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Tailoring larval diets and sugar sources to enhance development and adult survivorship of *Culex quinquefasciatus* (Say) (Diptera: Culicidae)

Penyesuaian pakan larva dan sumber gula untuk meningkatkan perkembangan dan kelangsungan hidup *Culex quinquefasciatus* (Say) (Diptera: Culicidae) dewasa

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ABSTRACT

Culex quinquefasciatus (Say) (Diptera: Culicidae) is a primary vector of lymphatic filariasis and various encephalitis viruses, posing significant public health threats. Optimizing mass-rearing protocols for this mosquito species, particularly through improved nutritional strategies, is crucial for enhancing vector control effectiveness and efficiency. This study evaluates the effects of larval diet composition and adult sugar diet on the development and survivability of *C. quinquefasciatus*. Larvae were fed five diets based on combinations of protein-rich dog food (DF) and carbohydrate-rich rice flour (RF): 100% DF, 100% RF, DF:RF (3:1), DF:RF (1:1), and DF:RF (1:3). Adult mosquitoes were provided with 10% sucrose, 10% dextrose, or 10% honey. Observed parameters included larval and pupal morphometry, pupation time and rate, adult wing length, and adult survival over 50 days. The results showed that the DF:RF (1:1) diet yielded optimal larval length, pupation rate, and adult female wing length, whereas the DF:RF (3:1) diet resulted in the shortest pupation time and largest larval width. The combination of the DF:RF (1:1) diet and honey was the most effective in promoting adult development and survival. These findings provide valuable insights for optimizing mass-rearing protocols, which are essential for research and vector control programs.

Key words: adult survival, *Culex quinquefasciatus*, larval development, nutrition, sugar

ABSTRAK

Culex quinquefasciatus (Say) (Diptera: Culicidae) merupakan vektor utama filariasis limfatik dan berbagai virus ensefalitis yang berpotensi mengancam kesehatan masyarakat. Optimalisasi pemeliharaan massal nyamuk ini, khususnya melalui perbaikan aspek nutrisi, menjadi langkah penting dalam mendukung pengendalian vektor yang efektif dan efisien. Penelitian ini mengevaluasi pengaruh komposisi diet larva dan jenis diet gula pada nyamuk dewasa terhadap perkembangan dan kesintasan *C. quinquefasciatus*. Larva diberi lima diet berbasis kombinasi pakan kaya protein (*dog food*, DF) dan karbohidrat (*rice flour*, RF): 100% DF, 100% RF, DF:RF (3:1), DF:RF (1:1), dan DF:RF (1:3). Nyamuk dewasa diberi diet 10% sukrosa, 10% dekstrosa, dan 10% madu. Parameter yang diamati meliputi morfometri larva dan pupa, waktu dan laju pupasi, panjang sayap dewasa, serta kesintasan nyamuk dewasa selama 50 hari. Hasil menunjukkan bahwa diet DF:RF (1:1) menghasilkan panjang larva, laju pupasi, dan panjang sayap nyamuk betina dewasa yang optimal, sementara DF:RF (3:1) memberikan waktu pupasi terpendek dan lebar larva terbesar. Kombinasi diet DF:RF (1:1) dan madu terbukti paling efektif untuk mendukung perkembangan dan kesintasan nyamuk dewasa. Temuan ini dapat memberikan informasi penting untuk mengoptimalkan protokol pemeliharaan massal dalam penelitian dan pengendalian vektor.

Kata kunci: *Culex quinquefasciatus*, gula, kelansungan hidup, nutrisi, perkembangan larva

INTRODUCTION

Culex quinquefasciatus (Say) (Diptera: Culicidae) is a nocturnal mosquito commonly found in tropic, subtropical, and temperate regions (Sudomo et al. 2010). This mosquito is often encountered in urban areas, particularly in locations with larval habitat support such as stagnant water and small reservoirs (Omotayo et al. 2022). The presence of *C. quinquefasciatus* in an area can pose serious health problems to humans as it serves as a major vector for various disease causing pathogens, such as *Wuchereria bancrofti* causing lymphatic filariasis, as well as West Nile virus (WNV) and Japanese encephalitis virus (JEV) causing neurological infections like encephalitis (Bhattacharya & Basu 2016).

Statistically, it is estimated that there are approximately 120 million cases of lymphatic filariasis infection globally, with around 40 million people suffering from severe disability or swelling, commonly known as elephantiasis. Additionally, case of encephalitis caused by WNV and JEV transmitted by *C. quinquefasciatus* are known to have high fatality rate, ranging from 20–30 %, and approximately 30–50% of survivors experience long term effects such as paralysis, seizures, and behavioral changes (Sudomo et al. 2010; Cheng et al. 2022; Garjito et al. 2018).

Given the significant health risks posed by diseases transmitted by *C. quinquefasciatus*, both fundamental and applied research on vector control and disease prevention in the laboratory has become crucial (Ong & Jaal 2018; Elora & Sarkar 2018). Through mass rearing, researchers can manage large mosquito populations and conduct experiments under controlled conditions, allowing them to obtain valid and representative research results (Bhujel & Saha 2023; Petersen et al. 2016).

Food quality during the larval and adult stages in mass rearing greatly affects the growth, development, and survival of mosquitoes. A comprehensive understanding of the nutritional requirements and roles of individual nutrient classes, such as carbohydrates, proteins (including essential amino acids), and vitamins, is critical for optimizing mass rearing protocols for *C. quinquefasciatus*. Carbohydrates serve as a primary energy source, essential for maintaining the metabolic activities of developing larvae, while proteins and their constituent amino acids support the synthesis of vital enzymes and structural components necessary for growth (Van Schoor et al. 2020). Vitamins, on the other hand, function as coenzymes in various metabolic pathways, assisting in the proper functioning of enzymes responsible for energy production and nutrient assimilation (Rivera-Pérez et al. 2017).

Various nutritional regimens have been studied to understand the development of larvae and the survival of adult mosquitoes. A previous study on *Aedes aegypti* (Linnaeus) reported that the dietary needs for growth, physical size, and nutrient storage in these mosquitoes depend on a relatively low yet essential protein-to-carbohydrate ratio to achieve optimal developmental outcomes (Van Schoor et al. 2020). Another study on *Anopheles gambiae* Giles demonstrated that females fed sucrose lived longer than those fed glucose, fructose, or a combination of both (Kessler et al. 2015). Similarly, studies have shown that using 100% fish food for larvae and honey as a sugar source for adults was more effective for rearing *Anopheles darlingi* Root under experimental conditions (Zanin et al. 2019).

Other studies on *A. gambiae* and *A. darlingi* have shown that nutritional regimens play a crucial role in mosquito survival and development. Females of *A. gambiae* that were fed sucrose exhibited longer lifespans compared to those fed glucose, fructose, or a combination of both (Kessler et al. 2015). Similarly, rearing *A. darlingi* larvae on a diet of 100% fish food, along with honey as a sugar source for adults, proved to be more effective under experimental conditions (Zanin et al. 2019).

Despite extensive research on other mosquito species, the optimal dietary regimen for enhancing the rearing productivity of *C. quinquefasciatus* remains unclear. Therefore, this study has two main objective: first, to assess the effect of larval dietary composition on the development of *C. quinquefasciatus*, focusing on key physiological parameters such as larval length, width, and pupation rate. Second, to identify the impact of various sugar sources on the survivability of adult mosquitoes, monitoring physiological parameters up to 50 days post-eclosion.

MATERIALS AND METHODS

Mosquito rearing

Egg rafts of *C. quinquefasciatus* (Diptera: Culicidae) were collected from the courtyard of a laboratory, Bandung, Indonesia (6°51′27″ S, 107°37′30″ E), using ovitraps. Five field ovitraps were deployed, each consisting of a black bucket containing dog food as an attractant and laboratory-reared water. The traps were placed in shaded areas around the laboratory and inspected every 2–3 days. Only egg rafts (not larvae) were collected, thereby minimizing the risk of contamination from other mosquito species.

The eggs used in the trial were the first batch collected from the field and were reared in the laboratory only up to the first generation (F_1) before being used in

experiments. Egg rafts were placed in plastic trays (29 cm × 19 cm × 6.7 cm) containing 1 l of deionized water. Rearing conditions were maintained at 27 ± 1.04 °C, 68.4 ± 9.19% relative humidity, and a 14:10 h light:dark photoperiod, simulating natural conditions conducive to larval development.

After 24 hours, 100 first-instar larvae (L_1) were transferred into 750 ml plastic cups containing 500 ml of deionized water, using a plastic pipette for precise handling. Water was replaced every three days. Upon pupation and emergence, adults were transferred to 30 cm × 30 cm × 30 cm cages for maintenance, and species identity was confirmed at the adult stage using morphological characteristics following standard taxonomic keys (WHO 2020). Only specimens matching the diagnostic features of *C. quinquefasciatus* were included in the study, ensuring that all individuals belonged to the same species

Larval morphometric, pupation time, pupation rate, and wing length of *C. quinquefasciatus* reared on different dietary nutritional compositions

To assess the effect of diet on *C. quinquefasciatus* development, we compared five dietary compositions of dog food (DF) and rice flour (RF). Due to the exploratory nature of this study and the absence of a universally recognized 'standard diet' for *C. quinquefasciatus* rearing, no control diet was used based on pre-established protocols. Instead, the five dietary treatments were considered distinct experimental conditions, enabling a comparative assessment of the developmental outcomes across different protein and carbohydrate sources. The primary goal was to optimize dietary formulations for mosquito development, with future studies potentially using the most effective diets identified here as baseline controls.

Five different larval diets were compared in this study (Table 1). The dog food (Royal Canin Puppy®) served as a protein-rich source, while rice flour (Rose Brand®) was used as a carbohydrate source. Both ingredients were ground into fine powder using a blender. The diets were formulated with different ratios of these two ingredients as follows: 100% RF, 100% DF, 25% DF and 75% RF (DF:RF = 1:3), 50% DF and 50% RF (DF:RF = 1:1), and 75% DF and 25% RF (DF:RF = 3:1). Each diet is represented by five trays with each tray representing a biological replicate consisting of 100 larvae. Each dietary treatment was represented by five trays, with each tray serving as a biological replicate consisting of 100 larvae. The larvae were fed once a day in the morning at a rate of 0.5 mg/larva/day (50 mg/100 larvae). For this experiment, we used F_1 generation larvae. No adaptation or acclimatization process was applied, as the eggs collected from the field were hatched directly in the laboratory. Consequently, the larvae developed entirely under controlled laboratory conditions from the beginning of their life cycle. They were never exposed to natural environmental conditions, and were therefore already accustomed to the laboratory's temperature, humidity, water quality, and food. Thus, a specific acclimatization procedure, typically necessary when transferring organisms from the field to the lab, was deemed unnecessary.

A total of 30 larvae and 30 pupae were randomly selected from each treatment replication. These were fixed in an 80% ethanol solution to preserve the specimens and halt biological processes that might alter size or shape. The morphometric parameters measured included larval width, total length, and pupal cephalothorax width. Larval length was defined as the distance from the anterior head to the posterior abdomen, and larval width was measured at the widest point of the abdomen (5th segment from the anterior).

For wing length measurement, 20 female and 20 male adult mosquitoes from each treatment were collected using a mechanical aspirator and transferred into 15-ml vials. The mosquitoes were anesthetized by placing them in a 4 °C refrigerator freezer to prevent movement during measurement. The right or left wing was detached, mounted on a glass slide, and covered with a cover slip. Wing length measurements were taken using a stereo microscope at 1.25x magnification, and images were captured with a USB digital microscope camera. The images were analyzed with ZenCore image processing software. Pupation duration was calculated from the first day of larval emergence (L1) to the day

Table 1. Larval diets composed of varying ratios of dog food (DF) and rice flour (RF)

Diet	Dog food	Rice flour
100% RF	-	100%
100% DF	100%	-
DF:RF (1:3)	25%	75%
DF:RF (1:1)	50%	50%
DF:RF (1:3)	25%	75%

of pupation. The success of pupation was assessed by counting the number of larvae that successfully developed into pupae.

Survival rate of adult mosquitoes with different sugar sources

Adult *C. quinquefasciatus* mosquitoes (n = 25 males and 25 females) from each larval diet treatment were placed in 30 cm × 30 cm × 30 cm cages maintained under controlled conditions (25 ± 0.7 °C, 78 ± 11.1% humidity, 14:10 (L:D) photoperiod) throughout the 50-day observation period. They had access to cotton soaked in one of three sugar source solutions, such as 10% sucrose, 10% dextrose, and 10% honey. These solutions were prepared by dissolving 100 g of Gulaku® sugar, 100 g of Lihua Starch® dextrose monohydrate, and 100 ml of Granova® honey in 1000 ml of water. Mortality was recorded daily, and dead mosquitoes were removed promptly for counting and sex identification.

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test. Data were considered normally distributed if $P > 0.05$. To analyze the significance between groups for larval and pupal morphometric data, wing length, and pupation time, the Kruskal-Wallis test was used due to the data not being normally distributed, followed by Dunn's test for post hoc analysis. The pupation rate was analyzed using the Chi-square test, with larvae grouped based on successful or unsuccessful pupation. Additionally, differences in adult mosquito survival across various combinations of larval diets and carbohydrates sources were analyzed using Kaplan-Meier survival curves, with the log-rank test used to identify significant differences between treatment groups.

Statistical analyses were conducted using GraphPad Prism (GraphPad Software, San Diego, CA, USA) for data visualization and survival analyses, and IBM SPSS Statistics (IBM Corp., Armonk, NY, USA) for additional statistical testing. Statistical significance was set at $p < 0.05$.

RESULTS

Larval morphometric, pupation time, pupation rate, and wing length of *C. quinquefasciatus* reared on different dietary nutritional compositions

The DF:RF (1:1) (6.36 ± 0.44 mm) and DF:RF (3:1) (6.35 ± 0.44 mm) diets resulted in the longest average larval lengths, with no statistically significant variation between these treatments. In terms of larval width, the DF:RF (3:1) diet yielded the largest value (0.90 ± 0.07 mm), comparable to 100% DF (0.89 ± 0.08 mm) and DF:RF (1:1) (0.89 ± 0.09 mm) treatments. Conversely, larvae fed with the DF:RF (1:3) diet exhibited the smallest larval width (0.81 ± 0.09 mm), which was significantly smaller than all the other treatments ($p < 0.05$) (Table 2).

Pupae reared on the DF:RF (3:1) diet exhibited the largest cephalothorax width (2.02 ± 0.024 mm), which was significantly different from the smallest cephalothorax width observed in the DF:RF (1:3) treatment (1.90 ± 0.03 mm). Notably, pupae fed 100% DF and DF:RF (1:1) diets showed similar cephalothorax widths with no significant differences between these treatments (Table 2).

The effect of larval feeds on the mean time to reach the pupal stage varied from 8.13 to 9.63 days between each group (Table 1; $\chi^2 = 279.86$, df = 4, $P < 0.001$). A significant difference was observed, with larvae fed 100% DF requiring the shortest time to pupate, while those fed DF:RF (1:3) took significantly longer compared to all other groups. Additionally, mosquito larvae reared on DF:RF (1:1) and DF:RF (3:1) did not differ significantly in pupation time; however, larvae in both groups took longer to pupate than those reared on the 100% DF treatment (Table 2).

The highest pupation rate was observed in pupae reared on DF:RF (1:1), reaching 100%, which was significantly higher than the 100% DF treatment (80.4 ± 6.6 %). However, no significant differences were observed between the DF:RF (1:1) treatment and the DF:RF (3:1) or DF:RF (1:3) treatments (Table 2).

This study revealed that females reared on DF:RF (1:1) diet exhibited the longest wing length (3.51 ± 0.17

Table 2. Larval morphometric, pupation time, pupation rate, and wing length of *Culex quinquefasciatus* reared on different dietary nutritional compositions

Larva feed	Larvae		Pupae cephalothorax width (mm)	Pupation time (Day)	Pupation rate (%)
	Length (mm)	Width (mm)			
100% Dog food	6.24 ± 0.60 ab	0.89 ± 0.08 a	1.96 ± 0.04 ab	8.13 ± 0.05 a	80.4 ± 6.6 bc
100% Rice flour	N/A	N/A	N/A	N/A	N/A
DF:RF (3:1)	6.35 ± 0.44 a	0.90 ± 0.07 a	2.02 ± 0.03 a	8,54 ± 0.05 b	99,4 ± 0.2 ab
DF:RF (1:1)	6.36 ± 0.44 a	0.89 ± 0.09 a	1.95 ± 0.03 ab	8,75 ± 0.07 b	100[a]
DF:RF (1:3)	6.04 ± 0.47 b	0.81 ± 0.09 b	1.90 ± 0.03 b	9,63 ± 0.07 c	97,8 ± 1.6 ab

mm), whereas those in the DF:RF (1:3) diet had the shortest (2.84 ± 0.07 mm). Among males, the longest wing length was observed in the 100% DF group (2.77 ± 0.04 mm), whereas the shortest was recorded in the DF:RF (1:3) group, with these differences being statistically significant (Table 3). These findings suggests that a balanced diet composition of DF and RF promotes greater wing development, particularly in females.

Survival rate of adult mosquitoes with different sugar sources

Over the 50-day observation period, the survival rates of male mosquitoes fed with honey and sucrose were the highest, with no significant difference between the two treatments (Chi-Square = 0.02, p = 0.88), while the dextrose group exhibited the lowest survival rates (Sucrose, Chi-Square = 12.791, p < 0.0001; Honey, Chi-Square = 11.62, p < 0.001) (Figure 1).

Regarding female mosquitoes, those fed sucrose demonstrated the highest survival rates during the first 30 days, maintaining a cumulative survival rate close to 90%, although gradual declines were observed thereafter. By day 50, the honey-fed group has the highest survival rate among the three treatments. In contrast, the dextrose-fed group exhibited the lowest survival rates, with a marked decline in cumulative survival beginning around day 20 (Figure 2).

Survival analysis of adult mosquitoes with both different dietary nutritional larval feeds and sugar sources

On day 50, male mosquitoes fed with the sucrose DF:RF (1:1) treatment showed the highest survival rate, with 80% still alive, closely followed by those treated with honey DF:RF (1:1), with no significant difference between the two. Additionally, the survival decline in the sucrose DF:RF (1:1) group was slower compared to the other treatments (Figure 3). In contrast, the lowest survival rate was seen in males fed with a dextrose (3:1) treatment, with only 12% of the mosquitoes surviving (Chi-square = 21.15, P < 0.001).

Among the female *C. quinquefasciatus* populations tested, those fed with honey DF:RF (1:1) exhibited the highest survival rate, with 88% surviving on day 50, and the survival curve showed a gradual, stable decline, indicating optimal survivability compared to other treatments (Figure 3). The survival curve for this treatment also exhibited a very slow and stable decline, indicating optimal survivability compared to the other treatments. In contrast, the treatments with Sucrose (100% DF) and Dextrose (1:3) showed the

Table 3. Results of wing length measurement of male and female adult mosquitoes

Larva feed	Wing length (mm)	
	Male	Female
100% Dog food	2.77 ± 0.04 a	3.35 ± 0.06 a
100% Rice flour	N/A	N/A
DF:RF (3:1)	2.60 ± 0.02 b	3.30 ± 0.05 ab
DF:RF (1:1)	2.61 ± 0.03 b	3.51 ± 0.17 a
DF:RF (1:3)	2.47 ± 0.05 b	2.84 ± 0.07 b

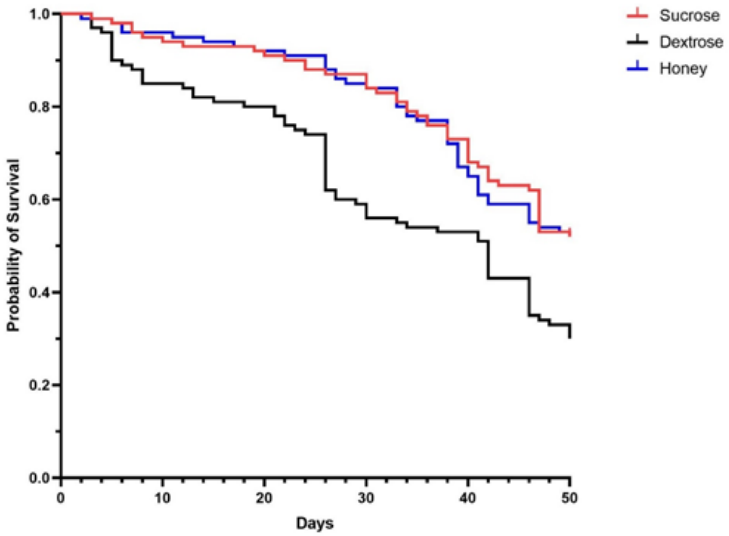

Figure 1. Kaplan-Meier survival curves and estimates for mosquito survival in three different carbohydrate sources (sucrose, dextrose, and honey) for male mosquitoes.

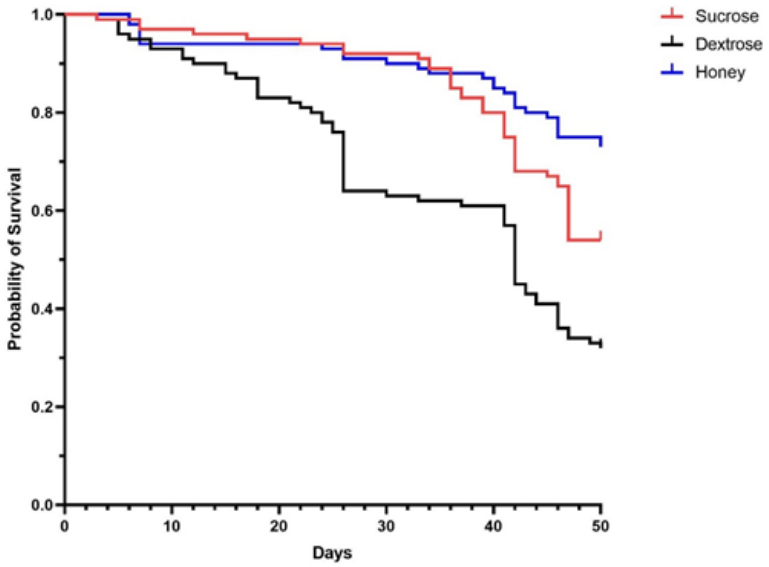

Figure 2. Kaplan-Meier survival curves and estimates for mosquito survival in each of the three different carbohydrate sources (sucrose, dextrose, and honey) for female mosquitoes.

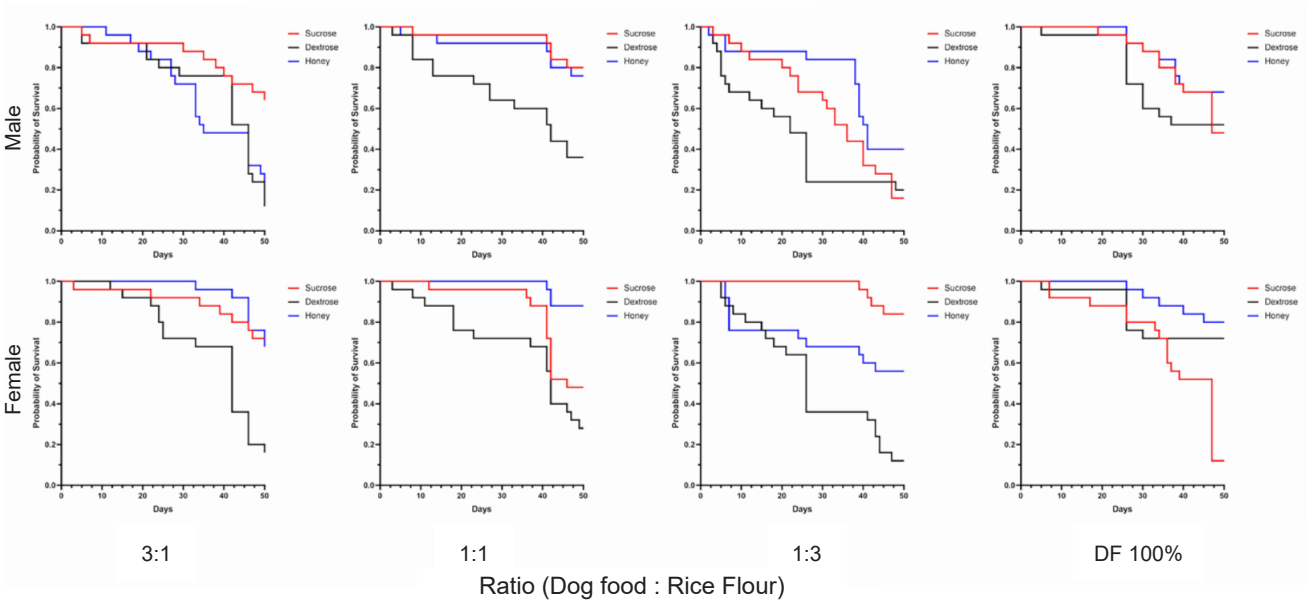

Figure 3. Kaplan-Meier survival curves and estimates for mosquito survival in various larval dietary compositions (100% DF, 100% RF, DF:RF (1:3), DF:RF (1:1), DF:RF (1:3)), and adult carbohydrate sources (sucrose, dextrose, honey).

lowest survival rates, with only 12% of the mosquitoes remaining alive on day 50. Notably, the dextrose DF:RF (1:3) group experienced a sharp survival decline around day 20, when seven mosquitoes died, leading to just 36% survival.

DISCUSSIONS

Larval morphometric, pupation time, pupation rate, and wing length of *C. quinquefasciatus* reared on different dietary nutritional compositions

Overall, the findings of this study indicate that differences in the ratio of protein to carbohydrate-based diets influenced the morphometric characteristics of larvae and pupae, pupation time, pupation rate, and wing length of *C. quinquefasciatus*. Nutritional requirements for mosquito development can be broadly categorized into macronutrients and micronutrients, with carbohydrates and proteins serving as the primary macronutrients essential for larval growth and development (Souza et al. 2019). Diets with high protein or a balanced protein-to-carbohydrate ratio were associated with optimal growth and development, highlighting the pivotal role of proteins in enzyme regulation, hormone production, and the formation of structural components vital for mosquito development (Van Achoor et al. 2020; Linenberg et al. 2016; Pooraiiouby et al. 2018).

The results of this study further highlight the ability of *C. quinquefasciatus* larvae to adapt their metabolic processes under dietary imbalances. High-protein

diets allowed larvae to sustain optimal morphometric size despite carbohydrate limitations, suggesting the activation of alternative metabolic pathways such as gluconeogenesis. This process enables the conversion of amino acids derived from dietary proteins into glucose, which can subsequently be utilized for trehalose synthesis and energy production. Such metabolic flexibility plays a critical role in supporting larval growth and development even when carbohydrate availability is low (Miyamoto & Amrein 2017). For instance, studies have shown that fruit flies and adult mosquitoes express genes associated with the gluconeogenesis pathway (Miyamoto & Amrein 2017). Similarly, larvae of *Manduca sexta* (Linnaeus) have demonstrated the ability to activate gluconeogenesis to fulfill their energy. However, it is important to note that although gluconeogenesis has been observed in other insect species, its spesific role in *C. quinquefasciatus* larvae remains speculative and warrants additional research (Thompson 1995; Thompson et al. 2002).

The absence of sufficient protein intake in mosquito larvae adversely affects several critical aspects of their life cycle. For instance, the results from this study indicate that larvae fed on a carbohydrate-rich diet exhibited the smallest larval and pupal morphometrics, highlighting the detrimental effects of protein deficiency. Such size reductions, resulting from inadequate protein consumption, can extend beyond larval and pupal stages, potentially influencing adult mosquito fitness. Previous finding demonstrated that smaller *A. aegypti* individuals have diminished feeding and lower metabolic reserves, factors that may ultimately compromise their survival and reproductive success. Therefore, the nutritional composition during the larval stages plays a crucial role in shaping the overall fitness of adult *C. quinquefasciatus*, reinforcing the importance of balanced diet intake (Souza et al. 2019; Paige et al. 2019; Carvajal-Lago et al. 2021). Furthermore, *C. quinquefasciatus* larvae fed on a 100% RF diet showed a very high mortality during the first and second instar stages. This outcome is attributed to the fact that diets with excessively high carbohydrate content are unable to provide the essential amino acids required for protein synthesis and the development of critical body structures, which are necessary for larval survival (Van Schoor et al. 2020).

Although high-protein diets yield favorable results in terms of growth and development parameters, larvae fed 100% DF diets exhibited the lowest pupation rate compared to other treatments. This is likely due to a protein-rich diet with low carbohydrates can lead to nutritional imbalances (Souza et al. 2019; Hellhammer et al. 2023). This phenomenon is closely tied to the concept of critical mass, which refers to the minimum size or weight larvae must achieve to progress to the next life stage. Reaching this threshold is crucial for triggering hormonal changes necessary for metamorphosis. Once larvae attain the critical mass, their bodies begin to increase the production of ecdysteroid hormones, a group of steroid hormones that play a key role in insect development and enables larvae to undergo the transformation into pupae.

Larvae fed diets with an imbalanced protein-to-carbohydrate ratio, such as those high in protein but low in carbohydrates, may fail to reach the required critical mass required for optimal ecdysteroid release. This disruption can lead to delayed or failed pupation, as seen in our study with *C. quinquefasciatus*. In essence, without reaching the critical mass needed to trigger this hormonal surge, larvae are unable to complete the molting cycle necessary for pupation (Souza et al. 2019; Hellhammer et al. 2023). Moreover, in these nutrient-restricted conditions, larvae may trade off their use of protein for physical growth, such as developing larger body structures, at the expense of successful survival to the pupal stage (Souza et al. 2019; Yan et al. 2021; Yan et al. 2023).

The smallest wing lengths observed in both male and female mosquitoes reared on the DF:RF (1:3) treatment are consistent with findings by Van Schoor et al. (2020), who reported that protein-deficient diets result in shorter wing lengths in both sexes. These results underscore the essential role of protein in supporting optimal wing size development (Van Handel & Day 1989). Furthermore, our study revealed that male mosquitoes are more efficient in utilizing protein for wing growth, whereas female mosquitoes appear to require a more balanced protein-to-carbohydrate ratio for optimal development, including the development of reproductive organs and wings. This suggests a potential sex specific difference in nutrient utilization.

Larger wing length is often associated with greater body size, which in turn contributes to stronger immune systems. Mosquitoes with larger bodies are typically more resilient to environmental stressors such as temperature variations, humidity, and population density, which is especially important in laboratory mass rearing. Additionally, mosquitoes with stronger immune systems are more effective for use in laboratory research related to vector competence and population control programs (Brown et al. 2019 ; Mackay et al. 2023).

Larger mosquitoes also exhibit enhanced reproductive capacity, particularly females, who have the potential to produce more eggs. This is highly

advantageous for mass-rearing programs in laboratories focused on studies related to vector population control, reproduction, and genetic (Mackay et al. 2023; Mamai et al. 2017). On the other hand, larger males may have an advantage in mating competition, which is crucial for mating behavior studies or maintaining male populations used in control programs, such as the sterile insect technique (SIT) (Aldridge et al. 2024; Contreras-Perera et al. 2023).

Survival rate of adult mosquitoes with different sugar sources

Based on the findings obtained regarding the survival rates of *C. quinquefasciatus* under different sugar diets, the results contrast with previous study where *A. aegypti* mosquitoes fed a dextrose-based diet exhibited the highest longevity, surviving an average of 14 days longer than those fed honey, highlighting species-specific variations in how carbohydrate sources impact longevity in different mosquito species (Posidonio et al. 2021).

In addition to sugars like sucrose and fructose, honey contains a variety of other essential nutrients, including proteins, vitamins, amino acids, minerals, and other micronutrients, each of which may contribute to its positive effects on longevity (Da Silva et al. 2016). This aligns with studies showing that providing additional amino acids to female *C. quinquefasciatus* resulted in a 5% increase in their survival rate, while no significant effect was observed in males (Vrzal et al. 2010). Similarly, an extended lifespan was observed in adult *An. darlingi* when fed complex nutrient sources, including amino acids (Zanin et al. 2019).

Survival analysis of adult mosquitoes with both different dietary nutritional larval feeds and sugar sources

Our findings suggest that survival and overall health of *C. quinquefasciatus* adults are closely tied to the nutritional balance of their larval diet, as an inadequate food supply during the larval stage can significantly increase adult mortality. A1:1 protein to carbohydrate ratio is ideal, providing the necessary protein for growth and development while ensuring adequate carbohydrates for energy, nutrient storage, and mitigating the risk of malnutrition (Shapiro et al. 2016). Furthermore, the presence of other essential nutrients, such as amino acids, vitamins, and lipids, is crucial for proper mosquito development, reproduction, and health. These factors, together with a balanced protein-carbohydrate diet, significantly influence mosquito growth, development, pupation, and survival (Vrzal et al. 2010; Merritt 1992; Sivanathan & Zairi 2012; Joy et al. 2010; Kivuyo et al. 2014).

The results of this study are consistent with previous findings by Elora & Sarkar (2018), who reported that a more balanced protein to carbohydrate ratio in larval diets, such as those containing soybean powder, led to lower adult mortality in *C. quinquefasciatus*. In contrast, diets with a higher protein to carbohydrate ratio, such as fish food, dog food, and dried fish, were associated with increased adult mortality. A similar trend was also observed in *A. aegypti*, as noted by Van Schoor et al. (2020). This could be attributed to the elevated protein levels, which may result in the accumulation of harmful ammonia. The breakdown of proteins and amino acids produces nitrogenous waste, and high ammonia levels from protein digestion are known to be toxic to mosquitoes (Van Schoor et al. 2020; Scaraffia et al. 2008; Dias et al. 2019).

While excessive protein intake can indeed be detrimental, our results also reveal that insufficient protein poses equally serious risks. The sharp decline in survival to only 36% after day 20 in the DF:RF (1:3) and dextrose treatment groups underscores the equally harmful impact of insufficient protein. Larvae reared on low protein diets exhibited slower growth and produced smaller adults with shorter wings, characteristics linked to reduced fitness and heightened vulnerability to environmental stress due to limited energy reserves and hemocyte numbers (Souza et al. 2019; Linenberg et al. 2016; Mackay et al. 2023). These findings reinforce that while nutrient excess is harmful, nutrient deficiency is equally detrimental. Thus, maintaining a balanced nutritional profile rather than minimizing or overloading any single macronutrient is essential for optimizing mosquito development and adult survivability.

Building on the importance of a balanced larval diet, sugar also plays a critical role in adult mosquito survival, particularly as their primary energy source. In temperate and subtropical climates, mosquitoes typically consume sugar within the first 2 to 5 days after emerging, as it provides the necessary energy for flight and is associated with increased survival and successful mating (Van Handel 1985; Foster 1995).

In nature, the primary sugar sources for mosquitoes are floral and extrafloral nectars, with nectar-derived honey serving as a complex and concentrated nutrient source rich in sugars, water, amino acids, and trace amounts of vitamins and minerals, which provide essential energy and nitrogen to support mosquito survival, longevity, and vector competence (Zanin et al. 2019; Vrzal et al. 2010; Foster 1995).

The relationship between nectar-derived honey and the success of mosquitoes as disease vectors is evident, as these compounds support prolonged survival, enabling mosquitoes to feed on multiple hosts and facilitating pathogen incubation (Woodring et al. 1996). Additionally, mosquitoes, like many insects, prefer carbohydrate sources enriched with amino acids, highlighting their significant role in shaping insect life histories and enhancing survival and reproductive success (Alm et al. 1990; Mevi-Schütz & Erhardt 2003).

In addition to amino acids, certain phytochemicals such as quercetin and p-coumaric acid have been identified in the nectar, honey, and pollen of various plant species. These compounds have been shown to influence sugar-feeding behavior in insects (Singaravelan et al. 2005). Specifically, dietary p-coumaric acid was found to extend the lifespan of adult worker honey bees by 14.1%, while quercetin increased the longevity of adult female *A. aegypti* mosquitoes by 30% (Liao et al. 2017; Nunes et al. 2016). Interestingly, the presence of these phytochemicals did not affect sugar intake, suggesting that the observed extension of lifespan was independent of sugar consumption. Both quercetin and p-coumaric acid function as antioxidants, and their role in enhancing longevity is thought to be linked to their ability to upregulate antioxidant enzymes and reduce the accumulation of reactive oxygen species (ROS), which are associated with cellular aging (Yue et al. 2019; Alugoju et al. 2018).

Information regarding these phytochemicals effect on fecundity was limited. However, previous studies have shown that the lifespan extension observed in mosquitoes consuming p-coumaric acid and quercetin was not associated with a trade-off between longevity and reproductive output, and was instead linked to the differential expression of genes involved in xenobiotic metabolism regulation, stress resistance, and longevity, suggesting that these phytochemicals contribute to lifespan extension through multiple biological pathways (Njoroge et al. 2021; Mao et al. 2013; Pallauf et al. 2017).

The results of this study reveal that while honey promotes longevity and survival in both male and female mosquitoes, sucrose primarily enhances the survival of males. This difference can be attributed to the distinct nutritional profiles of honey and sucrose. Sucrose, a disaccharide composed of glucose and fructose, serves as a straightforward energy source commonly used in laboratory-reared mosquitoes (Zanin et al. 2019). Since male mosquitoes rely exclusively on sugar for energy and do not require blood meals, they may metabolize sucrose more efficiently than females, benefiting from its simple composition (Barredo & DeGennaro 2020). In environments with higher concentrations of sucrose, the increased energy availability allows males to exhibit greater survivorship, as their metabolic needs are effectively met by sucrose alone (Vaidyanathan et al. 2008). In contrast, honey offers a more complex array of sugars, including glucose, fructose, and sucrose, alongside additional nutrients. These extra nutrients provide broader metabolic advantages, particularly for female mosquitoes, which have higher energy demands due to their reproductive and blood-feeding requirements (Foster 1995). The absence of these supplementary compounds in sucrose likely limits its ability to sustain female longevity, as females require more than just basic sugar intake for optimal survival (Barredo & DeGennaro 2020). This could explain why sucrose has a less pronounced effect on female mosquitoes, who need more than just simple sugars to thrive.

On the other hand, dextrose, a monosaccharide, is the simplest form of sugar, consisting solely of glucose. While glucose can be rapidly utilized by cells for energy, it provides a less sustained energy source compared to sucrose (Paulsen et al. 2019; Nayar & Sauerman 1971). This rapid energy spike may not support the long-term metabolic needs of mosquitoes, leading to lower survival rates when fed on dextrose alone.

Additionally, both the sucrose-fed group (DF:RF (1:1) and 100% DF) and the dextrose-fed group (DF:RF (3:1) and DF:RF (1:1)) exhibited a similar steep decline in survival rates after 40 days. This decline aligns with various studies reporting the mean adult longevity of *C. quinquefasciatus* males maintained at 25°C to be approximately 39.8 days and an average lifespan across different temperatures of 32.7 days, indicating that temperature significantly influences longevity and survival rates (Oda et al. 1999; Moser et al. 2023).

CONCLUSIONS

Culex quinquefasciatus larvae fed the DF:RF (1:1) diet exhibited optimal outcomes, including the greatest larval length, pupation rate, and female wing length. Notably, the combination of the DF:RF (1:1) diet and honey as the adult sugar source was the most effective regimen for both larval development and adult survival in mass-rearing programs. These findings have practical implications for vector control strategies, as they could enhance the efficiency of interventions such as the sterile insect technique (SIT) or Wolbachia-based programs by promoting the production of healthier, longer-lived mosquitoes. To build on these insights, future research should focus on investigating the metabolic pathways involved in nutrient processing and conduct long-term studies to assess the impact of nutrition on vector competence.

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