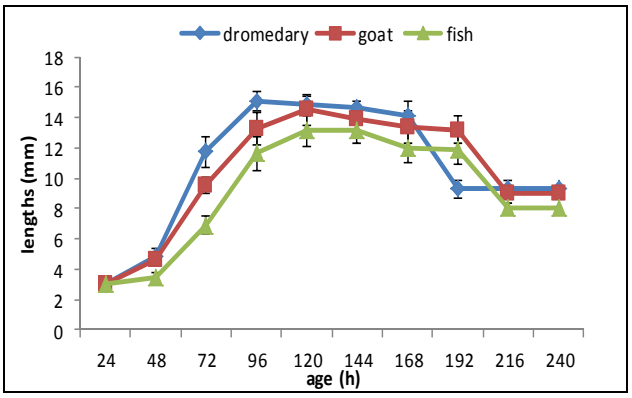


**Fig 3:** Mean lengths during the development period of *C. vicina* fed on camel and goat meat and fish at 20 °C

### At 20 °C temperature

Table 4 and figure 4: represent the average lengths during the development period of the *C. vicina* at 30 °C. The number of individuals is not similar (n ≠ 30) because mortality is noticed under this temperature. The results obtained show that the greatest length values are found in maggots fed camel of meat. The lengths of individuals fed of fish are smaller than the two meat-fed larvae. According to figure 4 representing the lengths at 30 ° C, it is observed that the highest values of the mean lengths are observed with all foods at 96 h: camel meat (14.05 ± 1.03 mm), goat meat (12.94 ± 0.80 mm) and fish (11.42 ± 0.93 mm). The Scheffe's post hoc test showed a significant difference between the mean lengths of maggots fed by fish, camel and goat meat at 72 h and 96 h, 120 h and 168 h (Scheffe's test: p = 0.000). No difference at 48 hours is noticed between fish with camel meat (p = 0.095) and goat meat (p = 0.963) on the one hand and between the two types of meat (p = 0.103) on the other hand. At 144 h, no significant difference between camel and goat meat is noticed (Scheffe's test: p = 0.970). However, between the fish and the two types of meat, the difference was significant (p = 0.000).

**Table 4:** Average lengths during the development period of the *C. vicina* fed on camel and goat fish and meat at 30 °C. (*: P≤0.05 significant; **: 0.05 <*p* <0.01 highly significant; ***: 0.001 <*p* <0.0001 very highly significant).

| Age (hour : h) | Type of food | Number of individuals (n) | Lengths (mm) Mean/ gap | F |
|---|---|---|---|---|
| 48 | Dromedary<br>Goat<br>Fish | 28<br>27<br>18 | (5.44±0.79) mm<br>(5.05±0.50) mm<br>(5.00±0.66) mm | 3.327** |
| 72 | Dromedary<br>Goat<br>Fish | 27<br>25<br>18 | (12.07±1.75) mm<br>(10.14±1.51) mm<br>(7.88±1.20) mm | 39.86*** |
| 96 | Dromedary<br>Goat<br>Fish | 27<br>25<br>13 | (14.05±1.03) mm<br>(12.94±0.80) mm<br>(11.42±0.93) mm | 35.672*** |
| 120 | Dromedary<br>Goat<br>Fish | 24<br>24<br>10 | (13.18±0.84) mm<br>(12.18±0.99) mm<br>(10.65±0.94) mm | 26.930*** |
| 144 | Dromedary<br>Goat<br>Fish | 18<br>24<br>5 | 8.66±0.48) mm<br>(9.54±0.64) mm<br>(6.70±0.44) mm | 54.097*** |
| 168 | Dromedary<br>Goat<br>Fish | 18<br>24<br>5 | (8.66±0.48) mm<br>(8.08±0.60) mm<br>(6.70±0.44) mm | 25.830*** |

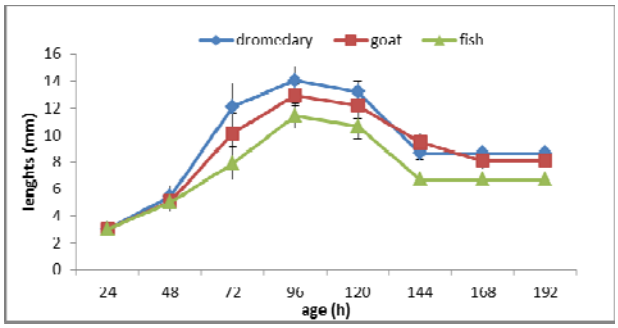


**Fig 4:** Average lengths during the development period of *C. vicina* fed on fish and camel and goat meat at 30 °C.

## Discussion

In this work, the *C. vicina* develops in the early larval stages (L1, L2) at the same time in all biotic and abiotic conditions. No remarkable difference is noticed in the duration of the initial stages on all the studied substrates. Under 20 °C, starting from the third larval stage (L3) a difference exists in longevity on all the substrates. In larvae fed of camel meat, longevity is one day shorter than those fed with goat meat and fish. Under this temperature, *C. vicina* can complete its development in an optimal way on all substrates and gives the emergence of adults. On the other hand, at 30 °C, the longevity differs a little. Larval development of individuals fed of camel meat and fish is one day shorter than the samples fed of goat meat. Unfortunately, their development they cannot be completed and stops at the pupae stage. Consequently, no emergence of adults is occured in all studied substrates. The experimental results obtained by [9] on *C. vicina* indicate that the development duration of larvae fed with brain, heart, lung or kidney of the pig is two days shorter than those placed on liver. The same type of result was obtained with larvae of *Calliphora augur* (Fabricius 1775) and *Lucilia cuprina* **(**Wiedmann 1830**)** [36]. Finally, the comparison of substrates derived from different animals indicates also a faster development of *Lucilia sericata* (Meigen, 1826) on pig meat than on beef and that larvae grow rapidly and significantly **[31].**

In this experiment, longevity of development stages under the two temperatures is daily observed. At 20 °C, *C. vicina* maggots, raised on camel meat develop more rapidly than other types of tissue. On the other hand, it is observed that the development of maggots fed on goat meat is slower than other types of substrates at 30 °C. This development may vary when larvae are raised on the lung and heart compared to the liver of the same animal; cow or pig. Therefore, for forensic entomologists, the location in the corpse from which larvae of blowflies are taken should be indicated. According to the study conducted by **[31]** on the growth rate of *L. sericata* on different tissues, it is impossible to definitively exclude organs structure as an affecting factor to this rate. Feeding substrate may have a significant effect on larval growth rate [2]. In this study, weight growth at 20 °C was higher than that at 30 °C, especially on meat (camel and goat) than fish. Moreover, the greatest weight value is marked in the camel group under the two temperatures. For *C. vicina*, [31] suggest that growth rates vary considerably among different tissues and that growth is lower in the liver than in other tested organs. Recent studies have also suggested that the tissues on which *C. vicina* larvae feed may also influence the growth rates [9]. The growth of *C. vicina* flies larvae on pig liver are significantly faster than on lung, kidney, heart and brain tissues. Potentially, this has major implications when larvae are fed on a food substrate (often liver) in the laboratory, in PMI determining in a judicial case [9]. Preliminary studies have shown that the highest growth rate on lamb hash liver comparing with those of beef or pig [2]. Byrd (in: Byrd & Castner, 2001) [20] has noticed that chicken live ris an inappropriate growth environment because it is easily liquefied at liquids access may prevent larvae of some species from developing naturally. However, stage duration of *L. sericata* fly larvae on the artificial diet (whole milk powder, dry yeast and wheat germ) was somehow longer than on the beef liver. This artificial diet is adapted for usage in laboratory experiments [37, 38] explained in other studies the possibility of chemical difference between animal and human tissues. In parallel, this work describes some simple experiments to study the growth of *C. vicina* larvae on different tissues of different animals. According to [39, 40]**,** the effects of drugs on the growth of necrophagous larvae may be affected by the choice of tissues used to feed the larvae. Paracetamol does not appear to affect the development of the blowfly larvae, particularly on days 2-4 of the development where growth is accelerated compared to the control group. This can generate a difference of about 12 hours in IPM estimation [9-39]. It is therefore necessary to collect only actively fed larvae from a corpse. *Calliphora* larvae have a remarkable ability to eliminate drugs in a short time and actively during pupation [41].

In this study, *C. vicina* larvae feed normally and voraciously on all the studied tissues (camel and goat meat, fish). Studies conducted by [42] on *Calliphora* have shown that the larva is capable of developing protein fats and accumulating them in fatty substances for use during metabolism and during metamorphosis. Larvae of many blowfly species produce a number of enzymes including proteases, phosphatases, lipases, and other metabolic products that break down the structure of the epidermis and dermis of the skin **[43]**. *Calliphora* species are known to produce proteases **[44]**.The proteolytic enzymes produced by the blowfly larvae also play a role in their diet, while larvae of *L. cuprina* produce proteolytic enzymes that degrade gelatin and casein**[45].**

In the obtained results, we noticed that maggots bred under the temperature of 30 °C are incapable of completing their development which stops at the pupal stage. A high mortality is noticed during feeding stages. However, no emergence of adults is noticed as the pupae are dried out and damaged inside due to the high temperature. On the other hand, at 20 °C maggots can complete their development until the emergence of adults and no mortality is noted during the development period. This temperature is perfect for a complete development of the species. Larval development at T°=30 °C is faster especially at feeding stages than at 20 °C, but it is not optimal for *C. vicina*. [16] noted that larvae may fail to reach the pupal stage if no shelter is found or if the humidity is very high. In the case of *C. vicina* (Robineau - Desvoid) at temperatures higher than 15 °C, eggs hatch in about 24 hours after which larvae begin to feed on body tissues **[2]**.The larvae died at 35 °C, but in **[1]**, they survived at this temperature but failed at pupation. However, [46] reported that species of the genus *Calliphora* appear to be more adapted to cold than other Calliphoridae [2].

[47] have determined that fluctuations related to constant temperatures delayed the larval development of *Proteophormia terraenovae* (Robineau-Desvoid) (Calliphoridae). In turn, **[11]** found that the development of blowflies bred under temperature fluctuations is delayed in comparison with those bred under constant conditions. The

most interesting is the inability of *C. vicina* to complete development at temperatures higher than 29 °C [4]. The maggots' development on fish and camel and goat meats was monitored and examined for *C. vicina*. The obtained results showed that the weight of individuals fed on these three substrates and under the two temperatures of 20 °C and 30 °C increases gradually to the prepupal stage. Starting from this stage, the weight begins to decrease until the pupal stage. [36]also observed that the weight of the species *L. cuprina* and *C. augur*( is decreased at the pupal stage on all used substrates: brain, liver and lamb meat at 25 ± 3.5 °C. While [9] have also observed that larvae grow on the pig's brain and heart, but they have noted a weight loss and size reduction during the prepupal stage until the pupal stage at 20 °C. The larval growth of *C. vicina* on different substrates differs considerably. Larvae fed on the brain and / or heart show noticeable weight loss and size decrease during the post-feeding stage.

In our case, camel meat-fed maggots reach the highest weights compared to other types of nutrient substrates during stage 3 under both temperatures 20 °C (110.90 ± 9.93 mg) and 30 °C (71.94 ± 15.17 mg ). Lighter pupae were obtained at 30 °C than at 20 ° C on all substrates and especially fish. The mean weights of the maggots raised on the different substrates at 20 °C are higher than those of 30 °C throughout the development period. Variance Analysis of the results indicates that the weight of fish- fed maggots remains low throughout development period compared to maggots fed of both types of meat. The Scheffe's post-hoc test indicates a significant difference between the three substrates. This is explained by the influence of the type of substrates on the weight of the individuals. *C. vicina* fed on the substrates studied in this study grows and increases in weight and size. This weight gain is attributed to the nutrients present in the substrates. Indeed, according to [31] the nutritive value of the substrate has a role in nutritional preferences by species as well as in size increase. Not all ingested tissues have the same nutritional value for larvae. On the other hand, [48] found no significant difference in average length and weight of *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) larvae on equine and porcine muscles.

The larvae of the fly *L. sericata* have been reared on a diet consisting mainly of whole milk powder, dry yeast and wheat germ. No significant difference is noted in weight of pupae between samples raised on this diet and those raised on beef liver [37]. In a study conducted on the development of the species *Chrysomya rufifacies* (Diptera: Calliphoridae), pupal length decreases progressively with decreasing temperatures. After migration begins, the length of the larvae decreases gradually until pupation [49]. The larval growth of *Hypopygiopsis violacea* (Macquart, 1835) (Calliphoridae) shows a rapid increase in length and then a decrease before pupation [50].

These experiments at 20 °C and 30 °C, after the egg hatching of *C. vicina* on all substrates, the larvae grow in size progressively and significantly ($p$ <0.05). However, the length of Stage 3 larvae decreased significantly after maggots enter the post-feeding phase ($p$ <0.05) and then a stable measure is noted of the resulting pupae. Larvae of *L. sericata* are significantly larger when raised on the brain and lungs than when raised on other tissues, but they are rather small when raised on the intestine compared to the brain or lung [51].After the variance analysis of this study, camel meat-fed maggots reached the highest length compared to other tissues during Stage 3 at both 20 °C (15.05 ± 0.62 mm) and 30 °C (14.05 ± 1.03 mm). The mean lengths of maggots during their development on the different substrates studied at 20 °C. are higher than those at 30 °C. On the other hand, *C. vicina* larvae are significantly larger when grown on camel meat than when are raised on other tissues under both temperatures. The length of fish-fed maggots is smaller compared to other tissues.

In the work of [52] on *Musca domestica* (Linnaeus, 1758) (Mucidae), the growth rate of flies that were fed on goat brain compared to those fed with liver and goat meat showed little differences ($P$ <0.05). On the other hand, flies grown on brain and flesh showed a more significant difference ($P$<0.05). [51] demonstrated that larvae of *L. sericata* were significantly larger on brain and cow lung than when raised on other tissues (heart, kidney, intestine and meat). In this same study, the larvae are developed on the brain faster and became larger compared than on liver and meat. These results are consistent with those obtained by [36] who also found significantly smaller *L. cuprina* pupae derived from larvae cultured on sheep liver.

[10] have determined the minimum sizes of larvae required for pupation for the Calliphoridae family found in southeastern Australia. With the help of the larvae grown under desert conditions, they found that two common species: *L. cuprina* (Wiedemann) and *Calliphora stygia* (Fabricius) form pupae of at least 60% and 73% of normal size [53].

## Conclusion

Our study corroborates somewhat the studies of these authors who show that the species *C. vicina* increases in weight and size in the early feeding stages than from stage 3 to the prepupal stage at 20 °C and 30 °C. It thus seems that the important factors contributing to the observation of differences in weight and size growth in necrophagous insects in general and *C. vicina* in particular are the nature of dietary tissues, the activity of proteolytic enzymes and the amount of soluble proteins. Other nutrients can also play a decisive role **[36].** It is clear that the animal tissue ingested by Calliphoridae larvae has a significant effect on growth rate and this must be taken into account more explicitly in the growth model calibration for the PMI estimation [31]. Temperature is not the only factor influencing the rate of insect development. Food availability may also play an important role [54]. The species *C. vicina* has a forensic importance and this study showed how the tissue consumed by the larvae affects its growth under different abiotic conditions. Thus, substrates and climatic conditions used in laboratory farming and the results obtained can contribute to the determination of the PMI

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