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Developmental time variation of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) colonization in two bacteriological culture media vs red meat of sheep

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Abstract

The present study was designed to examine the different time period of developmental stages of fly *Lucilia sericata* in different artificial diet like using common bacteriological media, blood agar and Nutrient agar in comparison to natural diet like red meat of sheep. The parental adult fly containing 10 female and 5 male flies per cages (30X30X30cm) and replicate of four cages for each type of diet were evaluated over one continuous generation. Recording the time required for egg hatching, larval developments, pupation and total time for egg-eclosion was performed every three hours intervals for eggs and every six hours intervals for larva and pupa. Total time period in days required for completion of the life cycle was recorded as 12.29±0.16 days for diet using red meat of sheep which was shortest time period as compared to blood agar (14.41±0.21) and nutrient agar (17.55±0.19). The bacteriological media are prepared in sterile condition and do not produce any offensive odour when kept for a longer time which is unlikely with natural meat. The present study revealed that though natural meat led the early development of larvae and other transformation stages of *L. sericata*, artificial laboratory media can also be used as an alternative to replace the natural meat as diet for laboratory colonization of *L. sericata* effectively.

Keywords: Artificial diet, agar, colonization, *Lucilia sericata*

1. Introduction

The sheep blow fly *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae) is a necrophagous fly that plays an important role in Forensic Science, using as a biological indicator in estimation of post-mortem interval (PMI) as their developmental times are predictable under particular environmental conditions^[1, 2, 3]. The time of death can be calculated by counting back the days from the state of insect colonization living on the corpse^[4]. In Medical Science, maggot therapy can be used in healing of incurable wound^[5, 6]. In the field of Veterinary Medicine also, now-a-days larval therapy is practised in small as-well-as in few large animals for faster healing of incurable infection^[7]. The larva of *L. sericata* used in wound therapy reduces the infection as well as improve the nutrition of tissues for rapid healing of the wound. The blow fly requires protein meal for ovarian development and typically lays egg 150-200 eggs per batch. The larvae of blow flies are necrophagous or polyphagous mostly feed on decomposing tissues and vegetal nectar. There is a growing trend of the use of maggots of blowflies to cleanse wounds and similar lesions that are difficult to treat by standard methods in human medicine and is usually called maggot therapy. It is also becoming more widely used in veterinary medicine. However, supply of good quality, and especially aseptic, maggots remains a problem for both these fields of clinical use. In laboratory condition larvae of different stages of *L. sericata* produced in mostly natural diet like beef liver. However, natural diets such as beef liver that are often used in laboratory rearing of flies produce offensive odours and contamination whereas artificial diet have pleasant odour, can be maintained in sterile condition and of consistent quality than natural food material for rearing of larvae^[8, 9, 10]. Therefore the present study encompassed to use natural diet like red meat of sheep and artificial diet like blood agar and nutrient agar for colonization of *L. sericata* under laboratory conditions to study the life table in comparison to a natural animal protein diet.

2. Materials and Methods

2.1. Collection of samples

The parental adult flies were collected from natural environment or field including garden, different meat/fish shop of the locality, the live-stock farm of the University, R.S. Pura, and Jammu. The nectar of flowers, cow dung in the field were served as bait in the garden; besides, chicken and fish viscera were also used as bait for collecting adult stage of the fly in the open area. Adult flies were catch with help of flytrap or hand trap or using entomological net and stored in a

glass beaker covering with muslin cloth in the top and brought to the laboratory. The collection was mostly done in the early morning and evening as the fly density was mostly observed more during the period than the rest of the daytime. Collection was continued till sufficient number of desired fly captured for colonization process. Morphological identification of adult fly was done following the criteria laid by previous worker^[11, 12].

Table 1: Duration of total time required in different stages of life-cycle of *Lucilia sericata* different diets in laboratory

Diet	Time(days), Mean \pm SEM				
	Egg period	Larval stage	Pupa stage	Emergence to egg laying	Total Life-cycle
Diet1(Red meat of sheep)	0.87 ^a \pm 0.02	4.01 ^a \pm 0.11	4.24 ^a \pm 0.05	3.15 ^a \pm 0.08	12.29 ^a \pm 0.16
Diet2(Blood Agar)	1.01 ^b \pm 0.03	4.67 ^b \pm 0.05	4.89 ^b \pm 0.12	3.85 ^b \pm 0.06	14.41 ^b \pm 0.21
Diet3(Nutrient Agar)	1.25 ^c \pm 0.04	5.99 ^c \pm 0.11	6.18 ^c \pm 0.09	4.13 ^c \pm 0.03	17.55 ^c \pm 0.19

Values were in Mean \pm SE, small different superscript denote significant difference from one another at 1% ($P < 0.01$)

2.2. Rearing of flies in the laboratory

Laboratory rearing of fly was performed from April to June 2015 for identification of candidate species *L. sericata*. The study used a total of 120 female and 60 male of *L. sericata* fly and divided them in to cages of four replicates at the rate of 10 female to 5 male per cage. The adult flies were kept in 30 \times 30 \times 30 cm cages and provided with 200gm of mutton per cage as meal and then covered with light coloured fine muslin cloth in the top of the cages in such a way that to avoid the entry of other insect species. Flies were maintained in the laboratory under controlled conditions of mean temperature of 27 \pm 2^oC and 70 \pm 10% RH with 12:12hrs photoperiod^[13] with an external net curtain to avoid the entry of other insect species. After laying eggs, the dead specimens were collected for morphological identification by using the defined taxonomic keys^[11, 12, 14] which was further confirmed by larval morphological study by preparing permanent mount and by using standard key for candidate species^[11]. The flies were reared on different diets, i.e. in red meat of sheep, blood agar and nutrient agar separately to acquire *ad libitum* amount of larvae for further study.

2.3. Laboratory colonization of *L. sericata* larvae in different diets

The method of colonization of larvae of *L. sericata* was followed by the method described by Elshehaby *et al.* (15) and Rueda *et al.* (5) to investigate colonization of larvae on three different diets i.e. diet I as red meat of sheep, diet II as blood agar and diet III as nutrient agar. The candidate species of adult *L. sericata* containing 10 female and 5 male flies per cages (30X30X30cm) and replicate of four cages for each type of diet were evaluated over one continuous generation. Each four cages of diet I was supplied with approximately 100gram of red meat of sheep in a petri dish with a wet tissue paper to avoid dryness of meat. Similarly, for diet II and diet III types, freshly prepared uncontaminated blood agar and nutrient agar in petri dish with wet tissue paper, respectively was placed in four separate cages each. As a means of carbohydrate source, an additional petri dish containing a cotton pad soaked in sugar solution was placed within the cages irrespective of the diet used in the study. A layer of moist cotton was placed in the bottom of all cages to prevent dryness inside the cages. Immediately after hatching of egg on first observation, the parent flies were shifted to another new cage. Supervision of rearing cages was essential for all the

cages to observe different developmental stages in the life-cycle of the fly. Upon emerging, the adults were placed in new cages and provided with essential diet. Recording the time required for egg hatching, larval developments, pupation, and total time for egg-eclosion was performed every three hours intervals for eggs and every six hours intervals for larvae and pupa^[15]. On each recording occasion, at least 10 individuals of each cage were checked by using a light microscope to observe egg lay time. Confirmation of candidate species was also done by making permanent slides of anterior and posterior spiracle of the third stage larva by using standard key^[11]. On evaluation of diet, life-cycle duration in different stages of development and total life-cycle was determined based on respective diets. The data recorded from the parameter of study was analysed by 't-test' to compare the life cycle stages in three different diets. Time required for different stages of life cycle starting from hatching of the egg to emerging of the adult to laying of an egg was recorded in days.

3. Results

The fly *L. sericata* was identified morphologically. The body of the fly was slender and about 8-10 mm in size. The filamentous arista was long with many long setae giving it a feather-like appearance that arises on the dorsal surface of a segment of the antenna and the orange palpus is also well visible. The stem vein of the wing was without bristle. There are no setae on the upper surface of the lower thoracic squama. Both the sexes are very similar in appearance but distinguished by the distance between the eyes, which are almost touching interiorly in males and separated in females.

3.1. Colonization of *L. sericata* in different diets

The life cycle of fly *L. sericata* which included four distinct stages egg, larval, pupal and imago are observed in the study in three different diet types and the duration of different developmental stage is given in the Table.1. It was observed that time required (days) to complete each stages of the life cycle was significantly different from one another in each diet. The duration (days) of an average egg period for diet I, using red meat was (0.87 \pm 0.02), which was shortest amongst the three diets as compared with diet II of blood agar (1.01 \pm 0.03) and in diet III (1.25 \pm 0.04) in nutrient agar. The average larval period observed as (4.01 \pm 0.11), (4.67 \pm 0.05) and (5.99 \pm 0.11) for diet I, II and III respectively. The total

time period in days required for completion of a life cycle was recorded as (12.29±0.16) days for diet I, using red meat of sheep which was the shortest time period as compared to diet II (14.41±0.21) days and diet III (17.55±0.19) days, used blood and nutrient agar respectively.

4. Discussions

Laboratory colonization of larvae depends on optimum environmental condition and larval diet¹⁶. In this study it is tried to compare the use of conventional natural diet like red meat of sheep with artificial diet like blood agar and nutrient agar for colonization of *L. sericata* as per recommended environmental temperature¹³. The present study showed adult flies maintained on red meat of sheep took shorter time period for every stages of development like the egg hatching, larval period, pupal stage and eclosion when compared to artificial diet like blood agar and nutrient agar. The total duration of the life-cycle from egg hatching to eclosion of an adult on red meat of sheep took a 12.29±0.16 days which was almost similar to finding of Elshehaby *et al*¹⁵. Where it was reported to be 11-15 days of time when liver and meat used as natural diet. The duration of the life cycle in an artificial diet i.e. blood agar and nutrient agar were in almost similar to the results of Ruedra *et al.*⁵, where it was recorded 14 days of duration from egg to adult in artificial diet for *L. sericata* fly. However, with varied temperature and environment, different researcher has recorded slightly longer duration of the life cycle for Calliphoridae fly *viz.* 32 days at 16 °C and 20 days at 21 °C⁽¹⁾; 26 days by Usaquen and Camacho¹⁷ and Nuorteva (18) recorded 23-28 days under field condition. The disparity between present results and other studies can be explained as the varied temperature used and the characterization of the local population studied as well as geographical distribution. So it can be stated that development of fly larvae is temperature dependent and in higher temperature, the rate of development increases and duration of development becomes shortened. The longer duration of the larval and pupal periods on artificial diets i.e. blood agar and nutrient agar than those on a natural diet like red meat of sheep might be due to the nutritional quality of diet for development of larvae. The longer duration of development of blow fly larvae in the diet mainly using artificial diet of different composition compared with natural diet was also reported in others experiments^{16, 19}. The egg to pupal transformation as well as adult emergence was observed similar in both natural diet (sheep meat) and in an artificial diet of agar medium. Thus the result suggests that agar medium can be useful to rear larvae. The advantages of agar medium over natural diets were that, it was easy to maintain sterile condition for growing of sterile eggs in agar. However, several studies on the blow fly's growth factor requirements concluded that protein, cholesterol, vitamin B and albumin were essential growth factor for potential blow fly development^{20, 21, 22}. The present study used sheep flesh, which contain more protein and fats²³ and a good source of energy which led an early development of larvae and other transformation stages as compared to development or transformation on agar medium. In commercial preparation of agar medium only 3% sheep RBC was added in blood agar medium and in nutrient agar, peptone was used as a protein source.

5. Conclusions

The present study was concluded that the colonization of *L.*

sericata larvae is possible on artificial media like blood agar and nutrient agar media and can be maintained in sterile condition for maggot debridement therapy. These diets can be used for mass rearing of blow fly larvae for several basic and applied studies in near future.

6. Conflict of interest

The authors declare that they have no conflict of interest to publish the paper in the Journal of Entomology and Zoology Studies.

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