

## THE EFFECT OF LARVAL AND ADULT NUTRITION ON SURVIVAL AND FECUNDITY OF DENGUE VECTOR *Aedes albopictus* SKUSE (DIPTERA: CULICIDAE)

By

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### Abstract

The effect of larval and adult nutrition on survival and fecundity of the dengue vector *Aedes albopictus* Skuse (Diptera: Culicidae) were studied under laboratory conditions, the energy for the physiological activities for both male and female mosquitoes is provided largely by their reserves during larval stage and affected by adult diets. Two groups of larvae (A, B) were reared at 27±3°C, 70-80% R.H. and DL. 12: 12. Group (A) with 200 larvae (high larval diet) and Group (B) with 600 larvae (low larval diet). *Ae. albopictus* exhibited increased fecundity and egg hatch success. Immature development was quick. Immature survival was high, with lowest rate in the pupal stage. Highest longevity was observed in large females fed water + 10% sucrose solution (29.571±0.415 days) while the lowest one was (1.3±0.132 days) in starved small females. Large females have significantly (P< 0.001) higher fecundity than smaller females, regardless of whether the females were provided 10% sucrose solution or not (524±0.203 eggs/group 159.714±0.1997 eggs/group), respectively. The addition of 10% sucrose solution significantly (P< 0.001) increase the fecundity regardless of whether large or small females (657.9±0.2198 eggs/group, 242.429±0.119 eggs/group), respectively.

**Key words:** Nutrition, Longevity, Fecundity, *Aedes albopictus*

### Introduction

*Aedes albopictus* is a daytime biting mosquito that transmits a variety of viruses. It has also been reported that it is capable of ovarian transmission, but in most *Aedes* vectors, the general route that transmits arboviruses to humans is through a female bite. Upon feeding on an infected host, the ingested virus needs a certain period of time to enter the midgut epithelium, replicate in it and after a while its viral particles are transferred to the salivary glands via the haemolymph. In dengue virus particles reach the salivary glands 10 days after an infectious blood meal is ingested. In case of the female does not live long enough, the virus will not complete development and cannot be transferred to a new host. Thus, the longevity of female is crucial factor for vector competence. Many factors come into play when the vectorial competence of a mosquito is considered. The ability to be a competent vector depends on various physical and physiological history. In

particular, stage specific survivorship, fecundity and abundance are critical to disease transmission. Foster (1995) studied the mosquito sugar feeding and reproductive energetic. Straif and Beier (1996) studied the effects of sugar availability on the blood-feeding behavior of *Anopheles gambiae* (Diptera: Culicidae). Gary and Foster (2001) studied the effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector *Anopheles gambiae* and reported that most mosquito species are known to use sugar as a necessary source of energy. Neto and Navarro-Silva (2004) studied development, longevity, gonotrophic cycle and oviposition of *Ae. albopictus* Skuse (Diptera: Culicidae) under cyclic temperature. Hashim *et al.* (2008) discussed the life table of immature *Ae. albopictus* Skuse (Diptera: Culicidae) During the wet and dry seasons in Penang, Malaysia. Aida *et al.* (2011) reported the biology and demographic parameters of *Aedes albopictus* in northern peninsular Malaysia and observed that

natural *Ae. albopictus* populations in Penang seem largely determined by quick development in combination with low immature loss and increased oviposition. Sugar feeding is a fundamental characteristic of mosquito life. Most evidence indicates frequent ingestion by both sexes and all ages of mosquitoes of sugar. Energetically, sugar and blood are interchangeable; females must need blood to develop eggs and sugar to survive, to fly seeking out their hosts and to enhance reproduction. Mosquitoes commitment to sugar is further illustrated by a wealth of behavioral, structural, and specializations for finding, feeding on, and processing it. Blood and sugar feeding activities are antagonistic and mutually exclusive, owing to conflicting demands, yet they support the same goals and often share the same activity period. The rules by which females make food-choice decisions have been inadequately explored. Sugar is the basic food of adult mosquitoes. It is the only nutrient consumed by males and probably the more common one for females, even if they need vertebrate blood to produce eggs. Therefore, our goal is generate the influence of larval and adult nutrition and also the important role of sugar on survival and fecundity of *Ae. albopictus* under laboratory conditions.

### Materials and Methods

*Ae. albopictus* mosquitoes were provided from the colonies already maintained for more than 15 generations in Zoo-Morphology, Cell biology and Parasitology Institute, Heinrich-Heine-Universitat, Düsseldorf, Germany. Two groups **A** & **B** of newly hatched *Ae. albopictus* larvae were reared under laboratory conditions of  $27\pm 3^{\circ}\text{C}$ , 70-80% R.H. and DL. 12: 12. GA with 200 larvae (High larval diet), GB with 600 larvae (Low larval diet) were reared in enamel pan (20cm in diameter) and the larvae were offered Tetramin as food until pupation. Similar food quantities were offered daily to group A, B. Newly formed pupae from each group were collected singly in specimen tubes (3.5x8cm). The effect of different

adult diets on survival and fecundity was carried out by using 9 groups of 20 newly emerged virgin males and females from individual separated pupae. The diets offered to the mosquitoes were: no food, Water, 10% sucrose solution, Water + 10% sucrose solution, Water + blood, 10% sucrose solution + blood, and 10% sucrose solution + water + blood. For blood feeding a Guinea pig was presented 3 times a week and the females were placed in a screened wooden cages provided with distilled water for oviposition. Dead females as well as those groups which could not lay eggs, were collected, dissected using binocular microscope in order to determine the degree of their ovarian development. Daily mortality males and females were recorded. Deposited eggs were collected and counted daily. All experiments were repeated at least three times.

Statistical analysis: mean life-span for males and females and mean fecundity ( $\pm$  SE) of reported larval and adult diets were compared using one-way ANOVA followed by multiple pair-wise comparisons by LSD test. Statistical analysis was done using SPSS software (Version 14.0 for Windows evaluation version).

### Results

Results showed that females fed on water, 10% sucrose solution, 10% sucrose solution + blood and water + 10% sucrose solution failed to deposit eggs. Dissection of these females revealed undeveloped ovaries except in case of females fed blood + 10% sucrose solution revealed developed ovaries but they failed to deposit eggs due to the absence of water as oviposition substrate. In case of water + blood and water + blood + 10% sucrose solution fed females succeeded to develop and lay eggs.

The mean life-span of the large females emerging from high larval diet when supplied with water only was  $5.615\pm 0.437$  days; meanwhile addition of blood prolonged the life span being  $8.368\pm 0.746$  days. The presence of 10% sucrose solution alone prolonged significantly ( $P < 0.001$ ) the life span

of both large and small females being  $13.429 \pm 0.249$  days and  $10.429 \pm 0.249$  days, respectively. Furthermore, the addition of blood to 10% sucrose solution increase significantly ( $P < 0.001$ ) the life span of large females being  $24.714 \pm 0.32$  days however there was an insignificant increase in life span of small females being  $9.714 \pm 0.32$  days, meanwhile the addition of water to sugar and blood resulted in a significant ( $P < 0.001$ ) increase in the life span of both large females being  $27.9 \pm 0.252$  days and small females being  $14.6 \pm 0.289$  days (Tab. 1).

The highest value of longevity recorded ( $29.571 \pm 0.415$  days) was when large females were fed 10% sucrose solution + water + blood while the lowest value recorded was ( $2.47 \pm 0.115$  days) in case of starved small females. The longevity of starved large males was  $1.3 \pm 0.132$  days while in case of small males it was ( $2.473 \pm 0.0667$  days).

Table (2a,b) showed the statistical analysis by multiple pair-wise comparisons by LSD test for both experimental groups, for GA high larval diet ( $d.f.=7.16$ ,  $F=23958.447$ ,  $P=0.000$ ) for males, ( $d.f.=7.16$ ,  $F=688174$ ,  $P=0.000$ ) for females, ( $d.f.=1.4$ ,  $F=79600.5$ ,  $P=0.000$ ) for fecundity meanwhile for group (B) low larval diet ( $d.f.=7.16$ ,  $F=31274.023$ ,  $P=0.000$ ) for males, ( $d.f.=7.16$ ,  $F=88786.508$ ,  $P=0.000$ ) for females and ( $d.f.=1.4$ ,  $F=12801.8$ ,  $P=0.000$ ) for fecundity, longevity of females fed water only increased significantly ( $P < 0.001$ ) by the addition of 10% sucrose solution, while by the addition of blood a non significantly decrease ( $P=0.92$ ) was observed.

Results also showed that the larval diet affect significantly ( $P < 0.001$ ) the adult longevity. Despite fed similar adult diets small adults from (low larval diet) whether females or males survive much significantly ( $P < 0.001$ ) less than others large ones (high larval diet). The longevity value of both

large and small females reared solitary and offered 10% sucrose solution recorded  $12.125 \pm 0.177$  days and  $9.333 \pm 0.927$  days, respectively, showing a significant difference ( $P < 0.05$ ) than when reared with males ( $13.429 \pm 0.249$  days &  $10.429 \pm 0.249$  days). Longevity recorded for solitary large and small males offered 10% sucrose solution was ( $11.71 \pm 0.191$  days,  $8.82 \pm 0.133$  days), respectively. The longevity decreased significantly ( $P = 0.014$ ) when reared with females recording ( $10.2 \pm 0.917$  days,  $8.7 \pm 0.917$  days), respectively.

The addition of blood to 10% sucrose solution did not significantly ( $P = 0.652$ ) increase the longevity in case of large and small males while significantly ( $P < 0.001$ ) increase the longevity of both large males and females. Multiple pair-wise comparisons by LSD test between the means longevity of large and small adults showed that the larval diet affect highly significantly ( $P < 0.001$ ) longevity of females and males regardless the type of adult diet except in some cases of the adult males not affected by the larval diets when fed some types of adult diets, when large males offered 10% sucrose solution alone have longevity not significantly ( $P = 0.652$ ) higher than that of small ones and the longevity of small males when reared solitary and fed 10% sucrose solution not significantly ( $P = 0.065$ ) differed from that of the large ones.

Females fed blood only are able to develop eggs in both experimental groups (A&B), but they were not able to lay eggs, due to the absence of water as oviposition substrate.

In presence of water of large females showed a high fecundity ( $524 \pm 0.203$  egg/group) compared to small ones ( $159.714 \pm 0.199$  egg/group). Moreover the addition of 10% sucrose solution increase significantly ( $P < 0.001$ ) the fecundity being  $657.9 \pm 0.219$  eggs/group for large females and  $242.429 \pm 0.119$  eggs/group for small ones.

Table 1: Effect of nutrition on survival and fecundity of *Ae. albopictus* (Skuse) males and females reared on high larval diet (200 larvae) and low larval diet (600 larvae) under laboratory conditions of 27±3°C, 70-80% R.H. and D: L (12:12).

Adult Nutrition	High Larval Diet (200 larvae) <sup>b</sup>			Low Larval Diet (600 larvae) <sup>b</sup>		
	Mean life-span <sup>a</sup>		Total No. of eggs / group <sup>a</sup>	Mean life-span <sup>a</sup>		Total No. of eggs / group <sup>a</sup>
	♂	♀		♂	♀	
Starved	1.9±0.233 <sup>A</sup>	2.955±0.0591 <sup>A</sup>	-	1.3±0.132 <sup>A</sup>	2.47±0.115 <sup>A</sup>	-
Water	4.615±0.241 <sup>B</sup>	5.615±0.437 <sup>B</sup>	-	3.167±0.171 <sup>B</sup>	4.11±0.103 <sup>B</sup>	-
10% sucrose	10.2±0.917 <sup>C</sup>	13.429±0.249 <sup>C</sup>	-	8.7±0.917 <sup>C</sup>	10.429±0.249 <sup>C</sup>	-
10% sucrose (♀♀ only)	-	12.125±0.177 <sup>D</sup>	-	-	9.333±0.027 <sup>D</sup>	-
10% sucrose (♂♂ only)	11.71±0.191 <sup>D</sup>	-	-	8.82±0.133 <sup>C+</sup>	-	-
Water + 10% sucrose	18.75±0.255 <sup>E</sup>	29.571±0.415 <sup>E</sup>	-	11.75±0.255 <sup>D</sup>	17.571 ± 0.415 <sup>E</sup>	-
Water + blood	4.976±0.435 <sup>B</sup>	8.368±0.746 <sup>F</sup>	524± 0.203 <sup>A</sup>	2.8±0.435 <sup>E</sup>	5.368 ± 0.746 <sup>F</sup>	159.714 ± 0.199 <sup>A</sup>
10% sucrose + blood	10.27±0.108 <sup>C</sup>	24.714±0.32 <sup>G</sup>	-	8.22±0.108 <sup>C</sup>	9.714±0.32 <sup>G</sup>	-
10% sucrose + Water + blood	19.077±0.164 <sup>E</sup>	27.9±0.252 <sup>H</sup>	657.9± 0.219 <sup>B</sup>	11.7±0.648 <sup>D</sup>	14.6±0.289 <sup>H</sup>	242.429 ± 0.119 <sup>B</sup>
<i>d. f.</i>	7.16	7.16	1.4	7.16	7.16	1.4
<i>F</i>	23958.447	688174	79600.5	31274.023	88786.508	12801.8
<i>P</i>	0.00	0.00	0.00	0.00	0.00	0.00

a. Means life-span for females and males from high and low larval diets with different letters were significantly different ( $P < 0.0001$ ) among groups fed a variety of adult diets according to ANOVA, multiple pair-wise comparisons by LSD test,

b. Number of replicates=3

## Discussion

The energy of the physiological activities for both male and female mosquitoes is provided by feeding sugar, the progress on the sugar-feeding behavior of mosquitoes and application in vector-borne disease control was reported by (Jiang *et al.*, 2012). The nutritional environment experienced by larvae strongly influences female fitness-related traits such as body size, general metabolic reserves and fecundity for both anautogenous and autogenous mosquitoes (Briegel, 1990a; Briegel, 1990b; Telang and Wells, 2004; Reyes and Villanueva, 2004). Although *Ae. albopictus* females require a blood meal in order to mature eggs, fecundi-

ty for the first ovarian cycle is determined by reserves derived from larval nourishment. High-reserve female *Ae. albo-pictus* produced significantly more eggs, after a blood meal, than blood-fed low-reserve females.

The results demonstrated that the energy reserves accumulated in adult females *Ae. albopictus* vary according to the type of food they imbibe, and their nutrient reserves at emergence (indicated by body size). Moreover, it appeared that adults *Ae. albopictus* can be kept alive and healthy on a diet consisting solely of 10% sucrose solution, as sucrose provides energy for maintenance and all activities, including flight. These results were consistent with those of Van

Handel (1965). Nayer and Van Handel (1971) during the laboratory studies on *Ae. taeniorhynchus* and *Ae. sollicitans* showed that glycogen and triglycerides are synthesized, from a mixture of glucose and fructose 1:1; furthermore, they mentioned that sugar and glycogen are utilized for flight activity while triglycerides are mostly used for general maintenance functions such as respiration, excretion, digestion. If maximal longevity is to be achieved in the case of female mosquitoes, triglycerides are the respiratory substrate, and they are synthesized from sucrose. Heavier mosquito females survive longer and have a higher blood feeding success rate than lighter ones. Similar results were reported by Nasci (1986) for *Ae. aegypti* females. Thus the increased parity rates in large female mosquitoes may be attributed to the increased survival rate of these females.

The reduction in the mean life-span of females of all examined *Ae. albopictus*, fed both sugar and blood as compared to those fed on sugar only might be due to the fact that the amounts and rate of glycogen and triglycerides synthesized from sucrose only was more than that synthesized from sucrose and blood. These results were in agreement to those of Liles and Delong (1960) in case of *Ae. aegypti* and Nayar and Sauerman (1971) for *Ae. taeniorhynchus* and with those of Gary and Foster (2001) for *An. gambiae*, they described that females (pooled and individual) given blood + sugar lived significantly longer than did those were given access to either no food (water only), 10% sucrose, human blood. Females given blood alone reduced survivorship.

These data indicate that female *An. gambiae* could replace sugar with increased blood feeding without suppressing the reproductive fitness. The increased blood feeding could, in turn, increase the rate of malaria transmission and may explain the unusual efficiency of this vector, but differ from that of Lavoipierre (1961) for *Ae. aegypti* and, *C. p. queenslandensis*. Van Han-

del (1965) showed that the rate of triglycerides synthesis is similar, whether sugar or a calorically equivalent amount of protein is fed, but glycogen is synthesized 10 times faster from sugar than from protein. Thus immediately after blood feeding, eggs start to mature, suggesting that most of the energy and protein from blood feeding is diverted to egg maturation, where it is trapped; only a small fraction of the energy is available for flight and general maintenance, probably minimal for survival, so the adult die soon. On the other hand, when feeding on sugar, most of the energy would be expended on general maintenance and flight. In sugar and blood-fed groups, most of the energy for maintenance is provided by sugar with the result that the survival is prolonged.

Survival of starved adults after emergence is dependent on the amount of energy reserves carried over from the larval stage; this was clearly evident from the data presented on survival however, when food is provided for other adults, survival is controlled by the type of diet available. Both **large** and small females supplied with water + blood were able to lay eggs, while the addition of 10% sucrose solution increases the ability and strength to produce eggs, even though it greatly increases the mean life-span. These results were consistent with that of Nayar and Sauerman (1971) for *Ae. taeniorhynchus*, they observed that females with sucrose supplementing blood produced 26% more eggs but increase the mean life-span more than 3 times that of females fed blood alone.

In the present study, it appeared that since survival and fecundity are interrelated phenomena, it appears that diet composed of blood and sucrose, water and sucrose, sucrose only sugar, water and blood, the sucrose supplied the majority of energy for general maintenance, where as blood is used almost exclusively for the production of eggs.

These results are quite comparable to those of Provost (1957) for *Ae. taeniorhynchus*

when observed in nature. He claimed that this occurs in nature under the pressures of different ecological factors, and so can be attributed to them, but if the same phenomenon occurs under the controlled conditions, as in the present study then it is apparently due to the ageing process accompanied by blood and sugar feeding, egg laying and survival of females of all examined mosquito. Straif and Beier (1996) observed that mosquitoes provided with sugar lived on average almost 3 d longer than females without sugar (19.0 vs. 16.2 d). After stratification by age, mosquitoes in the youngest (5-12 d) and middle (13-19 d) age strata showed no differences in blood-feeding patterns relative to the sugar availability.

However, the mosquitoes from the oldest age group and no access to sugar had more total blood feeds than the long-lived females (>or=20 d) with access to sugar (9.8 versus 6.5).

Furthermore, mosquitoes > or =20 d old and without sugar available had a higher blood-feeding frequency than females that

had sugar available (0.36 versus 0.25 blood meals per female per day). The enhanced blood-feeding capability among older sugar-deprived *An. gambiae* emphasized the close association between sugar-feeding and blood-feeding behavior and the potential consequences for transmission of malaria parasites and other pathogens. While these results were on the contrary with those of Gary and Foster (2001) for *An. gambiae*, observed that the daily fecundity was higher for females given blood alone than for those fed blood + sugar (13 versus 9 eggs per female daily). However, total fecundity and intrinsic rate of increase were not affected by sugar availability.

### Conclusion

It can be concluded that the high larval diet lead to large females having the highest longevity and highest fecundity, the combination of blood and 10% sucrose solution lead to the highest fecundity and the addition of 10% sucrose solution to water lead to the highest value of longevity ( $P < 0.001$ ).

Table 2a: Multiple pair-wise Comparisons by LSD test

Larval Diet	Adult Females Diet/ Adult Females Diet	P							
		Starved	Water	10% sucrose	10% sucrose (♀♀ only)	Water + 10% sucrose	Water + blood	10% sucrose + blood	10% sucrose + Water + blood
High Larval Diet	Starved		0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water	0.000		0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose	0.000	0.000		0.000	0.000	0.000	0.000	0.000
	10% sucrose (♀♀ Solitary)	0.000	0.000	0.000		0.000	0.000	0.000	0.000
	Water + 10% sucrose	0.000	0.000	0.000	0.000		0.000	0.000	0.000
	Water + blood	0.000	0.000	0.000	0.000	0.000		0.000	0.000
	10% sucrose + blood	0.000	0.000	0.000	0.000	0.000	0.000		0.000
	10% sucrose + Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
High Larval Diet	Males Diet/ Males Diet	Starved	Water	10% sucrose	10% sucrose (♂♂ only)	Water + 10% sucrose	Water + blood	10% sucrose + blood	10% sucrose + Water + blood
	Starved		0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water	0.000		0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose	0.000	0.000		0.000	0.000	0.000	0.652	0.000
	10% sucrose (♂♂ only)	0.000	0.000	0.000		0.000	0.000	0.000	0.000
	Water + 10% sucrose	0.000	0.000	0.000	0.000		0.000	0.000	0.000
	Water + blood	0.000	0.000	0.000	0.000	0.000		0.000	0.000

	10% sucrose + blood	0.000	0.000	0.652	0.000	0.000	0.000		0.000
	10% sucrose + Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Larva-Diet	Females Diet/ Females Diet	<i>P</i>							
		Starved	Water	10% sucrose	10% sucrose (♀♀ only)	Water + 10% sucrose	Water + blood	10% sucrose + blood	10% sucrose + Water + blood
Low Larval Diet	Starved		0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water	0.000		0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose	0.000	0.000		0.000	0.000	0.000	0.000	0.000
	10% sucrose (♀♀ Solitary)	0.000	0.000	0.000		0.000	0.000	0.000	0.000
	Water + 10% sucrose	0.000	0.000	0.000	0.000		0.000	0.000	0.000
	Water + blood	0.000	0.000	0.000	0.000	0.000		0.000	0.000
	10% sucrose + blood	0.000	0.000	0.000	0.000	0.000	0.000		0.000
	10% sucrose + Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Low Larval Diet	Males Diet/ Males Diet	Starved	Water	10% sucrose	10% sucrose (♂♂ only)	Water + 10% sucrose	Water + blood	10% sucrose + blood	10% sucrose + Water + blood
	Starved		0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water	0.000		0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose	0.000	0.000		0.014	0.000	0.000	0.652	0.000
	10% sucrose (♂♂ only)	0.000	0.000	0.014		0.000	0.000	0.000	0.000
	Water + 10% sucrose	0.000	0.000	0.000	0.000		0.000	0.000	0.92
	Water + blood	0.000	0.000	0.000	0.000	0.000		0.000	0.000
	10% sucrose + blood	0.000	0.000	0.652	0.000	0.000	0.000		0.000
10% sucrose + Water + blood	0.000	0.000	0.000	0.000	0.92	0.000	0.000		

Table 2b: Multiple pair-wise Comparisons by LSD test

Larval Diet	Females Diet/ Low Larval Diet	<i>P</i>							
		Starved	Water	10% sucrose	10% sucrose (♀♀ only)	Water + 10% sucrose	Water + blood	10% sucrose + blood	10% sucrose + Water + blood
High Larval Diet	Starved	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose (♀♀ Solitary)	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water + 10% sucrose	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	10% sucrose + Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.0000
High Larval Diet	Males Diet/ Low Larval Diet	Starved	Water	10% sucrose	10% sucrose (♂♂ only)	Water + 10% sucrose	Water + blood	10% sucrose + blood	10% sucrose + Water + blood
	Starved	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Water	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10% sucrose	0.000	0.000	0.000	0.000	0.065	0.000	0.000	0.652	0.000
10% sucrose (♂ only)	0.000	0.000	0.000	0.000	0.000	0.833	0.000	0.000	0.779
Water + 10% sucrose	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.944
Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10% sucrose + blood	0.000	0.000	0.575	0.000	0.000	0.000	0.000	0.000	0.000
10% sucrose + Water + blood	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

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