EFFECTS OF SUGAR CONCENTRATION ON FECUNDITY, BITING BEHAVIOR AND SURVIVABILITY OF FEMALE *AEDES* (STEGOMYIA) *ALBOPICTUS* (SKUSE)

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Abstract. This study was conducted to better understand the effect of different sucrose concentrations on *Aedes albopictus* fecundity, biting behavior and survival. Laboratory strain *Ae. albopictus* females were raised at four different sucrose concentrations (10%, 30%, 50%, and 70%) and their fecundity, host biting on and survival rates were determined. Mosquitoes fed on high (50% or 70%) showed higher mean fecundity rate compared to those on low (10% or 30%) sucrose concentration, and had higher daily biting rate. On the other hand, mosquitoes fed on the low (10% or 30%) sucrose concentrations recorded higher survival rate. These results suggest female mosquitoes deficient in nutrient intake during sugar feeding may regain nutrients needed during blood feeding, whereas those fed on high sucrose concentration have high fecundity due to high biting rate but have low survivability due to low sucrose intake during sugar feeding. Thus, *Ae. albopictus* females have a capability to regulate their metabolic needs based on sugar nutrient availability.

Keywords: *Aedes albopictus*, biting frequency, fecundity, sucrose concentration, survivability

INTRODUCTION

Asian tiger mosquito, *Aedes* (Stegomyia) *albopictus* (Skuse), is native to Asia and has been one of the most internationally invasive species in the past two decades (Benedict *et al,* 2007). It is a common mosquito species associated with sub-urban and rural habitats, is closely associated with water-holding containers around human dwellings and is a major vector of dengue virus (DENV), second only to *Aedes aegypti* (L).

According to data provided by the Malaysia Remote Sensing Agency (2015), 62,648 DENV cases were reported between January and 11 July 2015. The number of cases increased 34.2% (15,967 cases) compared to the previous year in which there were only 46,681 cases within the same period. This mosquito species is a potential vector for other viruses, such

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as Japanese encephalitis (JEV), West Nile (WNV) and Yellow Fever (YFV) viruses in humans (Shroyer, 1986; Mitchell, 1995). Laboratory experiments showed that the Southeast Asian strains of *Ae. albopictus* can transmit Zika virus (ZIKV), thus increasing the possibility for the species to be a local vector for ZIKV (Wong *et al*, 2013). Notwithstanding the role of this mosquito as a local vector for various human viruses, it is a major nuisance-biting pest (Moore and Mitchell, 1997; Rezza, 2012).

Aedes albopictus is an exophilic and exophagic mosquito usually found outdoors (Hawley, 1988). In natural environment, *Ae. albopictus* breeds in tree holes and other natural water reservoirs. Unfortunately, urbanization has led the mosquito to adapt breeding in artificial water containers, which in turn increases the risk for such mosquito-borne diseases to be transmitted to humans (Li *et al*, 2014).

Mosquitoes utilize plant-based sources of sugar for energy. Sugar is a necessity for both male and female mosquitoes as source of nutrition for survival and breeding. In a laboratory study, the ability of sucrose to enhance longevity of mosquitoes has been shown quite conclusively (Jahangir *et al*, 2003). The vast variety of plant species in the natural environment offers different sugar composition to mosquitoes. Mosquito resource-seeking behavior towards different sugar compositions was studied by Müller *et al* (2011), who demonstrated that *Ae. albopictus* is attracted only to certain species of flowers with specific sugar composition. The role of sugar feeding produces significant effects on reproduction capacity based on different mosquito species (Braks *et al*, 2006). However, blood meal is necessary for egg development in female mosquito, and both blood and sugar are interchangeable depending on mosquito needs.

Recently, research has been conducted on mosquito control through regulating sugar feeding behavior. For example, attractive toxic sugar bait (ATSB) using eugenol as active ingredient was implemented for controlling *Ae. albopictus* (Revay *et al*, 2014). Xue and Barnard (2009) reported that with availability of sucrose rate of mosquito responding to human host is reduced.

Thus, this study aimed to identify the effect of sugar concentration on fecundity, biting behavior and survival rates of adult female *Ae. albopictus* in a laboratory environment. An understanding of the pattern of mosquito sugar feeding behavior may assist in the development of surveillance or control technologies.

MATERIALS AND METHODS

Mosquito species and rearing

Eggs of a laboratory strain of *Ae. albopictus* were obtained from the insectarium of Vector Control and Research Unit (VCRU), Universiti Sains Malaysia. Larvae were reared with food applied onto the water surface every two days. Food was a mixture of cat food, beef liver, yeast and milk powder in the ratio of 2:1:1:1 by weight prepared in a fine powder form digestible to larvae. Larvae food was given as follows: 0.8 mg/larva for 1st - 2nd instars and 1.6 mg/larva for 3rd - 4th instars (Kassim *et al*, 2012). Newly emerged pupae were placed in a transparent plastic container containing 250 ml of dechlorinated water. The opening of the container was covered with a net and a hole was made for inserting or removing mosquitoes.

After 4-6 days, newly emerged adults were transferred to a cage (30 x 30 x 30 cm) and allowed access to a cotton pad soaked

in 10% sucrose solution with daily blood feeding on white mice. A filter paper was placed for females to oviposit their eggs, F1 generation of which were collected on the following day and were hatched and reared as described above.

Twenty newly emerged adult female mosquitoes (F1 generation) were transferred to a transparent Perspex cage (20 x 20 x 20) together with 20 adult male mosquitoes for mating. Mosquitoes were maintained under a photoperiod of 12 light:12 dark hours cycle at 29 ± 2°C and 67 ± 9% relative humidity.

Sucrose solution

A cotton wool ball soaked with a sucrose solution [10%, 30%, 50% and 70% (w/v)] was placed into three cages and was replaced for every two days to avoid fermentation.

Assessment of fecundity rate

Fecundity of female mosquitoes was assessed by identifying the maximum number of eggs produced from individual egg batches (Clements, 1992). A piece of filter paper was placed inside a petri dish filled with dechlorinated water as a platform for laying eggs. The filter paper was changed for every 2 days. Daily number of eggs produced was recorded once every two days for 10 days and the total number of eggs produced was noted.

Observation on biting behavior

The first blood-meal was given to female mosquitoes on day 4 after emergence. Female mosquitoes were allowed access to a white mouse restrained within a wire-netting, with the lower part exposed upwards for 20 minutes. Peak time for female mosquito biting activity was 06:00-08:00 PM (Delatte *et al*, 2010). A fully-engorged female resting on wall of the cage is considered as a single bite (Garrett-Jones, 1964). The blood feeding routine was repeated continuously and the frequency of mosquito bites was recorded for 10 days, after which the daily mean biting rate was determined.

Determination of survival rate

The number of surviving female mosquitoes was recorded daily and dead mosquitoes were removed to avoid any miscalculation of mortality on the following day. Total number of surviving female mosquitoes was recorded after 15 days.

Statistical analysis

Data of female *Ae. albopictus* fecundity were analyzed using Pearson correlation to determine the relationship between number of eggs produced by females fed on different sucrose solutions, and biting frequency and survivability of female *Ae. albopictus* using Kruskal-Wallis test to determine their relationship with different sucrose solution. Bonferroni correction test was used for determining any mistakes when rejecting null hypothesis. A p-value < 0.05 is considered statistically significant. All statistical tests were performed using IBM SPSS Statistics version 22.0 (IBM, Armonk, NY).

RESULTS

Fecundity of female *Ae. albopictus*

Ten-day mean (± SE) number of eggs produced by female *Ae. albopictus* fed on four different concentrations of sucrose solution ranged from 117 ± 21 eggs fed on 10% sucrose solution to 176 ± 34 on 70% sucrose solution (Fig 1). Pearson correlation analysis showed that the number of eggs produced is significantly correlated with sucrose concentration ($r = 0.215$, $p < 0.05$).

Biting frequency of female *Ae. albopictus*

Mosquito biting frequency was recorded as the presence of fully engorged

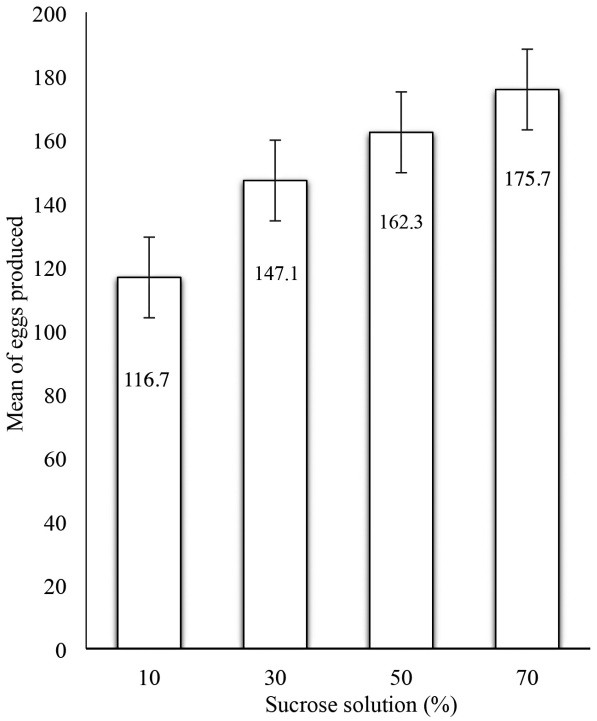

Fig 1–Eggs produced by female *Ae. albopictus* fed on different sucrose concentrations. Number and bar designate mean ±SE of triplicate experiments of eggs produced daily over a 10-day period.

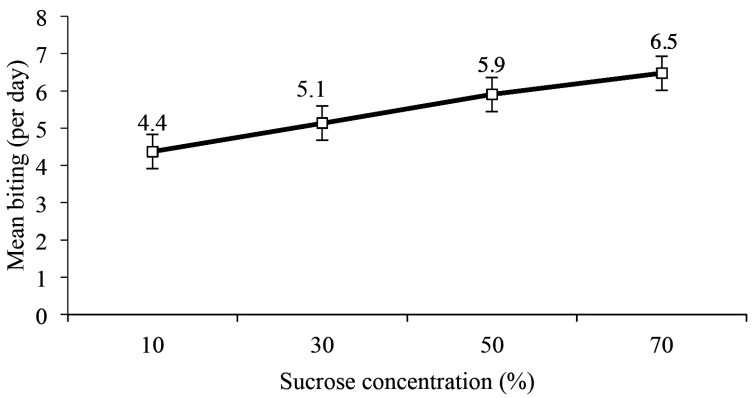

Fig 2–Biting frequency of female *Ae. albopictus* under different sucrose concentrations. Number and bar designate mean ±SE of triplicate experiments of daily biting of a mouse over a 10-day period.

female mosquitoes resting on cage walls of the cage after blood feeding on mice. Over a period of 10 days, mean (± SE) daily biting rate ranged from 4.4 ± 0.5 for mosquitoes fed on 10% sucrose solution to 6.5 ± 0.3 for those on 70% sucrose solution (Fig 2) Kruskal-Wallis test showed a significant difference ($\chi^2 = 21.635$, df = 3, $p < 0.05$) in the mean daily biting frequency of mosquitoes under different sucrose concentrations. Bonferroni correction test showed a significance difference for 10% and 50% ($p < 0.05$), 10% and 70% ($p < 0.05$) sucrose concentrations.

Survivability of female *Ae. albopictus*

Over a 15-day period mean percent mortality of female *Ae. albopictus* ranged from 0% fed on 10% and 30% sucrose solutions to 3% fed on 70% sucrose solution (Table 1). Pearson correlation analysis showed that the mortality of female mosquitoes is significantly correlated with the concentration of sucrose ($r = 0.167$, $p < 0.05$).

DISCUSSION

This study demonstrated that, while access to a high concentration of sucrose solution is significantly correlated with in-

Table 1
Mean percent daily mortality of female *Ae. albopictus* fed on different sucrose solutions over a 15-day period.

Sucrose solution (% w/v)	Mean percent mortality
10	0
30	0
50	2
70	3

creased blood feeding rate and fecundity, it also correlates with increased mortality of female *Ae. albopictus*. This result poses interesting questions regarding the importance of sugar meals in a natural setting and potential development of mosquito control initiatives, which are based on an understanding of sugar feeding behavior.

It was not possible to determine if it was the availability of increased sugar concentrations that assisted blood feeding success or the additional nutritional input from the sugar, which resulted in higher fecundity. Eliason (1963) suggested that mosquitoes may not be able to liquefy well highly concentrated sugar solution using their saliva. Thus, there might be a possibility that female mosquitoes fed on high sucrose concentrations have restricted carbohydrate nutrient. The results from our study correspondent to that by Scott *et al* (1997) who suggested that mosquitoes restricted in sugar feeding have higher daily fecundity, as they benefit more from blood meals to achieve adequate amount of nutrients required for energy reserves and ovarian maturation. This notion is supported by Nayar and Sauerman (1975) who stated that female mosquitoes lacking in sugar nutrient will utilize more nutrient from blood meal for both survival and eggs production.

Given the similarities between our results and those of previous publications, it raises the possibility that there are many factors influencing fecundity besides the level of nutrition obtained through a combination of blood and sugar meals. It may also be a species-specific response of mosquitoes to the relative proportions of blood and sugar needed in diet. Unravelling the role of sugar and blood feeding on fecundity is critical to the understanding of population dynamics of vector species, which may, in turn, influence mosquito control programs and assessments of mosquito-borne disease outbreaks.

Previous studies demonstrated that blood meal is more essential than sugar for enhancing the amount of eggs that a mosquito can produce. Gary and Foster (2001) reported mosquitoes that fed on blood alone produce more eggs than mosquitoes that provided both sugar and blood meals under standard larval nutrition. Joy *et al* (2010) concluded that mosquitoes given weekly blood meals produce more eggs compared to those provided with a single or no blood meal under a condition where all mosquitoes are offered a standard concentration of sugar solution but with different blood meal routine. The current study found out that females fed on high sucrose concentration have high biting frequency probably due to insufficient nutrient intake from the sugar feeding. The results are in agreement with those of Foster and Eischen (1987) stating that female mosquitoes biting frequency on host increases because they tend to seek for more blood meal as replacement for their sugar nutrient deprivation.

Sufficient sugar intake increases lifespan of mosquitoes (Gary and Foster, 2001). However, female mosquitoes fed on sugar

only do not live longer as those offered either blood meal and sugar or only blood meal (Styer *et al*, 2007a). Another study by Styer *et al* (2007b) confirmed that mosquitoes fed on both blood and sugar live significantly longer than mosquitoes fed on sugar alone or blood meal only. Female mosquitoes need an adequate amount of both blood and sugar intake for reproductive process and energy reserves to extend their lifespan. Deficiency in either of these requirements would affect their survivability. Different sugar quality also is believed to be a major factor affecting mosquito survivability.

In summary, the quantity of sugar intake by female *Ae. albopictus* influenced its fecundity, biting behavior and survivability. High amount of sugar intake is limited by the ability to ingest the sugar source. Consequently, female mosquitoes have the capability to regulate their metabolism needs based on sugar nutrient availability through changes in blood feeding behavior. This type of behavior understanding is crucial to explore novel control strategies for vector-control in the future.

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