

## Effect of Diet Regime on the Development and Survival of *Aedes albopictus* (Skuse) (Diptera: Culicidae)

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### ABSTRACT

A study was conducted to identify the responses of *Aedes albopictus* to different diet regime towards the development of juvenile and adult mosquitoes. Fish pellet was selected as standard diet in order to study the effect of diet regime on the development of *Ae. Albopictus*. Four different diet regimes (1.0 mg, 0.6 mg, 1.0 mg and 1.6 mg) were tested on 50 eggs of *Ae. albopictus* under laboratory conditions. Juvenile development until adult emergence was observed and recorded. Results indicated that the time taken to mature the mosquitoes was significantly affected by the diet regime. Furthermore, juvenile body size and adult wing size of *Ae. albopictus* were found to be greatly affected by diet regime exposed during juvenile stages. In summary, an increase of diet regime resulted in the decrease of developmental time and an increase in juvenile body size and adult wing size.

*Keywords:* *Aedes*, *Ae. albopictus*, diet, juvenile size, wing size.

### ARTICLE INFO

#### Article history:

Received: 1 September 2018

Accepted: 4 June 2019

Published: 21 October 2019

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### INTRODUCTION

Dengue fever (DF) is endemic and growing a public health problem which affecting the human health. (Getachew et al., 2015; Sairi et al., 2016; Rozilawati et al., 2017). Development activities, population growth, and increased individual movements have contributed to the emergence and reemergence of the disease (Chen et al.,

2009; Getachaw et al., 2015). Dengue viruses are the causative agents of DF which poses a serious health issues (Getachew et al., 2015; Vijayakumar et al., 2014). The viruses are transmitted through the bites of female *Aedes* mosquitoes. The main vectors in the transmission of dengue fever infection are *Aedes albopictus* (Skuse), otherwise known as known as the “Asian tiger mosquito” and *Aedes aegypti* (Linnaeus). *Ae. albopictus* prefers to breed in natural habitat and condition with more vegetation such as trees holes, rock pools and other natural sites (Rao et al., 2011; Ishak et al., 2015). However, deforestation activities, climate change and the increase in global trade have enabled this species to adapt with different environment. Few studies have observed the presence of this species in domestic and semi-domestic artificial container habitats (Dom et al., 2016a; Rao et al., 2011).

Many studies have documented that the rate of juvenile development and its survival are influenced by biotic and abiotic factor of the environment (Courret et al., 2014; Dom et al., 2016b; Koenraad & Harrington, 2008; Legros et al., 2009; Manorenjitha & Zairi, 2012; Rowbottom et al., 2015; Teng & Apperson, 2000). Limitation of food supply during juvenile cycle is a probable factor that may affect the adult survivorship and can contribute the body size of the adult mosquitoes (Jannat & Roitberg 2013). A recent study conducted by Araujo et al. (2012) stated that the survival of larvae increased with increasing food supply. This study also highlighted that improper food supply might delay the development of the larvae and thus decreased their survival rate during the fourth instar stages. Therefore, it is important to assess the influences of food supply on juvenile development in order to understand the dynamic of *Aedes* mosquito ecology; justifying the rationale of this study.

There is a group of researcher conducting a study on diet regime towards the development of *Aedes* mosquitoes. There are six diet ingredients used to study on the development of the *Aedes* mosquito species which are (a) beef liver powder, tuna meal, vitamin mix in water, (b) liver powder, (c) dog biscuit, beef liver, milk powder, (d) tuna meal, low heat desiccated non-defatted solid, Argentines beef liver powder, vitamin, (e) fish pellet and (f) dog food, dried beef liver, yeast, and milk powder. However, most of study used fish pellet formulated with necessary amino acid, protein, fiber, minerals and vitamin as their main ingredients and nutrition supply for development of juvenile mosquito (Arrivillaga & Barrera, 2004; Araujo et al., 2012; Courret et al., 2014; Manorenjitha & Zairi, 2012; Jannat & Roitberg, 2013; Jong et al., 2016; Yoshioka et al., 2012)

Study conducted by Araujo et al. (2012) had used fish pellet from TetraMin tropical flasks-spectrum brands as their main food ingredients. This food contained nutritionally balanced with selected vitamins, mineral and trace elements that required for juvenile development. Besides that, this study supplied the food regime (low, medium, high) based on the food amounts consumed by other species of culicidae. They concluded that the development time decreased with the increasing of food regime which the first and

fourth instar took longer time to develop when immature supplied with low and medium food amounts. Besides that, a study conducted in laboratory condition also found that development time of *Aedes albopictus* juvenile decreased with increasing of the diet level. They concluded that the longest pupation time was when larvae treated with 2.0 mg (14 days) diet regime while shortest time of pupation when larvae treated with 20.0 mg (8 days) (Yoshioka et al., 2012).

In addition, Arrivillaga and Barrera (2004) carried out a study that focussed on the food limitation in water storage container on development of *Ae. aegypti*. In this study, they used liver powder as their diet ingredients that supplied the main nutrition (carbohydrates, vitamin, mineral) that required for immature development. They found that immature supplied with lowest food level (0.01 mg / larval / day) showed adult emergence on day nineteen while immature reared under highest food level (1.6 mg / larval / day) emerged on day nine and produce adult with mass 2.338 mg. In 2016, study in Malaysia by Jong and colleagues focus on the minimum feeding regime required to produce viable and competitive adult *Ae. albopictus*. They had used combination of dog food, dried beef liver, yeast, and milk powder as food ingredient and stated that the duration of each immature stage was not affected by different feeding regimes except at the fourth instar. However, duration of the first instar was slightly prolonged with the reduction in feeding regime. Besides that, this study had followed standard feeding regime provided by VCRU in Universiti Sains Malaysia for rearing *Aedes* larvae which was 0.75 mg / larva / day as the control treatment.

Apart from that, three study conducted had focussed on the food and density influence on the juvenile development (Couret et al., 2014; Jannat & Roitberg, 2013; Manorenjitha & Zairi, 2012). This study observed effect of the overcrowding among the larvae to get the nutrition sources for development. As the finding, larvae reared under (1 mg) sub-optimal larval food condition had long development time (9 days) and for larvae supplies with optimal food (100 mg) took seven days to pupation (Manorenjitha & Zairi, 2012). Besides that, juvenile treated with high food treatment had low mortality rate which was twenty percent. While, juvenile with low food sources had highest mortality rate which is hundred percent (Jannat & Roitberg, 2013). Couret et al. (2014) had used combination of beef liver powder, tuna meal, vitamin and water as the main ingredient for food. This food contained all the important nutrition for the juvenile development including protein, amino acid, carbohydrates, vitamin and mineral and the diet regime was added daily based on the number of larvae alive in order to ensure amount of food offered per larva per day was kept constant throughout the juvenile development. However, high density of larvae with low food resources caused lowers development rates. There are major gap on the study of influence of diet on development of *Aedes* mosquito. The data on the effect of the diet on the life development of *Aedes* mosquito is still limited in this country. Thus, it

is very important to study the effect of diet on the development of this vector mosquito in the local environment using local strain in order to understand on the biology of mosquito.

## MATERIALS AND METHODS

This study utilized an experimental study design to investigate the effects of different diet regime on the development of *Ae. albopictus*. The comparative study was conducted by introducing laboratory strains of *Ae. albopictus* into four different diet regimes under laboratory setting. The experiment was completely randomized in fractional 4 x 2 replication (8 treatments). Independent variables consisted of four diet regimes (0.1 mg, 0.6 mg, 1.0 mg, and 1.6 mg) and two replicates. The data collected for each experiment were tabulated and analyzed using descriptive and statistical values by ANOVA. A laboratory strain F185 generation was used from Vector Control and Research Unit of Universiti Sains Malaysia to get F187 generation. Colonies were established and maintained in a thermostatically controlled insectary at  $25 \pm 2^\circ\text{C}$  and  $70 \pm 10\%$  relative humidity (RH) and 12:12 hours light: dark cycle. The rearing process was observed on a daily basis until F187 generation.

Pilot study was conducted in order to identify standard diet ingredient on juvenile development. This pilot study focused on observation of juvenile development until pupation. Three types of ingredient were used in this pilot study which are; (i) liver powder ( $D_1$ ), (ii) fish pellet ( $D_2$ ) and (iii) combination of cat biscuit, milk powder, yeast, chicken liver ( $D_3$ ). A total of 50 first larvae was placed into the plastic container with 300 ml of deionized water. Each day 0.75 mg of ( $D_1$ ) liver powder was added based on survival larvae. Cohorts were treated with a total larval diet of 22.5 mg (0.75 mg x 50 larvae) on day one. The larval diet was added daily until all larvae pupated. The procedures were replicated three times and repeated for other diet ingredient. The optimized diet ingredient that had high development rate and low morality rate from this pilot study was used for experimental phase. In this study fish pellet was selected as standard diet in order to study the effect of diet regime on the development of *Ae. albopictus*.

The observation on development on the *Ae. albopictus* with different diet regime (0.1, 0.6, 1.0, 1.6 mg / larvae / day) was conducted. Larval diet was offered once to each cohort throughout the experiment instead adding it from day one to day five, when the larvae were expected to pupate. Optimized diet ingredients from the pilot study were weighed using analytical electronic balance based on the calculation (mg x 50 larvae x 5 day), which were F1 (25 mg), F2 (150 mg), F3 (250 mg) and F4 (400 mg). For example, cohort F2 was treated with the feeding regime 0.6 mg / larvae / day were given a total diet of 150 mg (0.6 mg x 50 larvae x 5 days). Four plastic containers (10 x 10 x 10cm) were label with F1, F2, F3 and F4. A total of 50 eggs were submerged in plastic container with different label and the development was observed. Different diet regime was introduced to the egg in the water container. The experiments were conducted in replicates, twice for every diet regime. The

growth and survival of juvenile until adult emergence were monitored and recorded daily. The procedures were repeated for different diet regime is summarized in Figure 1.

Data collection was initiated when the first instar was introduced to a different diet. Visual observation was conducted every day at the same time (4.00 pm) to monitor the development of juvenile until adult emergence. Stages of the juvenile, time of pupation and days of adult emergence were recorded. The survival rate of each treatment was quantified by recording all survival juvenile and adult on a daily basis per container and all dead larvae were removed whenever they were observed. Data on 50% pupation was expressed by the day that half of the instar in the water container pupate. Juvenile body size was observed using standardized digital of random individuals every 24 hour starting on first instar. Measuring the size of juvenile was using Dino lite with open-access software DinoXcope version 1.18 (Madzlan et al., 2018). Adult emergence was collected and placed in a labeled universal bottle. This bottle was placed in a refrigerator at 4°C for two hours after adult collection in order to kill the adult. Wing was removed and mounted on microscope slide. These were then digitally scanned and measured under Dino-Lite using an open-access software DinoXcope version 1.18.

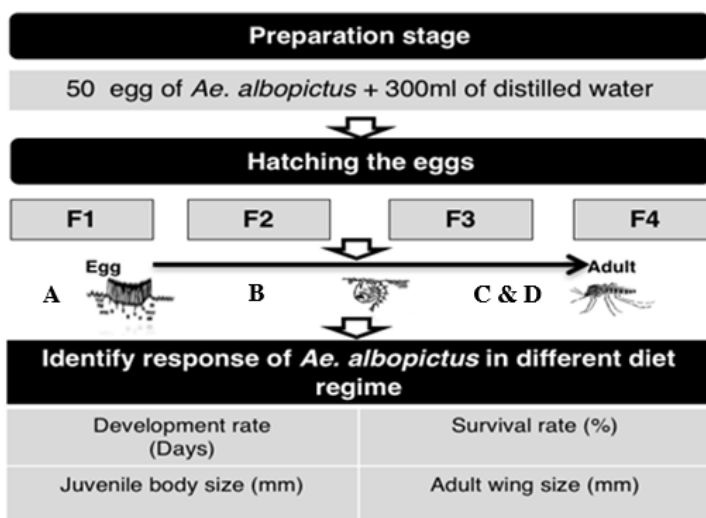


Figure 1. Flow process of the study on different diet regime.

\*Note: F1 (0.1 mg), F2 (0.6 mg), F3 (1.0 mg), F4 (1.6 mg). Several aspect on the development trend were monitored which are; (A) eggs hatching in days and percentage, (B) Fifty percent pupation in day, (C) fifty percent adult emergence and (D) hundred percent adult emergence

The wing size measurement of adult mosquito was also conducted. The dead mosquitoes were kept in the freezer to allow a measurement of wing lengths (Blackmore & Lord, 2000). The wing length was defined as the linear distance from the axillary incision to the apical margin excluding the fringe scales (Loetti et al., 2011; Neira et al., 2014; Schneider et al., 2004). To measure the length, the wings were detached proximal to the axillary notch, and

mounted on a microscope slide and covered with a cover slip (Schneider et al., 2004; Vidal & Suesdek, 2011). Only one wing of each adult was removed which was usually the left wing, unless it was damaged (Reiskind & Zarrabi, 2012).

In this study, the diameters of wing shapes measurement were used and this technique is unconventional to represent wing size/wing length (Figure 2). In mosquito studies (Culicidae), the wing is widely used for morphometric comparison because of its two-dimensional shape and because it contains veins that encompasses natural anatomical landmarks that are ideal for measurement (Lorenz et al., 2017). By measuring the shape, it is possible to observe the variation of wing shape. Besides that, this measurement technique is fast, inexpensive and simple which makes possible to compare shape with minimum interference from different size.



Figure 2. Measurement of the diameter of wing shape outline by red color of *Ae. albopictus*

The response of *Ae. albopictus* on different diet regime was determined by observing the response of eggs from hatching until adult emergence on four different diet regimes. Several aspect on the development trend were monitored which are; (A) eggs hatching in days and percentage, (B) Fifty percent pupation in day, (C) fifty percent adult emergence and (D) hundred percent adult emergence. For this study, the data on the effects of diet regime on the development of juvenile *Aedes albopictus* were analyzed with analysis of variance (ANOVA) through Statistical Package of Social Science (SPSS) software.

### **Effect of Diet on the Development Time and Survival of *Ae. albopictus***

Generally, the development time decreased with increasing of diet regime (Table 1). The day of the first egg hatching in different diet regime was recorded and labeled as stage A.



Eggs exposures to the highest diet regime showed the shortest period for hatching (1.6 mg:  $2.00 \pm 0.00$  days). In contrary, exposure to low diet regime (1.0 mg, 0.6 mg and 0.1 mg) showed longer period of egg hatching ( $13.00 \pm 0.00$  days;  $3.17 \pm 0.75$ ;  $3.83 \pm 0.41$  days respectively). Data on stages B was collected by recording the day that 50% of the larvae changed to pupae. It was observed that larvae supply with lowest diet regime took longest time for pupation (0.1 mg:  $9.17 \pm 0.75$  days). While, other diet regimes showed a shorter time for pupation (1.6 mg:  $7.50 \pm 0.55$  days; 1.0 mg:  $8.00 \pm 0.63$ ; 0.6 mg:  $8.50 \pm 0.55$  days).

Adult emergence was observed until 50% of the pupae emergence into adult and the data was collected to represent stages C. Fifty percent of adult emergence range from 8.83 to 11.00 days. Groups supplied with highest diet regime (1.6 mg) were found to be the fastest to emerge ( $8.83 \pm 0.41$  days) as compared to other diet regimes (1.0 mg:  $9.67 \pm 0.52$  days; 0.6 mg:  $10.33 \pm 0.52$ ; 0.1 mg:  $11.00 \pm 0.63$  days). Stages D was recorded when the entire juvenile emerged as adult mosquitoes in different diet regimes. During this stage, the group with lowest diet regime (0.1 mg) took the longest time to emerge ( $14.83 \pm 0.41$  days) compared to other group of diet regime. As for the increase of diet regime, the duration for full adult emergence also decreased (0.6 mg:  $14.00 \pm 0.63$  days; 1.0 mg:  $12.83 \pm 0.41$ ; 1.6 mg:  $11.50 \pm 0.84$  days). The ANOVA results was statistically significant, indicating that the development duration was influenced by diet regime,  $F(3,20)$ ,  $p < 0.05$ . Post hoc analysis with Tukey's HSD (using an  $\alpha$  of 0.05) revealed that juvenile supply with lowest diet regime ( $M = 14.83$ ,  $SD = 0.41$ ) had significantly longer the duration of development followed by diet regime 0.6 mg ( $M = 14.00$ ,  $SD = 0.63$ ), diet regime 1.0 mg ( $M = 12.83$ ,  $SD = 0.41$ ) and diet regime 1.6 mg ( $M = 11.50$ ,  $SD = 0.84$ ).

Table 1

*Duration of development of Ae. albopictus feed with four different diet regimes (0.1 mg, 0.6 mg, 1.0 mg and 1.6 mg) under controlled conditions ( $25 \pm 2^\circ\text{C}$  and  $70 \pm 10\%$  relative humidity (RH) and 12:12 hours light: dark cycle).*

| Stages | 0.1 mg          | 0.6 mg          | 1.0 mg          | 1.6 mg          | *p-values |
|--------|-----------------|-----------------|-----------------|-----------------|-----------|
| A      | $3.8 \pm 0.41$  | $3.2 \pm 0.75$  | $3.0 \pm 0.00$  | $2.0 \pm 0.00$  | <0.05     |
| B      | $9.2 \pm 0.75$  | $8.5 \pm 0.55$  | $8.0 \pm 0.63$  | $7.5 \pm 0.55$  | <0.05     |
| C      | $11.0 \pm 0.63$ | $10.3 \pm 0.52$ | $9.7 \pm 0.52$  | $8.8 \pm 0.41$  | <0.05     |
| D      | $14.8 \pm 0.41$ | $14.0 \pm 0.63$ | $12.8 \pm 0.41$ | $11.5 \pm 0.84$ | <0.05     |

*Note: Stages A: Eggs hatching; B: Fifty percent of larvae change to pupae; C: Fifty percent of pupae change to adult and D: Pupae change to adult.*

*Data are represented as Means  $\pm$  SD. \*There has significant difference found between duration of development of Ae. albopictus exposure with different diet regime  $p < 0.05$  based on one-way ANOVA.*

In addition, the survival rate of *Ae. albopictus* was also affected by different diet regime ( $p < 0.05$ ) (Table 2), which observed a decrease in numbers with increasing of diet regime. Group that was fed with highest diet regime (1.6 mg) showed the lowest survival rate in all stages (stage A:  $79.00 \pm 1.41\%$ ; stage B:  $62.50 \pm 3.53 \%$ ; stage C:  $50.00 \pm 0.00\%$ ; stage D:  $37.00 \pm 1.41\%$ ). However, the group exposed to lowest diet regime showed the highest survival rate during juvenile and adult stages (stage A:  $96.50 \pm 0.71\%$ ; stage B:  $87.50 \pm 0.71\%$ ; stage C:  $84.50 \pm 6.36\%$ ; stage D:  $71.50 \pm 0.71\%$ ). The ANOVA was statistically significant, indicating that survival rate was influenced by diet regime,  $F(3,20)$ ,  $p = < 0.05$ . Post hoc analysis with Tukey's HSD (using an  $\alpha$  of 0.05) revealed that juvenile supply with lowest diet regime ( $M = 71.50$ ,  $SD = 0.71$ ) had significantly highest survival rate followed by diet regime 0.6 mg ( $M = 68.5$ ,  $SD = 0.71$ ), diet regime 1.0 mg ( $M = 65.5$ ,  $SD = 0.71$ ) and diet regime 1.6 mg ( $M = 37.0$ ,  $SD = 1.41$ ). Generally, in duration of development aspect, the development time decreased with increase of diet regime. However, the survival rate decreased with an increase of diet regime.

Table 2

The survival rate of *Ae. albopictus* feed with four different diet regimes (0.1 mg, 0.6 mg, 1.0 mg and 1.6 mg) under controlled conditions ( $25 \pm 2^\circ\text{C}$  and  $70 \pm 10\%$  relative humidity (RH) and 12:12 hours light: dark cycle)

| Stages | Survival rate (%) $\pm$ SD, (n= 50/trials) |                 |                 |                  | p-values |
|--------|--|-----------------|-----------------|------------------|----------|
|        | Food amount                                |                 |                 |                  |          |
|        | 0.1 mg                                     | 0.6 mg          | 1.0 mg          | 1.6 mg           |          |
| A      | 96.5 $\pm$ 0.71                            | 92.5 $\pm$ 0.71 | 87.0 $\pm$ 1.41 | 79.0 $\pm$ 1.41  | <0.05    |
| B      | 87.5 $\pm$ 0.71                            | 82.5 $\pm$ 0.71 | 77.5 $\pm$ 0.71 | 62.5 $\pm$ 3.53  | <0.05    |
| C      | 84.5 $\pm$ 6.36                            | 72.5 $\pm$ 0.71 | 69.5 $\pm$ 0.71 | 50.00 $\pm$ 0.00 | <0.05    |
| D      | 71.5 $\pm$ 0.71                            | 68.5 $\pm$ 0.71 | 65.5 $\pm$ 0.71 | 37.0 $\pm$ 1.41  | <0.05    |

Note: Stages A: Eggs hatching; B: Fifty percent of larvae change to pupae; C: Fifty percent of pupae change to adult and D: Pupae change to adult.

Data are represented as Means  $\pm$  SD. \*There has significant difference found between duration of development of *Ae. albopictus* exposure with different diet regime  $p = < 0.05$  based on one-way ANOVA.

Previous studies have described that high diet condition correlates with shorter *Ae. albopictus* development time (Araujo et al., 2012; Manorenjitha & Zairi, 2012; Yoshioka et al., 2012). In the present study, all the groups exposed to different diet regime were able in completing the development cycles until adult. However, the shortest development period of *Ae. albopictus* (from eggs to pupae) was observed at highest diet regime. On the other hand, the longest development duration (14.83 days) was observed at lowest diet regime, probably as a result of insufficient energy to complete development (Arrivillaga & Barrera,



2004). Poor diet causes an extended development time and since immature spend 25% of their biomass and average moulting, mosquito larvae must require enough food supply for ecdysis (Araujo et al., 2012). In addition, this experiment showed that, survival rate decreased with an increase of diet regime. This result in line with previous study by Jannat and Roitberg (2013), where the authors reported that survival rate increased with decrease of food sources. Observation during the experiment showed that water with high diet regime produced scum and smell. The scum were produced from the gradual decomposition of organic matter (diet) and deposited as sediment and smell were produced due to the stagnant water condition which all the microorganisms using up all of the available oxygen.

### **Effect of Diet on the Juvenile Body Size of *Ae. albopictus***

The mean of juvenile body size of *Ae. albopictus* was significantly changed with diet regime (Figure 3). Adult wing size increased with the amount of food supplied during juvenile stages. Mean of juvenile body size varied between  $10.94 \pm 0.35$  mm to  $13.07 \pm 0.55$  mm. Juvenile body size of *Ae. albopictus* raised under a diet regime 0.1 mg/larva/day and below was shorter than 11.00 mm, while those under diet regime more than 0.1 mg (0.6, 1.0, 1.6 mg/larva/day) were longer than 11.00 mm. The ANOVA was statistically significant, indicating that juvenile body size was influenced by different diet regime,  $F(3, 8)$ ,  $p = 0.003$ . Post hoc analysis with Tukey's HSD (using an  $\alpha$  of 0.05) revealed that juvenile supply with lowest diet regime ( $M = 10.94$ ,  $SD = 0.35$ ) had significantly lower the adult wing size followed by diet regime 0.6 mg/larva/day ( $M = 11.40$ ,  $SD = 0.54$ ), diet regime 1.0 mg/larva/day ( $M = 12.43$ ,  $SD = 0.54$ ) and diet regime 1.6 mg/larva/day ( $M = 13.07$ ,  $SD = 0.55$  juvenile body size). As predicted, juvenile body size increased proportionally with increase in diet regime with the highest diet regime (1.6 mg) resulting in the largest mean juvenile body size. While, lowest diet regime (0.6 mg) produced smaller juvenile body size.

The differences in adult wing sized of *Ae. albopictus* were noted between group supply with different diet regime from 0.1 mg to 1.6 mg. The mean adult wing size between four different diet regimes (0.1 mg, 0.6 mg, 1.0 mg, 1.6 mg) was found significantly different (ANOVA,  $F = (3, 20)$ ,  $p = 0.004$ ) (Figure 4A). Adult wing size from the higher fed group (1.6 mg) was found to be large as compared to adult wing size from lower fed group (0.1 mg) which was smaller in size (Figure 4B). Wing size has been commonly used as a morphometric measurement of body size and weight of mosquito. Result indicated that different diet regimes correlated with adult wing size. Each group of diet regime produced adult wing size more than 11.00 mm. This result is in agreement with a previous study by Jong et al. (2016), which stated the adult wing size larger with an increase of diet regime. Besides that, additional data juvenile body size was observed and measured in this experiment to identify their significance with different diet regimes.

Wing size has been commonly used as a morphometric measurement of body size and weight of mosquito. Result obtained in this study indicated that different diet regime correlated with adult wing size. Each group of diet regime produce adult wing size more than 11.00 mm. This result is similar to a previous study conducted by Jong et al. (2016) which stated the adult wing size was larger as increase of diet regime. Additional data juvenile body size was observed and measure in this experiment to identify their significant with different diet regime. Furthermore, juvenile body size increased proportionally with increase in diet regime with the highest diet regime (1.6 mg) resulting in the largest mean juvenile body size. While, lowest diet regime (0.6 mg) produced smaller juvenile body size.

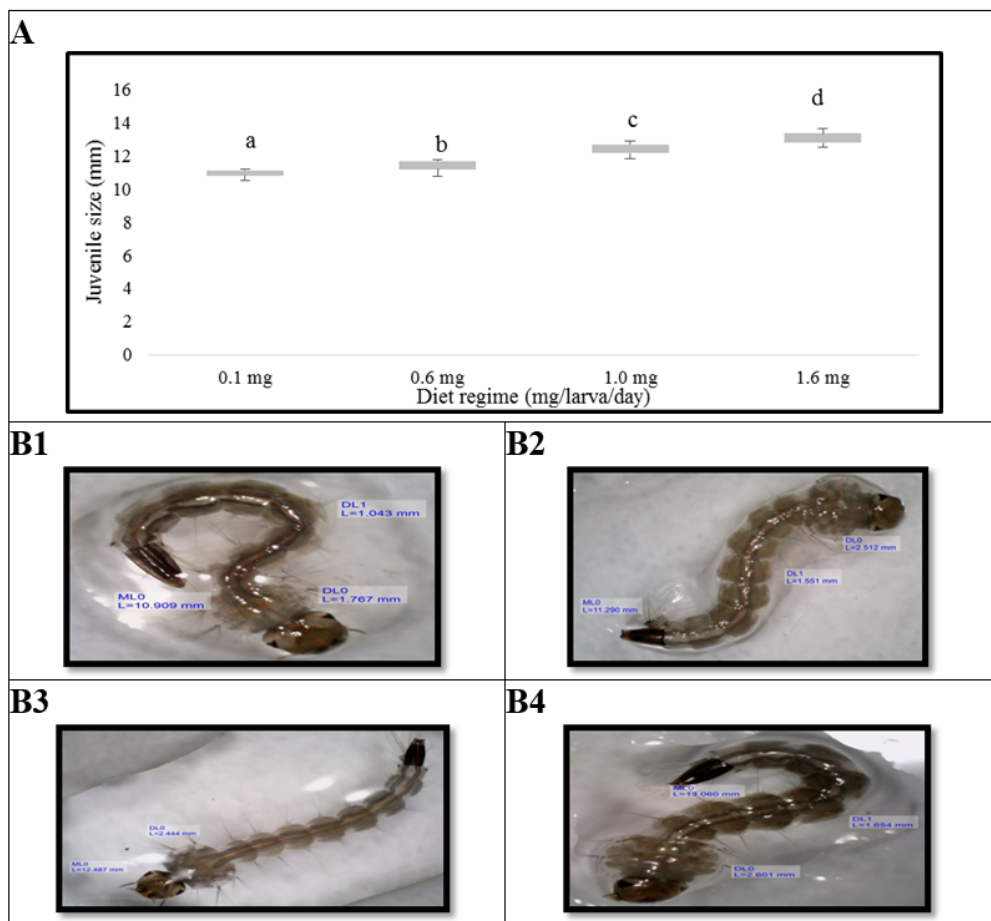


Figure 3. Effect of diet on juvenile body size of *Ae. albopictus*. (A) Data show juvenile size (mm) of *Ae. albopictus* under different diet regime (mg/larva/day). \*Different letter (a, b, c, d) represent significant different between column. (B) Size of juvenile supply with different diet measure under Dino-lite.

\* Note Juvenile exposure with different diet are labeled with B1,B2,B3 and B4 for 0.1 mg, 0.6 mg, 1.0 mg and 1.6 mg.

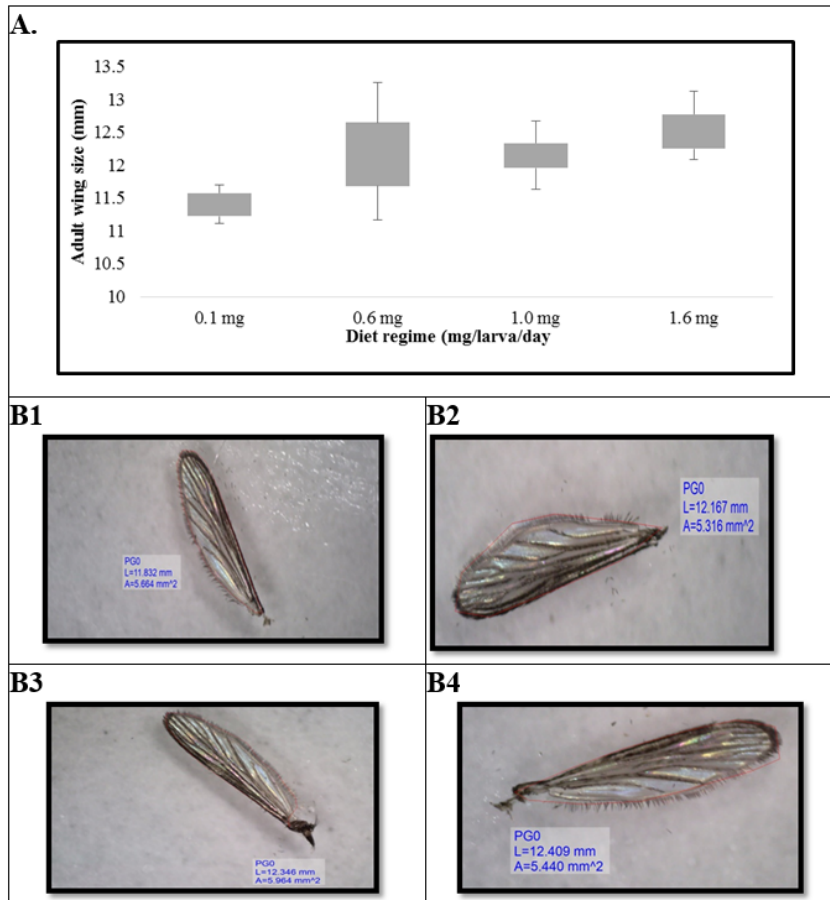


Figure 4. Effect of diet on the morphology of *Ae. albopictus*. (A) Box plot show a size of adult wing (mm) of *Ae. albopictus* under different diet regime (mg/larva/day). Shows adult wing size that supply with different diet during juvenile and measure under Dino-lite \* Note Juvenile exposure with different diet are labeled with B1,B2,B3 and B4 for 0.1 mg, 0.6 mg, 1.0 mg and 1.6 mg.

Nutrition is one of the factors that influences the development and survival of mosquitoes (Arrivillaga & Barrera, 2004; Couret et al., 2014; Jong et al., 2016). Supply of the nutrition during juvenile stages affects the development rate from egg until adult stage (Jong et al., 2016 ; Manorenjitha & Zairi, 2012). The result showed wide phenotypic variation in development duration, survival rate, hatching rate, juvenile size and adult wing size to *Ae. albopictus* that reared with fish pellet under different diet regime in laboratory. The range of diet regime used in this experiment covered a wide range of possible feeding conditions in natural environment.

The rearing of *Aedes* mosquitoes is complex and demanding for several reasons. *Aedes* larvae are affected by temperature, density and available nutrition (Dom et al.,

2016a; Madzlan et al., 2016; Madzlan et al., 2018). Our results showed that the fish pallet ingredient impacted the hatching rates, development times and survival of *Aedes albopictus*. Therefore this finding proposes delicate screening work and precise methods to be used for controlled laboratory strain. The advantage of this ingredient is it is readily available, convenient to treat, easy to preserve and cheap.

This study was designed purposely to determine the impact of diet regime on the development of local *Ae. albopictus*. The limitation of this study is that it only focused on few aspect on the development trend such as hatching period, pupation period and adult emergence without differentiating the developmental stages of the larval according to the stages of mosquito instar. Therefore, it is quite difficult to conclude that the effects of diet regime are related to the development period of *Aedes albopictus* due to non-consideration of stage of mosquito instar.

## CONCLUSION

An understanding of the natural factors that regulate natural populations of *Ae. albopictus* mosquitoes can improve control and reduce the incidence of dengue fever cases. In general, diet regime and available breeding water container will encourage the development of *Ae. albopictus* and also reduce their development time for adult emergence. Thus, studies focusing on the monitoring effect of diet regime on the development of mosquito are essential to controlling the transmission of the dengue disease. The findings show that a higher amount of diet regime can shorten the development rate of the mosquitoes. The outcome of this study can be utilized as baseline data to provide further information on the population dynamic of the mosquito.

## ACKNOWLEDGEMENTS

The authors sincerely thank to the contributor of research funding from Universiti Teknologi MARA (UiTM) - (600-IRMI/DANA 5/3/LESTARI (0093/2016), and Ministry of Higher Education (MOHE) Malaysia.

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