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www.biotaxaorg/RSEA. ISSN 1851-7471 (online) Revista de la Sociedad Entomológica Argentina 82 (3): 42-45, 2023

An effective artificial feeder for breeding *Aedes aegypti* (Diptera: Culicidae)

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Received *30 - III - 2023* | Accepted *02 - VIII - 2023* | Published *30 - IX - 2023*

https://doi.org/10.25085/rsea.820307

Un alimentador artificial eficaz para la cría de *Aedes aegypti* (Diptera: Culicidae)

RESUMEN. Los mosquitos, al igual que otros insectos hematófagos, requieren alimentarse de sangre para producir huevos. En la cría de colonias de laboratorio, generalmente se utilizan animales vivos, mientras que para ensayos experimentales se han desarrollado diversos alimentadores artificiales con características distintas. En este estudio, se seleccionó un alimentador artificial de vidrio previamente desarrollado y probado en infecciones con patógenos, y se evaluó su eficacia en la cría de *Aedes aegypti* (Linnaeus). Este dispositivo utiliza tripa bovina como membrana y sangre humana como fuente de alimentación, manteniendo una temperatura constante de 37 °C gracias a un baño termostático que permite la circulación de agua alrededor del alimentador. Durante un período de 4-5 semanas, se analizaron la eclosión de los huevos, las tasas de alimentación y fertilidad de tres cohortes de un linaje parental (P) y sus respectivos linajes filiales (F_1). Los resultados demostraron la eficacia de esta metodología para la cría de una colonia de *Ae. aegypti* hasta la segunda generación, con tasas de eclosión de huevos promedio de P= 0,92 y F_1= 0,80, tasas de alimentación promedio de P= 0,54 y F_1= 0,73, y una cantidad de huevos obtenidos de P= 1809 y F_1= 8579.

PALABRAS CLAVE. Alimentación artificial. Mantenimiento de colonia. Mosquitos.

ABSTRACT. Mosquitoes, like other hematophagous insects, need to feed on blood to produce eggs. For laboratory colony maintenance, live animals are generally used, while various artificial feeders with different characteristics have been developed for experimental assays. In this study, a previously developed and pathogen-tested glass artificial feeder was selected and evaluated for rearing *Aedes aegypti* (Linnaeus). The device utilizes bovine gut as a membrane and human blood as a food source, maintaining a constant temperature of 37 °C through a thermostatic bath that enables water circulation around the feeder. Egg hatching, feeding rates, and fertility were analyzed for three cohorts of a parental lineage (P) and their respective filial lineages (F_1) over a period of 4-5 weeks. The results demonstrated the efficacy of this methodology for rearing an *Ae. aegypti* colony up to the second generation, with average egg hatching rates of P= 0.92 and F_1= 0.80, average feeding rates of P= 0.54 and F_1= 0.73, and egg quantities obtained of P= 1809 and F_1 = 8579.

KEYWORDS. Artificial feeding. Colony maintenance. Mosquitoes.

Aedes aegypti (Linnaeus) is the main urban vector of many flavivirus involved in human diseases (Artsob et al., 2017). For this reason, it is subject of study all over the world where mosquito colonies are well established in insectaries (Kuno, 2010). As a blood meal is required by female mosquitoes for egg production, this is a critical step while rearing a colony (Kauffman et al., 2017). For this purpose, human volunteers or live animals are usually employed (Styer et al., 2007; Clemons et al., 2010). The implementation of live animals to feed mosquitoes and other insects represents an advantage as the insects are attracted by some signals emitted by the host, such as CO_2 and heat, and blood is available at the right temperature in natural conditions (Friend & Smith, 1985; Kasap et al., 2003). On the other hand, it is expensive, it requires space to breed the host and feed the colony, it is often unpleasant and the animals need to be anesthetized or immobilized during the alimentation (Friend & Smith,

1985). Hence, it is important to have an effective artificial feeder, which can supplant the use of animals, and, at the same time, that can be safely employed for infection experiments. Many methodologies that differ in the composition and temperature of the meal, the time required for an efficient alimentation, and the device employed for the feeding have been developed so far. Besides, the devices present different features: material, membrane type, capacity, and method of temperature regulation (Cosgrove et al., 1994; Tseng, 2003; Aldana et al., 2005; Costa-da-Silva et al., 2013; Luo, 2014; Marti et al., 2015). In this study, we demonstrate that it is possible to maintain a colony of *Ae. aegypti* by using an artificial glass feeder, which simulates natural feeding, previously employed for virus infection in our laboratory (Bonica et al., 2019; Marti et al., 2020). The device, which resulted from the combination of those developed by Luo (2014) and Marti et al. (2015), is made of glass with a capacity of 5-6 ml, it uses a thermostatic circulator bath to regulate temperature, it employs a cattle gut membrane that mimics human skin, and human blood with sucrose as meal (Fig. 1). The human blood used for our trials was provided by the Hemotherapy Institute of La Plata City.

Fig. 1. Artificial feeder. The glass feeder evaluated simulates natural feeding. It employs cattle gut membrane that mimics human skin, it has a capacity of 5-6 ml, the temperature is regulated in order to keep the blood at 37 °C through a thermostatic circulator bath, and it employs human blood with sucrose as meal source.

We evaluated the efficiency of the artificial feeder by measuring three reproductive parameters in a parental (P) and a filial (F_1) lineage: egg hatching rate (EHR), blood-feeding rate (BFR), and total number of eggs laid by group during the whole life. The EHR is equivalent to the number of larvae/eggs and was evaluated by using an X-square test. The BFR was calculated as the number of engorged females/number of females exposed to blood, and was evaluated by using a Fisher test (Conover, 1999; Ott & Longnecker, 2010). A Student test was conducted to evaluate the number of eggs laid by females in both P and F_1. These fitness parameters were evaluated in P and F_1, both artificially fed, to determine the fertility of the offspring in order to reinforce the effectiveness of the apparatus.

For this purpose, three parental cohorts (P_1, P_2 and P_3) of a hundred eggs each, collected from an established colony of *Ae. aegypti* in La Plata (Argentina) were evaluated. The established colony is maintained inside a screened cage (55 x 55 x 55 cm) and fed weekly on an immobilized chicken. The eggs were placed into a tray with dechlorinated water and 10 mg of yeast for hatching. Egg hatchability was recorded after 48 hours. Finely ground rabbit food was added to the trays to feed the larvae. The emerged adults were counted and sexed, and subsequently placed into cardboard cages (25 x 32 cm diameter), and fed with raisin and water. After 3-5 days from adult emergence a blood meal (4.25 ml human blood + 0.25 ml sucrose 50 % w/v) was offered to each cohort once a week for 30 minutes during 4 weeks (Fig. 1). Adult mortality was registered daily until the last specimen. Engorged females were counted over the total number of exposed females to determine the BFR. Wet cotton and a filter paper in a Petri dish were placed inside each cage to allow oviposition. The eggs laid during each week were counted and kept on their filter paper inside a plastic bag to maintain humidity for at least 5-7 days to allow embryogenesis before hatching. From each P cohort, one hundred eggs were collected and placed in a tray for hatching to obtain three filial cohorts (F_1.1, F_1.2 and F_1.3). The same procedures carried out for P were repeated for F_1. Both assays were maintained in an environment chamber set to simulate a curve of fluctuating daily temperatures according to the mean temperatures for *Ae. aegypti* in Argentina (20-30 °C, photoperiod 8:16 hours dark:light, 70±10 % RH) (Muttis et al., 2018).

All parameters evaluated for each cohort are detailed in Table I. Upon examining the P lineage in its entirety, it was observed that the mean total EHR was 0.92, while for the F_1 generation, it was 0.80. Significant differences between the two generations were identified (X-square= 16.16, df= 1, p= 5.81e-05). The mean BFR for each generation was calculated as 0.54 for P and 0.73 for F_1. The BFR was compared between P and F_1 along four weeks, and were found to be significantly different (X-square= 12.89, df= 1, p= 0.0003) only during the first week (Fisher test, p= $5.733e^{-8}$) while there were not significant differences in the other weeks.

Table I. Egg hatching rates (EHR), blood-feeding rates (BFR) and total eggs laid by *Aedes aegypti* females in the parental (P) and filial (F_1) cohorts during four weeks.

Lineage	Cohort	EHR (N=100)	Week 1				Week 2				Week 3				Week 4				Tot eggs laid by cohort	Tot mean eggs/♀
			N° ♀	BFR	Tot eggs	Mean Egg/♀	N° ♀	BFR	Tot eggs	Mean Egg/♀	N° ♀	BFR	Tot eggs	Mean Egg/♀	N° ♀	BFR	Tot eggs	Mean Egg/♀		
P	P1	0.98	36	0.44	226	6.3	25	0.64	277	11.1	8	0.88	208	26.0	3	0	64	21.3	775	21.5
	P2	0.89	18	0.94	397	22.1	15	0.53	256	17.1	3	0	8	2.7	0	-	-	-	661	36.7
	P3	0.89	29	0.21	2	0.1	20	0.55	144	7.2	15	0.33	165	11.0	4	0.75	62	15.5	373	12.9
F_1	F_1.1[a]	0.93	41	0.63	496	12.1	36	0.42	824	22.9	28	0.36	272	9.7	28	0.93	460	16.4	2052	50.0
	F_1.2	0.68	28	1	934	33.4	24	0.92	1134	47.3	11	0.82	79	7.2	6	-	142	23.7	2289	81.8
	F_1.3[a]	0.80	34	0.97	916	26.9	32	0.38	1313	41.0	29	0.79	781	26.9	25	0.96	253	10.1	3263	96.0

[a] F_1.1 and F_1.3 lived one more week and laid 26 and 949 more eggs, respectively.

The total amount of eggs laid by all females (83) of the P lineage for a period of four weeks (1809) was lower that the number of eggs (8579) laid by F_1 females (103) during five weeks (Student test, t= 2.3995, df= 23, p= 0.02491). Interestingly, two cohorts of F_1 lived one more week than the rest of the cohorts. Two females belonging to F_1.1 laid 26 more eggs during the fifth week, while 24 other females of the F_1.3 laid 949 eggs, scaling the number of total eggs laid by those cohorts to 2078 and 4212, respectively.

The experiment showed that the glass feeder evaluated could be employed to maintain an *Ae. aegypti* colony. While Marti et al. (2015) tested the feeder in colonies of triatomines, Luo (2014) limited his studies to the feeding of P of *Ae. aegypti* and *Ae. albopictus* (Skuse), and the analysis of some parameters of the development of the F_1. Herein, we extended the analysis of fitness parameters to the F_1 colony of *Ae. aegypti*, which is fundamental for breeding. We found high rates of hatchability in both P and F_1. The high *Ae. aegypti* BFR obtained may be due to the nature of the membrane, since high percentages were also shown in the works presented by Luo (2014) of 89.7 % and Cosgrove et al. (1994) of 67.5 % that employs collagen cattle gut membrane. Latex and Parafilm-M® membranes were tested with our glass feeder but, despite the results previously obtained with Parafilm-M® (Sri-In et al., 2020), the BFR were lower than those obtained with the cattle gut tissue layer, therefore they were discarded from the trial. Additionally, it was observed that the BFR remained notoriously high during the four offerings of blood meals to the same females during the whole experiment. This emphasizes the effectiveness of this method of artificial feeding. In addition, the high BFR and fertility obtained in this study might be due to the employment of human blood, as it was found that blood meal sources may affect reproduction in *Ae. aegypti*, being human blood more effective than others (Gunathilaka et al., 2017). Finally, the amounts of eggs laid by P and F_1 females during their whole life were evaluated. The analysis of the number of eggs per female per week, based on the number of females at the beginning of the experiment, shows that the relation between the number of eggs in F_1 and in P (F_1:P) is around 3:1. Additionally, it is interesting to highlight that F_1 lived one more week than P. Further fitness studies might determine if this method of alimentation could be having an effect on adult longevity.

The use of an artificial feeder in our study, composed of cattle gut membrane and human blood mixed with sucrose, offers a significant contribution to *Ae. aegypti* research. While the concept of an artificial feeder is not entirely novel, our study conducts a comprehensive analysis of fitness parameters in both the parental (P) and F_1 generations. To the best of our knowledge, this study represents one of the few attempts to investigate the reproductive success and fitness of F_1 mosquitoes using such an artificial feeder. This novel approach expands our understanding of the breeding protocols for *Ae. aegypti*, a species of utmost importance in the context of disease transmission. Moreover, the employment of human blood mixed with sucrose in the feeder provides a unique opportunity to study the effects of various viruses and pathogens (Bonica et al., 2019). As previously noted, this methodology presents several advantages, including the replacement of live animals for pathogen-free blood, the ability to perform experiments under clean and sterile conditions, and the precise control of pathogen dosage. Additionally, the handmade glass apparatus, with its capacity and high contact surface (2.5 cm diameter), not only allows for mass rearing but also permits a high number of insects to be exposed to the same physiological conditions. The glass feeder is easily maintainable and cost-effective, with an estimated cost of 10-12 USD for the apparatus and approximately 8 USD for 4 meters of cattle gut membrane, providing sufficient material for 80 feedings. It is worth noting that no blood spills or membrane ruptures were recorded during the experiments.

ACKNOWLEDGMENTS

This study was supported by grants from CONICET (PIP 2021-584), Agencia Nacional de Promoción Científica y Técnica, Argentina (PICT N° 2018-1545). We also want to thank Ms. Minardi G. for the statistical analyses.

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