

Komunikasi Pendek/Short Communication

Development of a Forensically Important Fly, *Megaselia scalaris* (Loew) (Diptera: Phoridae) on Cow's Liver and Various Agar-based Diets (Perkembangan Lalat Berkepentingan Forensik, *Megaselia scalaris* (Loew) (Diptera: Phoridae) Pada Hati Lembu dan Pelbagai Diet Berasaskan Agar)

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ABSTRACT

In forensic entomology practice, it is more common to use raw animal tissue to breed dipteran larvae and it often brings unpleasant odour in the laboratory. Few studies suggested the use of synthetic diets, mainly agar-based media, as alternatives to animal tissue but it is rarely being practiced in forensic entomology laboratory. The present study observed the growth of a forensically important fly, *Megaselia scalaris* (Loew) on raw cow's liver, nutrient agar, casein agar and cow's liver agar. A total of 100 *M. scalaris* eggs were transferred each into the different media and placed in an incubator at 30°C in a continuous dark condition. Data on length and developmental period were collected by randomly sampling three of the largest larvae from each rearing media, twice a day at 0900 and 1500 hours until pupariation. *M. scalaris* larvae reared on raw cow's liver recorded the highest mean length (4.23 ± 1.96 mm) followed by cow's liver agar (3.79 ± 1.62 mm), casein agar (3.14 ± 1.16 mm) and nutrient agar (3.09 ± 1.11 mm). Larval length in raw liver and liver agar were significantly different from those in nutrient and casein agar ($p < 0.05$). Larvae bred in liver agar and raw liver recorded the shortest larval duration before entering the post-feeding stage (89 hours), followed by nutrient agar (119 hours) and casein agar (184 hours). Total developmental time from oviposition until adult emergence for *M. scalaris* in liver agar and raw liver was approximately 163 hours. All puparia in nutrient agar and casein agar failed to hatch. This research highlighted the potential use of cow's liver agar as an alternative diet of raw liver to culture *M. scalaris* in laboratory.

Keywords: *Megaselia scalaris*, Forensic entomology, Development, Food type

ABSTRAK

Amalan entomologi forensik lazimnya menggunakan tisu haiwan untuk memelihara larva lalat dan ia selalunya menghasilkan bau yang busuk di makmal. Beberapa kajian mencadangkan penggunaan diet sintetik, terutamanya media berasaskan agar sebagai alternatif kepada tisu haiwan tetapi ia jarang diamalkan di makmal entomologi forensik. Kajian ini bertujuan untuk menentukan kadar perkembangan lalat berkepentingan forensik, *Megaselia scalaris* (Loew) pada hati lembu mentah, agar nutrien, agar kasein dan agar hati lembu. Sebanyak 100 biji telur *M. scalaris* dipindahkan secara berasingan ke dalam media dan diletakkan di dalam inkubator pada suhu 30°C tanpa pencahayaan. Data saiz dan tempoh perkembangan larva dikutip melalui persampelan tiga larva bersaiz paling besar daripada setiap media peliharaan, dua kali sehari pada jam 0900 dan 1500 sehingga pupariasi berlaku. Min saiz larva *M. scalaris* yang dibiakkan pada hati lembu adalah tertinggi (4.23 ± 1.96 mm) diikuti agar hati lembu (3.79 ± 1.62 mm), agar kasein (3.14 ± 1.16 mm) dan agar nutrien (3.09 ± 1.11 mm). Panjang larva yang dibiakkan pada hati lembu dan agar hati lembu adalah berbeza secara signifikan dengan larva yang dibiakkan pada agar nutrien dan agar kasein ($p < 0.05$). Larva pada hati lembu dan agar hati lembu merekodkan tempoh larva paling singkat sebelum memasuki peringkat pasca-pemakanan (89 jam), diikuti agar nutrien (119 jam) dan agar kasein (184 jam). Jumlah tempoh perkembangan daripada peringkat telur sehingga lalat dewasa pada agar hati dan hati mentah ialah kira-kira 163 jam. Semua pupa di dalam agar nutrien dan agar kasein gagal untuk menetas. Kajian ini mendapati bahawa agar hati lembu berpotensi digunakan sebagai diet alternatif kepada hati lembu mentah untuk pemeliharaan larva *M. scalaris* di makmal.

Kata kunci: *Megaselia scalaris*, entomologi forensik, perkembangan, jenis makanan

INTRODUCTION

Megaselia scalaris (Loew) or commonly known as scuttle fly, is a cosmopolitan species and has been recorded in

many forensic cases worldwide (Disney 1994, 2008). This species belongs to the family Phoridae and can be easily distinguished from other forensically important species by having thick costal vein, humpbacked appearance and

frequently running in an erratic manner (Disney 1994). Other distinctive features for this species includes having a shorter and broader sixth tergite on female adult and a single strong bristle on left side of epandrium on male adult (Disney & Sinclair 2008). *M. scalaris* and other phorid species are generally smaller compared to other forensically important species such as blow flies or flesh flies. This feature provides advantage for *M. scalaris* to enter enclosed environments as this species were usually found indoors. In forensic entomology, *M. scalaris* was frequently discovered on bodies in human premises and could be used as the reference to estimate time of death or post mortem interval (PMI) (Campobasso et al. 2004; Reibe & Madea 2010; Thevan et al. 2010).

This species has been reported feeding on a broad spectrum of food source including decaying organic matter (Disney 2008). In laboratory, this species has been reared on different types of media including agar-based diets such as chocolate and blood agar (Biery et al. 1979), cornmeal agar (Harrison & Cooper 2003), deer blood agar (Tumrasvin et al. 1977) and agar mixed with snail tissue extract (Idris et al. 2001). In forensic entomology laboratory procedure, animal tissue such as liver or meat are commonly being used as food source because it provides sufficient nutrient for larval growth (Davies & Ratcliffe 1994; Grassberger et al. 2003; Grassberger & Reiter 2001). However, the use of decomposing animal tissues often brings unpleasant odour and unsterile (Sherman & Tran 1995).

This paper report our preliminary findings on *M. scalaris* development using different food types, mainly using agar based diets. We also describe the methodology used to prepare the colony of *M. scalaris* in laboratory as reference for other researchers and students to use this species as an insect model in an entomological laboratory.

PREPARATION OF COLONY

Baited traps for capturing *M. scalaris* were made by referring to designs by different authors (Amoudi et al. 1989; Disney 2005; Moretti et al. 2009). Approximately 25 g of raw cow's liver was placed in four 4×3 cm plastic cups placed inside 8.5×6 cm semi-translucent cylindrical plastic containers. The opening of the cylindrical plastic container was covered with 1×1 mm plastic wire mesh thereby allowing the entry of scuttle flies. Traps were placed adjacent to the window opening in Forensic Entomology Laboratory, School of Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia for a duration of two days.

Phorid fly eggs recovered from the baits were transferred into a new plastic container for rearing. Approximately 50 g raw cow's liver was supplied as food source. The rearing container was placed inside a 33×23×18 cm plastic aquarium as a breeding cage. The opening of the aquarium was covered with cotton cloth and modified for the ease of sampling and supplying food for

live specimens. Rearing took place at room temperature and relative humidity (26.0-27.0°C, 55-65% RH) in 12:12 hours light and dark periods. Sugar granules and water were given *ad libitum* (Greenberg & Wells 1998) and minced cow's liver including its liquid exudates were supplied as protein source for the flies (Ames & Turner 2003).

To identify the species, some live adult specimens were withdrawn randomly from breeding cage, killed using chloroform and preserved in 70% ethanol. They were fixed on glass slides (Disney 1994) and few were chemically dried using hexamethyldisilazane (HMDS) (Triplehorn & Johnson 2005). Species identification was done by using scuttle fly taxonomic keys and descriptions (Disney 1989, 1994; Disney & Sinclair 2008; Brown & Oliver 2007; Tumrasvin et al. 1977). It was found that reared eggs and trapped adults consist of a single species of scuttle fly, *M. scalaris*.

PREPARATION OF DIETS

A total of four types of diets were prepared in this study: nutrient agar (control), casein agar, raw cow's liver and cow's liver agar. Nutrient agar was prepared by mixing 3 g of nutrient agar powder (Merck, Germany) into 75 ml distilled water and casein agar was prepared by adding 5.0 g glucose, 3.0 g starch, 3.2 g yeast, 3.0 g agar, 6.0 casein and 0.04 g multivitamin into 75 ml distilled water. For cow's liver agar, a total of 7 g of powdered cow's liver were mixed with 3 g nutrient agar in 75 ml distilled water. Agar mixtures were mixed in 250 ml beakers using magnetic stirrer on a hot plate at 90°C for 1 minute. All homogenized agar was autoclaved at 121°C for 15 minutes and allowed to cool down to room temperature before used. Raw liver media was prepared by cutting 100 g of cow's liver into approximately 1 × 1 × 1 cm cubes and placed into a 250 ml beaker layered with sterile wood shavings.

DATA COLLECTION

In order to obtain *M. scalaris* samples, minced cow's liver were introduced into breeding cage and oviposition was allowed within 2 hour period (Greenberg & Wells 1998). A total of 100 eggs were subsequently transferred using fine tip forceps each into different media as prepared above. All beakers were sealed with paper towel to avoid larvae escaping and to allow ventilation. Beakers were placed in incubator (IB-05G, Jeio Tech, Korea) set at 30°C in a continuous dark condition. Data were collected twice a day at 0900 and 1500 hrs until pupariation. Three of the largest larvae were randomly with drawn from each rearing media and measured its length using Leica Application Software™ attached to stereomicroscope Leica EZ4D™. During pupa stage, a total of 3 puparia were collected from each media. Their length and weight were measured and they were transferred into 50 ml universal container sealed with cloth to obtain pupa developmental time. Statistical analysis was conducted by using PASW® Statistics 18 software.

M. scalaris third instar larvae reared on raw cow's liver recorded the highest length value (4.23 ± 1.96 mm), followed by cow's liver agar (3.79 ± 1.62 mm), casein agar (3.14 ± 1.16 mm) and nutrient agar (3.09 ± 1.11 mm) respectively. The range of length were recorded as: 0.62-4.84 mm (nutrient agar), 1.12-5.83 mm (casein agar), 0.82-6.09 mm (liver agar) and 1.13-6.91 mm (raw liver). There were variations in the data of length of *M. scalaris* larvae causing violation of normal distributions even after data transformation. Kruskal-Wallis test was performed to determine significant differences between those diets. It was discovered that there was a significant difference between nutrient agar (mean rank = 87.27), casein agar (mean rank = 87.70), liver agar (mean rank = 120.52) and raw liver (mean rank = 130.61), $H = 20.55$, $df = 3$, $N = 204$, $p < 0.05$, Cohen's $f = 0.34$ (medium effect size).

Further analysis using Mann-Whitney *U* test discovered larval size on liver agar and raw liver were significantly different from nutrient agar and casein agar. Significant difference between nutrient agar (mean rank = 39.91) and liver agar (mean rank = 59.09) recorded as, $U = 684.00$, $z = -3.36$, $p < 0.05$, two-tailed test; between nutrient agar (mean rank = 38.20) and raw liver (mean rank = 56.88), $U = 593.50$, $z = -3.33$, $p < 0.05$, two-tailed test; between casein agar (mean rank = 50.19) and liver agar (mean rank = 68.09), $U = 1028.00$, $z = -2.82$, $p < 0.05$, two-tailed test; between casein agar (mean rank = 47.40) and raw liver (mean rank = 67.58), $U = 833.00$, $z = -3.20$, $p < 0.05$, two-tailed test. There was no significant difference detected between larval size of *M. scalaris* in nutrient agar (mean rank = 63.17) and casein agar (mean rank = 61.11), $U = 1793.00$, $z = -0.32$, $p = 0.75$, two-tailed test; and between liver agar (mean rank = 37.34) and raw liver (mean rank = 45.14), $U = 659.50$, $z = -1.49$, $p = 0.14$, two-tailed test.

Feeding larva, pupa and adult period were almost equal in liver agar and raw liver. Liver agar and raw liver media recorded shortest larva period, approximately 89 hours before entering post feeding stage. Puparia in liver agar and raw liver hatched after approximately 163 hours and adult longevity for male and female *M. scalaris* in both diets was approximately 71 hours. Total developmental time from oviposition until adult emergence for *M. scalaris* in liver agar and raw liver was approximately 163 hours. Larva period in nutrient agar were recorded approximately 30 hours longer compared to liver agar and raw liver, and entered post feeding stage after 119 hours. Longest larva development occurred in casein agar, which took approximately 184 hours. However, all puparia in nutrient agar and casein agar did not hatch.

This research highlighted the growth of *M. scalaris* on different diet types. Findings by Idris et al. (2001) was more relevant to be compared to our result because of similar methodology being utilized in diets preparation. Idris et al. (2001) recorded highest mean larval length in casein agar but in our study, larval length in casein agar was significantly lower than raw liver and liver agar. In contrast to developmental result by Idris et al. (2001), all *M. scalaris*

puparia in nutrient agar and casein agar did not successfully hatched into adults. In both of our studies, casein agar was a modification of brewer's yeast which was commonly used to culture dipterans colony in laboratory. However, we could not ascertain the possible cause for this dissimilarity although almost a similar approach was used.

Several authors highlighted the importance of synthetic diets using yeast component because it seemed to enhance larval growth (Amorim & Ribeiro 2001; Ribeiro & Zuben 2010; Daniels et al. 1991; Green et al. 2003; Leal et al. 1982; Leal et al. 1991; Sherman & Tran 1995; Singh 1977) but there were inconsistencies in their findings. For example, studies by Singh (1977) found larval survival could be influenced by the value of yeast being used. In another study, Leal et al. (1991) discovered combination of casein and yeast in diets was more effective than using pure casein because yeast could provide ribonucleic acid (RNA) to promote larva growth. However, according to Daniels et al. (1991), larval weight increased when yeast was added in diet consisting of agar and horse blood but they could not survive in pure yeast agar culture. These findings indicate further studies are required to understand the effect of yeast on larval growth, particularly on forensically important species.

This research also discovered that *M. scalaris* developmental rates in both raw liver and liver agar were approximately equal. Sterile liver agar was prepared as an alternative form to raw liver used in laboratory rearing of this species. Sherman and Tran (1995) recorded developmental and survival rate of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) larvae in mixed cow's liver puree and agar were better than using raw liver. In addition, liver agar gives us more advantages compared to raw liver because it does not emit foul odour, sterile and could be stored for a longer period.

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