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Evaluation of Larval Diets for Mass Rearing of *Aedes aegypti* L. (Diptera: Culicidae)

Glenda B. Obra, Abigaile Mia J. Hila*,
Arvin O. Dimaano and Sotero S. Resilva
Atomic Research Division
Department of Science and Technology – Philippine Nuclear Research Institute
(DOST-PNRI), Quezon City, 1101 Philippines
avjavier@pnri.dost.gov.ph

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Abstract

The ability to mass-produce quality insects in the laboratory is vital in any sterile insect technique (SIT) program. In mass-rearing Aedes aegypti, optimizing larval diet and conditions is essential as they directly affect adult traits that are important in future sterile male releases. This study aimed to evaluate different mosquito diets and determine the effects of changing parameters in the diet on the growth and development of Ae. aegypti (Old Balara strain). Different diets, densities, diet concentrations and the addition of brewer's yeast (BY) were evaluated in rearing mosquito larvae. The cat food (CF) diet showed a more synchronized day of pupation at five days, where 80% of males already pupated regardless of larval density. In all three diets, a larval density at 2 larvae/mL of water was comparable to 1 larva/mL, saving more space and reducing the water requirement of Ae. aegypti without affecting its quality. The addition of BY resulted in significantly reduced time to pupation, increased pupation and adult emergence percentages. Among the diet concentrations, 0.2 mg/larva was the ideal concentration for CF and International Atomic Energy Agency diets and 0.3 mg/larva for the commercial fish meal due to shorter larval durations, longer adult longevity and longer adult wing lengths. In rearing Ae. aegypti, it is best to use CF with BY at 0.2 mg/larva/day at 2 larvae/mL.

***Keywords**:* artificial blood feeding system, mosquito rearing, quality control, sterile insect technique

1. Introduction

Aedes aegypti is known as the primary vector of dengue and has been ranked as one of the top-most invasive species worldwide (Gratz, 2004; Lambrechts *et al*., 2010). This vector is also responsible for transmitting yellow fever, Chikungunya and Zika virus (Powell, 2018). From January 1 to October 19, 2019, 371,717 dengue cases, including 1,407 deaths, were reported through

the Philippines' Department of Health (DOH) routine surveillance system. The number of cases was 106% higher than the previous year's record (DOH, 2019). Hence, a National Dengue Epidemic in the Philippines was declared last August 6, 2019. In 2021, as of July 3, a total of 32,555 dengue cases with 119 deaths have been reported in the Philippines (World Health Organization [WHO], 2021).

In the absence of an efficient vaccine to protect humans from mosquito-transmitted diseases including dengue, vector control is one of the few proven ways to reduce the risk of transmission (Benedict and Robinson, 2003). Currently, the primary method of controlling mosquitoes relies on the use of chemical insecticides. Although there have had some remarkable successes, control has not been sustainable in the long term due to improper use, which leads to insecticide resistance, problems on health and environmental concerns (Cuervo-Parra *et al*., 2016).

An alternative for mosquito control is the sterile insect technique (SIT). SIT is a biological control method of suppressing the mosquito population by releasing overwhelming numbers of mass-reared sterile males which will produce no offspring when mated with females in the wild. With the continuous release of sterile males, the population of the same species is expected to decrease. When integrated well with other control tactics, SIT has been proven to be an effective tool to control various insect species of agricultural and medical importance (Knipling, 1955; Dyck *et al*., 2005; Hendrichs and Robinson, 2009).

Mass-producing the target pests in the laboratory is important in any SIT program. Application of the SIT involves the colonization of the target species and the large-scale rearing of viable and competitive sterile males for release (Puggioli *et al*., 2013). In mass-rearing, the quality of the larval diet and larval rearing conditions have a direct and irreversible effect on adult traits (Benedict *et al*., 2009). Other than nutrients, the cost and availability of diet components should also be considered.

The Food and Agricultural Organization (FAO) and International Atomic Energy Agency (IAEA) laboratory have formulated and recommended the use of IAEA diet comprised of tuna meal, bovine liver powder and BY for rearing *Aedes.* A diet composed of 80% cat food (CF) (Friskies®), 14% of brewer's yeast (BY) and 6% commercial fish meal (CFM) (Tetramin®) showed longer longevity in *Ae. albopictus* than the IAEA diet when given with water as food

(Puggioli *et al.,* 2013). A laboratory rodent diet (LDR) resulted in comparable percent pupation and adult emergence with the IAEA diet. Interestingly, LDR showed larger males and females with higher fecundity and fertility than *Ae. aegypti* fed with the IAEA diet (Bond *et al.,* 2017). Low-cost diets such as CF and rodent diets can be explored more for mosquitoes as they are effective and locally available.

This study mainly aimed to establish optimal *Ae. aegypti* rearing conditions on larval diet evaluation. Specifically, it identified a cost-effective diet for rearing *Ae. aegypti* and determined the appropriate larval density, the effect of BY addition and the suitable daily larval feeding regime on the growth and development of *Ae. aegypti* in the laboratory.

2. Methodology

2.1 Laboratory Maintenance of Ae. aegypti Colonies and Ethical Consideration

Female mosquitoes were fed using live mice obtained from a source recognized by the Department of Science and Technology (DOST) – Industrial Technology Development Institute following the standards needed for using laboratory animals for experiments. Another mosquito colony was fed using pig blood collected in a nationally-authorized pig slaughterhouse in Valenzuela City, Metro Manila, Philippines, which is recognized by the Bureau of Animal Industry and National Meat Inspection Service with the highest possible standards and strict adherence to the Philippine laws and regulations.

The stock colony of *Ae. aegypti* at the Philippine Nuclear Research Institute (PNRI) used in the study was the Old Balara strain, established from collections made in Old Balara, Quezon City (Obra and Javier-Hila, 2021). *Ae. aegypti* larvae were fed using the CFM (Tetramin®, Tetra, Germany) and later shifted to the IAEA diet. Adults were provided with 10% sugar solution on cotton sticks placed in a 100-mL plastic container. Adult females were blood-fed using two blood sources twice a week. Two colonies were maintained: one colony with live mice as blood source, while another colony with pig blood as a blood source. Fresh pig blood was used as a blood source for female mosquitoes to replace the use of live animals such as mice. Eggs were collected three times a week using an egging cup with water, lined with white filter paper. The larvae were reared in laboratory conditions with a mean temperature of 27±1 °C, relative humidity of 70±5%, and photoperiod of

12:12 (L: D). Upon pupation, pupae were collected and separated daily by size and placed in cups (n = 50 adults per cup). Adults were sexed by checking the antennae. They were later transferred in Bug dorm rearing cages (1 ft^3) maintained at a 1:1 sex ratio with 3,000 adults per cage. Pupal mortality per cup was also checked before transferring adults into the cages. The number of adults that were transferred was recorded daily.

2.2 Quality Control Test using Pig Blood Colony

Quality control tests were done per generation of *Ae. aegypti* that was fed with pig blood for 37 generations to monitor and maintain the quality of the mosquitoes in the laboratory. The mechanically defibrinated fresh pig blood (Puggioli *et al*., 2013) was contained in a fabricated stainless container with a capacity of 20 mL covered with pig intestine held in place using a rubber band to serve as a feeding membrane. The setup was warmed to 40 °C in a hot water bath for 3 min; a heated pouch placed underneath the container served as an additional heat source for the pig blood. Quality control tests for pig blood colony (F2 to F38) were conducted from the F30 generation *of Ae. aegypti* previously blood-fed with live mice for 30 generations. About 1,500 mosquitoes at 1:1 (male: female) sex ratio were introduced in the cage for each generation. Three sets of 100 eggs were set aside to monitor the quality of mass-reared pig blood mosquito colony for every generation. Hatch (%), male and female larval durations (days), time to pupation (days), pupation (%), pupal recovery from eggs (%), adult recovery from eggs (%), adult emergence (%) and male-sex ratio (male/female) of the 37 generations of pig blood colony were monitored and recorded. The baseline data obtained helped in understanding the effect of colonization and adaptation of the insect.

2.3 Evaluation of Different Diet Sources in Rearing Ae. aegypti

To identify a suitable rearing for *Ae. aegypti*, three diets, namely CF (Whiskas®, Mars Incorporated, United States), IAEA diet and CFM (Tetramin®) were compared using different parameters at different larval densities. The CF diet comprised of 85% (85 g) CF and 15% (15 g) BY, while the IAEA diet comprised of 50% (50 g) tuna meal, 35% (35 g) bovine liver powder and 15% (15 g) BY (IAEA, 2017) mixed with 1-L distilled water to produce 1% solution. CF and IAEA diets were given as liquid solutions, while CFM was weighed and given as powder. Eggs were hatched using nutrient broth (M002-500G, HiMedia Laboratories, India) (0.36 g) with BY (0.07 g) dissolved in 1-L dechlorinated water that was stored for 16 h before use (Obra and Javier-Hila, 2021). After 4 h, 100 hatched larvae were counted and placed

in a rectangular plastic container measuring 10 x 15 x 3.5 cm with 100-mL dechlorinated water. They were allowed to develop in an incubator (IPP30, Memmerth GmbH +Co.KG, Germany) set at 28 °C. Pupae were collected every day, and developmental parameters such as male and female larval durations, pupation (%), adult recovery (%), adult emergence (%), male-sex ratio (M/F), adult longevities and adult wing lengths were determined. The treatments were replicated three times.

To determine longevity, upon adult emergence, males and females previously separated by size were checked daily and placed separately either in Mylar film cages (n = 11 and above) or plastic cups (n = 10 and below) with sugar solution on cotton balls as a food source. Dead adults were also checked daily and removed to determine male and female longevity. Cotton balls with sugar solution as a food source were replaced as necessary.

To measure wing length, either the left or right side of the wing of males and females was detached and mounted on a glass slide in a drop of saline solution. Wing lengths were measured using a stereomicroscope (SZG1TR, Olympus, Japan) and an ocular micrometer beginning from the apical notch to the axillary margin excluding wing fringe (Figure 1) as measured by Mohammed and Chadee (2011). The size of adults was measured as the mean of their wing length, which strongly correlates with the weight of mosquitoes (Koella and Lyimo, 1996) and is widely used as a good estimate for adult size.

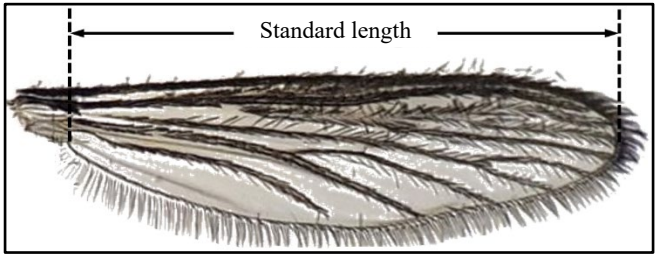

Photo by: A. M. J. Hila

Figure 1. Standard length measurement for *Ae. aegypti* wing

2.4 Evaluation of Effect of Different Larval Densities in Rearing Ae. aegypti

The three diets were compared using different densities of mosquito larvae at 1, 2 and 3 larvae/mL, corresponding to 100, 200 and 300 wrigglers, respectively. The hatched larvae were placed in a rectangular plastic container, same as in section 2.3 with 100-mL dechlorinated water, and were allowed to

develop in an incubator set at 28 °C. Similarly, the larvae received 0.2 mg/larva/day of the diet. Pupae were collected every day, and developmental parameters such as male and female larval durations, pupation (%), adult recovery (%), adult emergence (%), male-sex ratio (M/F), adult longevities, and adult wing lengths were determined. The treatments were replicated three times.

2.5 Evaluation of Effect of Addition of BY as Larval Diet Ingredient

CF diet at 1, 2, 3, 4 and 5 g with and without 15% BY was mixed with 100-mL distilled water corresponding to 1, 2, 3, 4 and 5% diet. The hatched larvae (n = 100) were placed in a rectangular plastic container with 100-mL dechlorinated water and were allowed to develop in an incubator set at 28 °C. The larvae were fed daily with 2 mL of the diets at different concentrations, namely 0.2, 0.4, 0.6, 0.8 and 1.0 mg/larva at a larval density of 1 larva/mL. Each container received 0.02, 0.04, 0.06, 0.08 and 0.10 g of diet daily. Pupae were collected every day, and developmental parameters such as male and female larval durations, pupation (%), adult recovery (%), adult emergence (%), male-sex ratio (M/F) and adult longevities were determined. The treatments were replicated three times.

2.6 Evaluation of Suitable Larval Feeding Regime

The three diets were compared using different diet concentrations for mosquito larvae set at 1 larva/mL density. About 0.5, 1 and 1.5 g of the CF and IAEA diets were mixed with 100-mL distilled water, corresponding to 0.5, 1 and 1.5% diets. The larvae were fed daily with 2 mL of three diets at different concentrations, namely 0.1, 0.2 and 0.3 mg/larva at a larval density of 1 larva/mL. Each container received 0.01, 0.02 and 0.03 g of diet daily (n = 100 larvae). Meanwhile, CFM was given to the larvae as powdered food (0.01, 0.02 and 0.03 g). The larvae were allowed to develop in an incubator set at 28 °C. Pupae were collected daily, and developmental parameters such as male and female larval durations, pupation (%), adult recovery (%), adult emergence (%), male-sex ratio (M/F), adult longevities and adult wing lengths were determined. The treatments were replicated three times.

2.7 Statistical Analysis

The data on larval diet evaluation were analyzed using two-way analysis of variance (ANOVA) to determine the relationship of two factors on the different parameters using Statistical Tool for Agricultural Research (STAR) version 2.0.1. Normality of residuals was tested using the Shapiro-Wilk test,

while homogeneity of variances was tested using Bartlett's test. Post hoc tests either by Tukey's honest significant difference (HSD) or Fischer's least significance difference (LSD) tests using the STAR software was used for further analysis to know the significant differences among treatments. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1 Quality Control per Generation of Mosquito Colony

Hatch and adult recovery of the pig blood colony were 89 and 85%, respectively (Table 1, Figure 2). Many artificial feeding systems were already developed to feed mosquitoes effectively. These feeding systems share common features: blood is placed in a thin membrane, on which the female mosquitoes will penetrate their proboscis to access and imbibe the blood, and a heating device or material is added to mimic vertebrate blood temperature (Romano *et al*., 2018). Costa-da-Silva *et al.* (2013) reported that although live animals are still the preferred blood source for laboratory colonies, many artificial feeders are available.

Table 1. Different parameters measured for the quality control tests of *Ae. aegypti* (pig blood colony) for 37 generations

Parameter	Mean ± SE
Hatch (%)	89.24±0.84
Male larval duration (days)	6.37±0.28
Female larval duration (days)	7.20±0.33
Time to pupation (days)	6.78±0.30
Pupation (%)	97.88±0.41
Pupal recovery (%)	87.38±0.94
Adult recovery (%)	85.17±0.98
Adult emergence (%)	97.47±0.37
Male-sex ratio	1.05±0.02

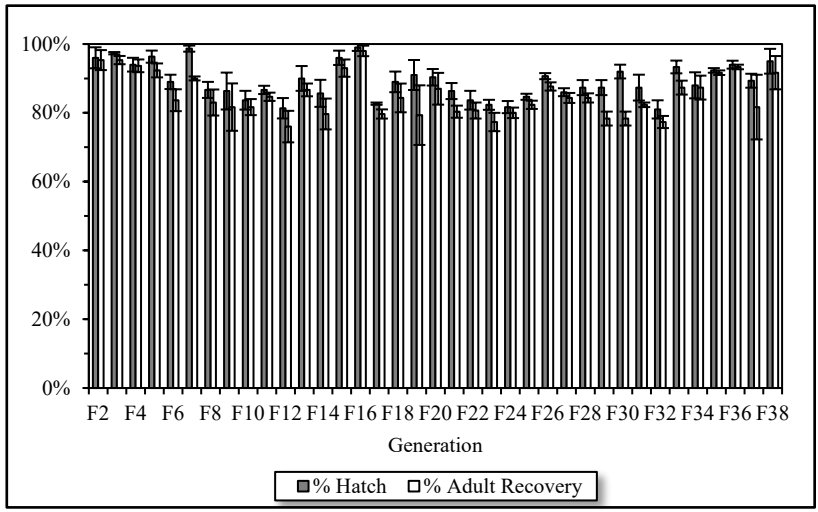

Figure 2. Hatch and adult recovery from eggs of laboratory reared population of *Ae. aegypti* fed with pig blood for 37 generations

3.2 Effect of Different Diets

Male larval duration, pupal and adult recoveries, adult emergence, sex ratio, adult longevity and female wing length did not significantly differ among the three diets. Female larval duration was significantly fastest ($F = 26.83$; $df = 2$; $p < 0.01$), and male wing length was significantly longest ($F = 13.87$; $df = 2$; $p < 0.01$) when larvae were fed with CF, followed by IAEA. Feeding larvae with CFM showed the longest larval development and shortest male wing length (Table 2).

3.3 Effect of Different Larval Densities

During the mosquito larval development, wrigglers in nature may experience periods of nutrient restriction and competition for resources like food and space (Reiskind and Lounibos, 2009). Generally, larval density is directly proportional to the female larval duration and inversely proportional to male wing length (Table 2). Male larval duration, pupal and adult recoveries, adult emergence, sex ratio, adult longevity and female wing length did not significantly differ among the three larval densities. The two lowest larval densities (1 and 2 larvae/mL) showed significantly shorter female larval duration than 3 larvae/mL ($F = 7.09$; $df = 2$; $p < 0.01$), while rearing larvae at 1 larva/mL exhibited the longest male wing length ($F = 7.12$; $df = 2$; $p < 0.01$) (Table 3).

Table 2. Different parameters of laboratory reared population of *Ae. aegypti* fed with different diets

Diets	MLD (days)	FLD[1] (days)	Pupation (%)	AR (%)	AE (%)	MSR	ML (days)	FL (days)	MWL[2] (mm)	FWL (mm)
CF	5.23[a]	6.24[a]	96.80[a]	92.87[a]	95.93[a]	1.16[a]	22.22[a]	21.43[a]	2.03[a]	2.62[a]
IAEA	5.32[a]	6.54[b]	96.52[a]	92.22[a]	95.78[a]	1.28[a]	19.99[a]	21.29[a]	1.94[b]	2.56[a]
CFM	5.44[a]	6.86[c]	96.93[a]	92.31[a]	95.23[a]	1.23[a]	17.88[a]	19.77[a]	1.88[c]	2.54[a]

MLD – male larval duration, FLD – female larval duration, AR – adult recovery, AE – adult emergence, MSR – male-sex ratio (M/F), ML – male longevity, FL – female longevity, MWL – male wing length, FWL – female wing length; [1]means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Tukey's HSD test; [2]means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Fischer's LSD test.

Table 3. Different parameters of laboratory reared population of *Ae. aegypti* with different larval densities

Larval density	MLD (days)	FLD[1] (days)	Pupation (%)	AR (%)	AE (%)	MSR	ML (days)	FL (days)	MWL[2] (mm)	FWL (mm)
1 larva/mL	5.24[a]	6.42[a]	96.56[a]	93.11[a]	96.40[a]	1.20[a]	19.54[a]	20.18[a]	2.01[a]	2.61[a]
2 larvae/mL	5.31[a]	6.50[a]	96.83[a]	92.44[a]	95.69[a]	1.26[a]	21.78[a]	21.63[a]	1.94[b]	2.57[a]
3 larvae/mL	5.43[a]	6.72[b]	96.85[a]	91.85[a]	94.84[a]	1.20[a]	18.77[a]	20.67[a]	1.90[b]	2.54[a]

MLD – male larval duration, FLD – female larval duration, AR – adult recovery, AE – adult emergence, MSR – male-sex ratio (M/F), ML – male longevity, FL – female longevity, MWL – male wing length, FWL – female wing length; [1]means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Tukey's HSD test; [2]means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Fischer's LSD test.

Using the CF diet, rearing *Ae. aegypti* males exhibited a more synchronized day of pupation at five days where 80% of males already pupated regardless of larval density (Figure 3). It took longer days for males to pupate when reared at 3 larvae/mL for both IAEA and CFM diets (Figure 3).

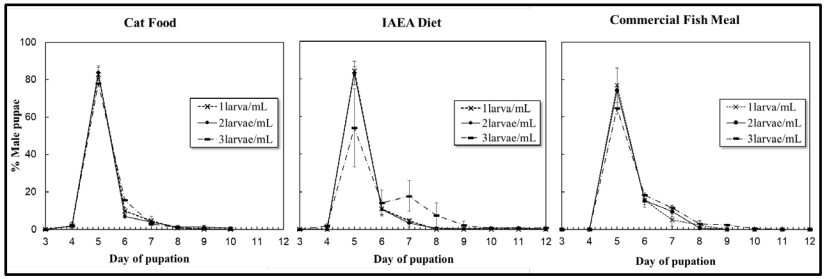

Figure 3. Daily male pupation of *Ae. aegypti* reared using different larval densities with CF, IAEA diet and CFM as larval diets

Interaction of diet and larval density was not significant in all parameters except for female longevity at 3 larvae/mL when the diet was CFM ($F = 3.19$; $df = 4$; $p = 0.04$). At 3 larvae/mL, rearing larvae through CFM obtained the shortest female longevity (Table 4).

Table 4. Female longevity (days) of laboratory reared population of *Ae. aegypti* fed with different diets and larval densities

Diet	Larval density		
	1 larva/mL	2 larvae/mL	3 larvae/mL
CF	19.31[a]	21.04[a]	23.93[a]
IAEA diet	20.27[a]	22.54[a]	21.07[a]
CFM	20.98[a]	21.30[a]	17.03[b]

Larval density	Diet		
	CF	IAEA diet	CFM
1 larva/ mL	19.31[b]	20.27[a]	20.98[a]
2 larvae/ mL	21.04[ab]	22.54[a]	21.30[a]
3 larvae/ mL	23.93[a]	21.07[a]	17.03[b]

Means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Fischer's LSD test.

Increasing larval density yielded longer mosquito larval development for female mosquitoes. Gilles *et al*. (2011) and Yoshioka *et al*. (2012) reported

that increasing larval density at the same lower diet levels prolonged the mosquito larval development time. Increasing larval density, despite giving the same amount of food, means reduced space available to each larva when a threshold is reached (Juliano, 2009; Epopa *et al*., 2018).

Overcrowding larvae scenarios are rarely seen in nature except in the period of drought. However, because rearing laboratories often lack space, a good balance should be seen among the larval density, the size of rearing trays and the amount of food provided for the larvae to ensure an optimal emergence rate of competitive male adults that are used for sterile male releases (Epopa *et al*., 2018).

In the present study, results suggest that male wing lengths were significantly lower in the CFM diet and significantly higher in the CF diet. The use of locally available and low-cost diet ingredients such as CF may reduce the cost of rearing *Ae. aegypti.* The adult body size in mosquitoes is usually equated with its wing length. Larger and consequently heavier mosquitoes can fly higher (Honek, 1993), and therefore may mate more and distribute more eggs. They also have longer life spans and are more likely to spread the etiologic agents of parasitic infections (Ameneshewa and Service, 1996).

In mass rearing *Ae. aegypti* for future SIT releases, it is ideal to maximize resources/spaces without affecting the quality of the males produced. In all three diets, using a larval density of 1 and 2 larvae/mL of water did not significantly differ from each other. However, using 2 larvae/mL would save more space and reduce the water requirement of *Ae. aegypti* without marring its quality.

3.4 Effect of Brewer's Yeast Addition as Larval Diet Ingredient

Male and female larvae reared on CF using different concentrations did not differ in adult emergence, male-sex ratio and male longevity. Diet concentrations and presence of BY showed independent relationships for pupal recovery ($F = 15.86$, $df = 4$, $p < 0.01$; $F = 4.88$, $df = 1$, $p = 0.04$, respectively), adult recovery ($F = 17.06$, $df = 4$, $p < 0.01$; $F = 5.21$, $df = 1$, $p = 0.03$, respectively), male larval durations ($F = 3.55$, $df = 4$, $p = 0.02$; $F = 14.45$, $df = 1$, $p < 0.01$, respectively) and female larval durations ($F = 8.79$, $df = 4$, $p < 0.01$; $F = 6.89$, $df = 1$, $p = 0.02$, respectively). Pupal and adult recoveries were highest when larvae were fed with 0.2 and 0.4 mg/larva of diet followed by 0.6 and 0.8 mg/larva, while lowest when larvae were fed with too much food at 1.0 mg/larva. Meanwhile, male larval duration was fastest when fed with 0.8 mg/larva and slowest with the lowest concentration at 0.2 mg/larva.

Meanwhile, the addition of BY showed higher pupal and adult recoveries and faster male and female larval durations (Table 5).

Table 5. Different parameters of laboratory reared population of *Ae. aegypti* fed with five diet concentrations of CF diet with and without BY

Diet concentrations	AE (%)		MSR		Male longevity (days)	
	with BY	without BY	with BY	without BY	with BY	without BY
0.2 mg/larva	99.31[a]	97.55[a]	1.23[a]	1.24[a]	22.86[a]	28.92[a]
0.4 mg/larva	99.65[a]	99.58[a]	1.17[a]	1.06[a]	26.02[a]	30.72[a]
0.6 mg/larva	97.26[a]	99.58[a]	1.13[a]	0.89[a]	27.39[a]	27.75[a]
0.8 mg/larva	97.43[a]	97.56[a]	1.08[a]	1.21[a]	24.47[a]	28.45[a]
1.0 mg/larva	97.81[a]	97.06[a]	1.58[a]	1.17[a]	26.32[a]	27.28[a]

Diet concentrations	PR (%)	AR (%)	MLD (days)	FLD (days)	Female longevity (days)	
					with BY	without BY
0.2 mg/larva	96.00[a]	94.50[a]	6.12[a]	6.75[a]	22.53[a]	25.01[a]
0.4 mg/larva	95.17[a]	94.50[a]	5.91[ab]	6.22[b]	24.46[a]	26.39[a]
0.6 mg/larva	84.67[b]	83.33[ab]	5.90[ab]	6.04[b]	24.90[a]	22.96[ab]
0.8 mg/larva	78.33[b]	76.50[b]	5.65[b]	5.96[b]	23.16[a]	26.37[a]
1.0 mg/larva	56.50[c]	55.00[c]	5.90[ab]	6.08[b]	25.56[a]	20.25[b]

Presence of BY	PR (%)	AR (%)	ML (days)	FLD (days)
with BY	86.13[a]	84.80[a]	5.75[b]	6.09[b]
without BY	78.13[b]	76.73[b]	6.05[a]	6.34[a]

Presence of BY	Female longevity (days)				
	0.02 g	0.04 g	0.06 g	0.08 g	0.10 g
with BY	22.53[a]	24.46[a]	24.89[a]	23.15[a]	25.58[a]
without BY	25.01[a]	26.40[a]	22.96[a]	26.37[a]	20.25[b]

Means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Tukey's HSD test; PR – pupal recovery, AR – adult recovery, MLD – male larval duration and FLD – female larval duration.

Interaction of diet concentrations and the presence of BY was not significant in all parameters except for female longevity. When larvae were fed without BY, female longevity was shortest when larvae were overfed at 1.0 mg/larva

(F = 5.41, df = 4, $p < 0.01$). Moreover, the addition of BY revealed longer female longevity (26 days) when larvae were fed only with 1.0 mg/larva of diet (Table 5).

Yeast can be used as an additional protein source to a diet to improve diet quality. Souza *et al*. (2019) demonstrated that larvae of *Ae. aegypti* fed with yeast diet developed faster than when fed with bacteria and microalgae diets.

When the concentration was increased at 0.4 mg/larva and higher, the water became turbid since the water in the trays was not replaced daily. Waste products presumably increased as the diet concentration is augmented resulting in the death of larvae at the end of rearing. Hence, there were lower pupal and adult recoveries as concentration was increased. Improvement of the survival rate due to the increasing food concentration may result in faster development durations in *Ae. aegypti*. However, an excess diet can negatively affect larval survival due to microorganisms involved in proliferating unconsumed food in rearing (Gilles *et al*., 2011). Therefore, the optimized concentration of the larval diet without affecting mosquito quality is essential in mass rearing.

3.5 Effect of Larval Diets and Diet Concentrations

Pupation, adult recovery, adult emergence, male-sex ratio and female longevity did not significantly differ among the three diet concentrations. Feeding larvae at a diet concentration of 0.2 mg/larva resulted in males with longer wing lengths (F = 5.67, df = 2, $p = 0.01$) (Table 6).

Table 6. Mean values of different parameters of laboratory reared population of *Ae. aegypti* fed with different diets and diet concentrations

Diet concentration	Pupation (%)	AR (%)	AE (%)	Male-sex ratio	Female longevity (days)	Male wing length (mm)
0.1 mg/larva	96.78^{a}	94.22^{a}	97.34^{a}	1.16^{a}	17.27^{a}	1.80^{b}
0.2 mg/larva	96.00^{a}	94.00^{a}	97.92^{a}	1.13^{a}	17.39^{a}	1.90^{a}
0.3 mg/larva	98.11^{a}	94.89^{a}	96.68^{a}	1.05^{a}	17.30^{a}	1.85^{ab}

Means followed by the same letter(s) within the same column of each parameter are not significantly different at 5% level of Tukey's HSD test; AR – adult recovery; AE – adult emergence.

Interaction of larval diet and concentrations was significant in male larval duration (F = 5.40, df = 4, $p < 0.01$), female larval duration (F = 3.90, df = 4, $p = 0.02$), male longevity (F = 5.29, df = 4, $p < 0.01$), and female wing length (F = 3.32, df = 4, $p = 0.03$). The shorter male larval duration was observed using CF at 0.1 mg/larva. The female larval duration was longer when fed with

IAEA diet at 0.1 and 0.2 mg/larva; while at 0.3 mg/larva, larval durations were significantly similar in all diets. Meanwhile, in terms of diet concentration, generally, male larvae reared at 0.1 mg/larva took the longest time to pupate among all diets (Table 7).

Table 7. Male and female larval durations (days), male longevity (days) and female wing length (mm) of laboratory reared population of *Ae. aegypti* fed with different diets and diet concentrations (DC)

Treatments	Male larval duration			Female larval duration		
Diet		DC			DC	
	0.1 mg/larva	0.2 g mg/larva	0.3 g mg/larva	0.1 mg/larva	0.2 mg/larva	0.3 mg/larva
CF	6.21[c]	5.53[b]	5.51[a]	7.81[b]	6.69[ab]	6.63[a]
IAEA Diet	7.66[a]	6.31[a]	5.38[a]	8.99[a]	7.42[a]	6.36[a]
CFM	6.95[b]	5.18[b]	5.00[a]	8.19[b]	6.31[b]	6.14[a]

DC	Diet			Diet		
	CF	IAEA	CFM	CF	IAEA	CFM
0.1 mg/larva	6.21[a]	7.66[a]	6.95[a]	7.81[a]	8.99[a]	8.19[a]
0.2 mg/larva	5.53[a]	6.31[b]	5.18[b]	6.69[b]	7.42[b]	6.31[b]
0.3 mg/larva	5.51[a]	5.38[c]	5.00[b]	6.63[b]	6.36[c]	6.14[b]

Treatments	Male longevity			Female wing length		
Diet		DC			DC	
	0.1 mg/larva	0.2 g mg/larva	0.3 g mg/larva	0.1 mg/larva	0.2 mg/larva	0.3 mg/larva
CF	17.10[a]	18.04[ab]	15.31[ab]	2.59[a]	2.73[a]	2.73[a]
IAEA	15.28[a]	18.59[a]	13.33[b]	2.23[b]	2.61[a]	2.58[a]
CFM	14.81[a]	15.69[b]	16.52[a]	2.60[a]	2.64[a]	2.67[a]

DC	Diet			Diet		
	CF	IAEA diet	CFM	CF	IAEA diet	CFM
0.1 mg/larva	17.10[ab]	15.28[b]	14.81[a]	2.59[a]	2.23[b]	2.60[a]
0.2 mg/larva	18.04[a]	18.59[a]	15.69[a]	2.73[a]	2.61[a]	2.64[a]
0.3 mg/larva	15.31[b]	13.33[b]	16.52[a]	2.73[a]	2.58[a]	2.67[a]

Means followed by the same letter(s) within the same column are not significantly different at 5% level of Tukey's HSD test.

Overall, male and female larvae reared on CF and IAEA at 0.2 and 0.3 mg/larva took the shortest time to pupate, wherein most males pupated at the 5th day after hatching. For the CFM diet, males reared at 0.1, 0.2 and 0.3 mg/larva, mostly pupated at day seven, six and five after hatching, respectively (Figure 4).

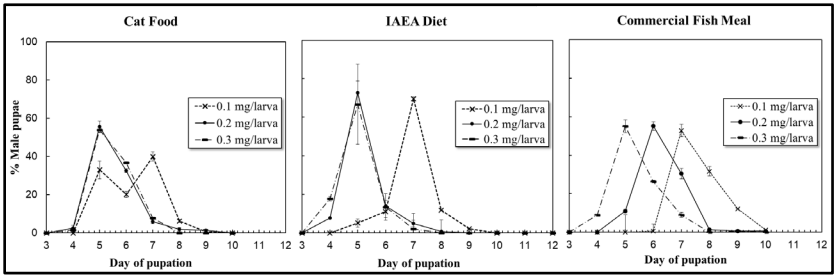

Figure 4. Daily male pupation of *Ae. aegypti* reared using different diet concentrations with CF, IAEA diet and CFM as larval diets

The length of rearing time is a crucial aspect for mass rearing as it also affects the production costs (Gunathilaka *et al*., 2017). Generally, the fastest development, which was reflected by larval duration, was observed from the larvae fed with a 0.3 mg/larva of diet (the highest concentration tested in the experiment). However, this did not significantly differ from 0.2 mg/larva for both CF and IAEA diets. Gilles *et al*. (2011) and Yoshioka *et al*. (2012) underscored that at higher diet concentrations, the time to pupation was shorter. At the lowest concentration of 0.1 mg/larva, longer time larval durations were recorded because of the intra-specific competition that negatively affects the larval development (Gilles *et al*., 2011). Chambers and Klowden (1990) stated that the ability of larvae to pupate is influenced by the nutritional reserves acquired during larval feeding. Therefore, larvae reared with higher diet concentration probably attained their critical weight earlier than those reared under lower diet concentration. Also, female larvae should attain a higher critical weight than males (Chambers and Klowden, 1990), as reflected in the current study with longer wing lengths recorded in females (2.23-2.73 mm) than in males (1.80-1.90 mm) (Tables 6 and 7).

Diet and diet concentration did not affect female adult longevity. Male adult longevity was positively affected by food concentration when given the IAEA diet. Meanwhile, optimizing adult concentration at 0.2 mg/larva for both CF and IAEA diets is important since there was a decrease in male longevity when the diet was only 0.1 mg/larva and when there was overfeeding at 0.3 mg/larva.

The two highest concentrations (0.2 and 0.3 mg/larva) showed the longest male wing lengths in all three diets. The wing length is another important factor to consider in rearing because it reflects body size, which is related to the survival and reproductive success of an adult mosquito (Clements, 1992). Wing length is used to estimate body size as it is easily measured relatively

stable character (Siegel *et al*., 1994). In addition, the body size of a female mosquito may also affect the number of eggs laid and mating success (Araujo *et al*., 2012). The small adult size of males can affect reproduction reducing spermatogenesis and testis size. Likewise, small females had fewer ovarioles per ovary, resulting in reduced fecundity (Medici *et al*., 2011).

Commercial animal diet products have traditionally been the key components of mosquito rearing diets because they are cheaper, locally available, more homogeneous than natural food sources and manageable in large quantities (Gerberg, 1970; Benedict *et al*., 2009; Damiens *et al*., 2012).

4. Conclusion

The current study demonstrated that mass rearing of *Ae. aegypti* can be optimized using CF with 15% BY at a larval density of 2 larvae/ mL, and a daily diet concentration of 0.2 mg/larva. When used in the future, this larval density and diet concentration will save more resources for the requirement of *Ae. aegypti* without compromising its quality. The addition of BY resulted in shorter larval durations, higher pupal and adult recoveries, and more extended female longevity. The findings of this study will contribute to developing an effective technique for mass rearing *Ae. aegypti* based on locally available materials.

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