Jurnal Sains Kesihatan Malaysia **16**(1) 2018: 29-33
DOI : http://dx.doi.org./10.17576/JSKM-2018-1601-04

Komunikasi Pendek/*Short Communication*

The Effect of Burned Liver on the Length, Weight and Development of *Megaselia scalaris* (Loew) (Diptera: Phoridae) – A Preliminary Assessment and Implications in Forensic Entomology

(Kesan Penggunaan Hati Lembu Dibakar terhadap Panjang, Jisim dan Perkembangan *Megaselia scalaris* (Loew) (Diptera: Phoridae) – Penilaian Awal dan Kesannya di Bidang Entomologi Forensik)

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ABSTRACT

Development of insects in laboratory for minimum post mortem interval estimation (mPMI) or time of colonisation (TOC) in forensic entomology can be affected by the type and quality of food consumed during larval period. Since mPMI estimation also involves analysis of larval specimens collected from burned human remains, it is important to study if burned tissues could affect growth of sarcosaprophagous larvae. This study investigated the effect of burned tissues on the size and developmental period of Megaselia scalaris (Loew) (Diptera: Phoridae), a species of forensic importance. Development of M. scalaris on 75 g burned cow's liver was compared with control liver in three study replicates. Mean larval length (2.87 ± 0.11 mm) and weight (0.81 ± 0.08 mg) of M. scalaris larvae in burned liver diets were significantly lower than larval length (5.03 ± 0.15 mm) and weight (2.85 ± 0.21 mg) of control liver diets ($p < 0.001$) whilst mean pupal length (2.53 ± 0.06 mm) and weight (0.92 ± 0.06 mg) in burned liver diets were significantly lower than pupal length (3.52 ± 0.06 mm) and weight (2.84 ± 0.16 mg) in control liver diets ($p < 0.001$). Development of larvae in burned liver was 5-9 hours slower than those feeding on control liver based on single observation. Although the assessment is preliminary, the findings indicate physical growth of larvae feeding on burned animal tissues was affected and entomological specimens recovered from burned remains should be evaluated carefully to avoid errors in mPMI/TOC estimation. Limitations and suggestions for further research are also presented herein.

Keywords: Burned liver; Megaselia scalaris; length; weight; developmental rate; minimum PMI; diet types

ABSTRAK

Perkembangan serangga di makmal bagi penentuan jarak waktu pasca kematian minimum (mPMI) atau masa kolonisasi (TOC) di bidang entomologi forensik boleh dipengaruhi oleh jenis dan kualiti makanan ketika peringkat larva. Oleh sebab anggaran mPMI turut melibatkan analisis spesimen larva yang dikutip daripada mayat dibakar, adalah penting untuk mengkaji sama ada tisu yang dibakar dapat mempengaruhi perkembangan larva sarkosaprofagus. Kajian ini menentukan kesan tisu dibakar terhadap saiz dan tempoh perkembangan Megaselia scalaris (Loew) (Diptera: Phoridae), spesies lalat berkepentingan forensik. Perkembangan M. scalaris pada 75 g hati lembu dibakar dibandingkan dengan hati lembu kawalan dalam tiga replikasi kajian. Min panjang larva (2.87 ± 0.11 mm) dan jisim larva (0.81 ± 0.08 mg) M. scalaris pada diet hati lembu dibakar adalah lebih rendah secara signifikan berbanding panjang larva (5.03 ± 0.15 mm) dan jisim larva (2.85 ± 0.21 mg) pada diet hati kawalan ($p < 0.001$) manakala panjang pupa (2.53 ± 0.06 mm) dan jisim pupa (0.92 ± 0.06 mg) pada diet dibakar adalah lebih rendah secara signifikan berbanding panjang pupa (3.52 ± 0.06 mm) dan jisim pupa (2.84 ± 0.16 mg) pada diet hati kawalan ($p < 0.001$). Berdasarkan satu pemerhatian, perkembangan larva pada hati dibakar adalah 5-9 jam lebih perlahan berbanding larva yang memakan hati kawalan. Walaupun penilaian ini masih di peringkat awal, keputusan menunjukkan perkembangan fizikal larva dipengaruhi tisu haiwan dibakar dan pengendalian spesimen entomologi yang dikutip daripada mayat dibakar perlu dilakukan dengan teliti untuk mengelakkan ralat dalam anggaran mPMI/TOC. Limitasi dan cadangan untuk kajian lanjutan turut dihuraikan.

Kata kunci: Hati dibakar; Megaselia scalaris; panjang; jisim; kadar perkembangan; PMI minimum; jenis diet

In forensic investigation, the time of death of decomposing human remains is usually inferred from the minimum post mortem interval (mPMI) or time of colonisation (TOC) by insects (Tomberlin et al. 2011). Development of insects, which mainly consists of sarcosaprophagous dipteran larvae, can be used as biological reference to estimate mPMI or TOC. Reference specimens collected from human remains are usually compared with the developmental rate of the same species developed in the laboratory or in conditions where it is almost similar to the

natural environment of the reference specimens (Amendt et al. 2007; Hall et al. 2012). This procedure commonly involves techniques that require breeding of reference species on decomposing animal tissues such as cattle liver (Grassberger & Reiter 2001, 2002).

However, in some cases, entomological specimens for mPMI estimations were collected from burned human bodies (Kumara et al. 2012; Nor Afandy et al. 2003). It was proposed earlier that burned flesh delayed the arrival of insects to the body (Catts & Goff 1992) but studies using burned pig and rabbit carcasses in more recent years showed no delay of insect colonisation compared to normal carcasses (Avila & Goff 1998; Gruenthal et al. 2012; Mahat et al. 2016) with inconsistencies of findings on the decomposition rates, insect succession and ovipositional patterns on burned carcasses (Beckerdite et al. 2014; Heo et al. 2008; Pacheco 2015; Vanin et al. 2013).

Since dipteran larvae could be found feeding on burned decomposing human and animal remains, it is important to know whether consumption of burned tissues by insect larvae could affect their life and size. To test this hypothesis, a developmental study using burned animal tissue (cow's liver) was conducted on *Megaselia scalaris* (Loew) (Diptera: Phoridae) a species of forensic importance particularly in concealed environments (Campobasso et al. 2004; Reibe & Madea 2010). It has also been recorded feeding on wide spectrum of food source including decomposing organic materials such as vertebrate and invertebrate carcasses (Disney 1994, 2008).

In this study, *M. scalaris* eggs were obtained from f_2 to f_3 adult colony maintained in Forensic Entomology Laboratory, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur. This colony originated from *M. scalaris* adults ovipositing on cow's liver bait placed in the laboratory. Adult *M. scalaris* flies were cultured in a plastic rearing container (33 cm × 8 cm × 26 cm) at room temperature (25-26°C) and relative humidity (65-75%) with 12 h light-dark photoperiods. Sugar granules and water were provided *ad libitum* for adult *M. scalaris* as food source while cow's liver exudates were provided as protein source for gravid females. To obtain the eggs, approximately 30 g cow's liver were introduced into the rearing container for 2 hrs as oviposition medium.

Cow's liver in this study was purchased from a local market and originated from single cattle. It was cut into 1 cm × 1 cm × 1 cm cubes and burned by using Bunsen burner at 500-550°C for 3 minutes in manual rotation, resulting dry-burned surfaces but medium cooked internally. After cooling down to room temperature, an approximately 75 g burned liver was put inside each six replicates of 300 ml plastic rearing container which was quarter-filled with sterile wood shavings. A total of 100 eggs were transferred randomly on the burnt liver surface. This procedure was conducted carefully using fine tip forceps with an aid of dissecting microscope to avoid damaging the eggs. Another six replicates were also prepared using fresh cow's liver cut into 1 cm × 1 cm × 1 cm cubes as controls.

All twelve rearing containers were subsequently covered with paper towel and fastened with rubber bands to avoid contamination by other insects and to prevent larvae from escaping the containers. They were placed in an open but shaded area, adjacent to the laboratory. Hourly ambient temperature and relative humidity were recorded using temperature and relative humidity data logger, EL-USB-2 (Lascar Electronics, UK).

Larval sampling was carried out at 4-hour intervals from 0700 to 1900 daily until they reach pupal stage. During each sampling occasion, three larvae were randomly collected from each container and washed by using distilled water. They were dried on a piece of tissue paper towel and weight was measured individually on a digital analytical balance. To measure the lengths, larvae were killed in near boiled water (≈80°C) for 30 seconds and measured by using Leica EZ 4D™ stereomicroscope and Leica Application Software (Leica Microsystems, Switzerland).

The length and weight of the *M. scalaris* pupae were measured by collecting three live specimens randomly from each rearing container. The length and weight of pupae were measured individually and then transferred into a plastic container covered with Kimwipes (Kimtech, Canada) tissue paper until they became adult flies. Observation of the pupae was conducted every 6 hours to determine eclosion time.

Megaselia scalaris larvae feeding on burnt liver in all study replicates had significantly lower larval length and weight compared to those in control liver (Table 1). Larval length in burned liver diets (2.87 ± 0.11 mm) was significantly lower than larval length in control diets (5.03 ± 0.15 mm), $t\,(108) = 11.47$, $p < 0.001$ (replicate 1). Similar results were also observed in replicate 2, $t\,(90) = 29.54$, $p < 0.001$, replicate 3, $t\,(93) = 14.01$, $p < 0.001$. Mann-Whitney U non-parametric test (SPSS® Ver. 18) was conducted to determine the difference of larval weight in burned and control liver diets. The effects were similar to the larval lengths which significant differences were detected between these two diets, $U = 362.00$, $z = -6.74$, $p < 0.001$ (replicate 1); $U = 0.00$, $z = -6.57$, $p < 0.001$ (replicate 2); and, $U = 2.50$, $z = -6.94$, $p < 0.001$ (replicate 3).

The effect of diet types on *M. scalaris* pupae could only be observed during replicate 1 as larvae in replicate 2 and 3 could not survive until pupal stage (Table 2). Pupal length in burned liver diets (2.53 ± 0.06 mm) was significantly lower than the pupal length in control diets (3.52 ± 0.06 mm), $t\,(49) = 11.45$, $p < 0.001$, while the weight in burned liver diets (0.92 ± 0.06 mg) was significantly lower than those in control media (2.84 ± 0.16 mg), $t\,(49) = 11.45$, $p < 0.001$. The total developmental passed for the larvae to complete larval stage was slightly slower in burned liver diet (104 hours) compared to those in control diets (95 hours). Feeding activity and movement of larvae in burned liver diets was in relatively slower rate compared to those in control diets.

TABLE 1. Length and weight of *M. scalaris* larvae in control (c) and burned liver (b) diets. All results between test groups were statistically significant ($p < 0.001$) for larval length (*t-test* for independent samples) and weight (Mann-Whitney *U* test)

Study Replicate	Mean Temperature/°C	*n*	Mean Length/mm	Length Mean Difference/mm	*t*	Mean Weight/mg	Weight Mean Difference/mg	*U*
1	31.06 ± 0.25	c = 54 b = 54	c = 5.03 ± 0.15 b = 2.87 ± 0.11	2.16	11.47	c = 2.85 ± 0.21 b = 0.81 ± 0.08	2.04	362.00
2	30.62 ± 0.21	c = 72 b = 18	c = 4.48 ± 0.09 b = 1.48 ± 0.04	3.00	29.54	c = 2.39 ± 0.19 b = 0.10 ± 0.00	2.29	0.00
3	30.23 ± 0.23	c = 72 b = 21	c = 4.65 ± 0.10 b = 1.78 ± 0.16	2.88	14.01	c = 2.41 ± 0.16 b = 0.18 ± 0.03	2.23	2.50

TABLE 2. Length and weight of *M. scalaris* pupae in control (c) and burned liver (b) diets. Statistically significant difference between test groups was in study replicate 1 ($p < 0.001$) for pupal length and weight (*t-test* for independent samples)

Study Replicate	Mean Temperature/°C	*n*	Mean Length/mm	Length Mean Difference/mm	*t*	Mean Weight/mg	Weight Mean Difference/mg	*t*
1	31.06 ± 0.25	c = 36 b = 13	c = 3.52 ± 0.06 b = 2.53 ± 0.06	0.99	11.45	c = 2.84 ± 0.16 b = 0.92 ± 0.06	1.91	11.54
2	30.62 ± 0.21	c = 75 b = 0	c = 3.59 ± 0.04 b = -	-	-	c = 2.75 ± 0.10 b = -	-	-
3	30.23 ± 0.23	c = 72	c = 3.48 ± 0.04 b = 0	-	-	c = 2.59 ± 0.10 b = -	-	-

The findings above showed consumption of burned tissues affected size and developmental rate of *M. scalaris* larvae. When compared to normal liver tissues, larvae in burned liver had significantly lower length (mean difference = 2.16-3.00 mm) and weight (mean difference = 2.04 – 2.29 mg). In terms of developmental periods, it took longer for larvae in burned liver to reach pupal stage compared to those in control liver, and this was observed based on one study replicate. In study replicate 2 and 3, larvae failed to develop to pupal stage.

Several findings previously indicated that different type of animal tissues being used in rearing could affect morphological sizes and developmental rate of blow fly larvae (Harnden & Tomberlin 2016; Warren & Anderson 2009). For example, development of *Calliphora vicina* (Robineau-Desvoidy) larvae was faster and the size was bigger when reared using tissues from lung, kidney, brain and heart than liver (Kaneshrajah & Turner 2004). It was also reported that using pig (*Sus scrofa* L.) tissue gave to faster and larger size of *Lucilia sericata* (Meigen) larvae compared to cow tissue (Clark et al. 2006). On *M. scalaris*, the most recent development studies were conducted by Thomas et al. (2016), Zuha et al. (2012) and Zuha and Omar (2014). Thomas et al. (2016) compared the developmental effect of *M. scalaris* using bovine and porcine liver as food source and found no significant difference between the food types. In Zuha et al. (2012), it was reported that using different condition of cow's liver such as fresh liver and its agar form affected developmental rate of *M. scalaris* at a range of 23-36°C.

However, variations from the effects on larvae development could also be due to factors other than type of tissues. Population genetics of flies and methodologies being employed in rearing could be the contribution factors to these variations (Flores et al. 2014; Thomas et al. 2016). In other findings, feeding behaviour and impacts on development might be species-specific (Niederegger et al. 2013) and condition of larval crowding could also implicate their growth (Ireland & Turner 2006). One of the most overlooked factor is the nutritional content of the food for larvae which commonly consists of carbohydrate, protein and fat (Bursell 1970; Speight et al. 2008). In forensic insects for instance, it has been reported that fat content in diet influenced size, mortality and developmental time of blow fly (Li et al. 2014). Therefore, it is important in future research on diet effects towards development to analyse the nutritional content of the diets.

In this study, although similar amount of normal liver and burned liver were used (75 g), physical changes in burned liver might have caused the differences in size and rate of development of *M. scalaris.* This results can be considered preliminary but forensic entomologists are advised to be cautious when estimating mPMI using larvae from burned tissue. It is recommended to conduct further investigation using different fly species and animal tissues with various degrees of burn. Since it is difficult to simulate burned liver tissues to those of burned human remains, we propose findings from this study to be extended and validated using decomposing burned animal remains in the field.

ACKNOWLEDGEMENT

We would like to thank the School of Diagnostic and Applied Health Science, Faculty of Health Science, Universiti Kebangsaan Malaysia, for providing facility and equipment in this research.

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Received: November 2016
Accepted for publication: October 2017