Optimization of the Feeding rate of *Anopheles Farauti* Colony Mosquitoes in Direct Membrane Feeding Assays

Lincoln Timinao (lincoln.timinao@my.jcu.edu.au)
Papua New Guinea Institute of Medical Research https://orcid.org/0000-0001-8295-8316
Rebecca Vinit
Papua New Guinea Institute of Medical Research
Michelle Katusele
Papua New Guinea Institute of Medical Research
Thomas R Burkot
Australian Institute of Tropical Health and Medicine, James Cook University
Louis Schofield
Australian Institute of Tropical Health and Medicine, James Cook University
Stephan Karl
Australian Institute of Tropical Health and Medicine, James Cook University

Research

Keywords: Direct membrane feeding assay, Anopheles farauti, Papua New Guinea

Posted Date: March 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-312197/v1

Version of Record: A version of this preprint was published at Parasites & Vectors on July 7th, 2021. See the published version at https://doi.org/10.1186/s13071-021-04842-y.

Abstract

Background: Direct membrane feeding assays (DMFA) are an important tool to study parasite transmission to mosquitoes. Mosquito feeding rates in these artificial systems require optimization as there are a number of factors that potentially influence the feeding rates and there are no standardized methods that apply to all Anopheline species.

Methods: A range of parameters prior to and during direct membrane feeding (DMF) were evaluated for their impact on *Anopheles farauti* feeding rates; including the starving conditions and duration of starving prior to feeding, membrane type, DMF exposure time, mosquito age, illumination level, blood volume, mosquito density and temperature of water bath.

Results: The average successful DMFA feeding rate for *An. farauti* colony mosquitoes increased from 50 % to 85 % when assay parameters were varied. Overnight starvation and Baudruche membrane yielded the highest feeding rates but rates were also affected by blood volume in the feeder and the mosquito density in the feeding cups. Availability of water during the pre-feed starvation period did not significantly impact feeding rates, nor did the exposure duration to blood in membrane feeders nor, the age of mosquitoes (3, 5 and 7 days post-emergence), illumination during feeding or the temperature (34 °C, 38 °C, 42 °C and 46 °C) of the water bath.

Conclusion: Optimal feeding conditions in *An. farauti* DMFA were to offer 50 female mosquitoes in a cup (with a total surface area of ~340 cm^2 with 1 mosquito / 6.8 cm^2) that were starved overnight 350-500 µL of blood (collected in heparin coated vacutainers) per feeder in feeders with a surface area ~5 cm^2 (with a maximum capacity of 1.5 mL of blood) via a Baudruche membrane, for at least 10-20 min.

Introduction

Transmission from humans to mosquitoes is a vital step in the malaria parasite life cycle. Transmission through the mosquito vector represents a bottleneck where parasite populations shrink from millions in the human body to as few as one in the mosquito [1]. Thus, malaria transmission is vulnerable to interruption when transiting from the human to the mosquito host [2]. This transition can be studied by direct membrane feeding assays (DMFA). During DMFA, mosquitoes feed through a membrane on blood kept warm via water-jacketed glass feeders [3] including blood harvested from humans with circulating malaria parasites, [4] to either study the parasites development in the mosquito [4] or to test interventions that disrupt their development hence, interrupting transmission. [5]

The mosquito blood feeding rate, i.e., the proportion of mosquitoes that successfully ingest blood is an important determinant of overall infection success. The success rate of ingesting blood from a membrane feeder can vary depending on the mosquito species, whether the mosquitoes were collected in the wild [6] or reared in a colony [7], as well as the level of adaptation of the colony.

Blood feeding rates also depend on the experimental conditions under which the DMFAs are conducted including i) the duration of starvation before exposure, ii) the starving conditions (access to water or no access to water), iii) the type of membrane used, iv) the amount of time mosquitoes are allowed to feed v) the mosquito age, vi) illumination level, vii) the blood volume in the feeder, viii) the density of mosquitoes attempting to feed and ix) water bath temperature during DMFA (Fig. 1).

However, membrane feeding studies have been conducted with a range of conditions and with varied feeding success [8, 9]. Thus, there is a need to optimize DMFA conditions for each colony mosquito species.

Starving conditions is a key component that greatly impacts mosquito feeding rates and a balance needs to be established between starving the mosquitoes for too long, thereby increasing mosquito mortality or affecting their fitness [10], and not starving for long enough so mosquitoes only partly feed or not at all.

Most studies describe dry starving for durations from 5–36 h [11–14] while other studies performed starving where the mosquitoes had access to water for 12 h [15, 16]. A study conducted by Coulibaly and colleagues compared the feeding rates of mosquitoes dry starved 8 h, 14 h and 20 h and concluded that mosquitoes starved 8–14 h yielded significantly higher feeding rates than mosquitoes starved 20 h. [10] However, most studies did not directly report the impact of starving on the feeding rate.

Membranes take the role of an artificial skin in the feeding experiments. An ideal membrane will yield the highest feeding rates in the shortest period of time. Parafilm and natural membranes such as Baudruche, sausage casing, chicken skin or rat skin have been used [6, 17]. Natural membranes which closely mimic the skin resulted in the highest feeding rates followed by Baudruche membrane which is derived from bovine cecum and finally Parafilm, a wax synthetic membrane [3]. Most studies reported using Baudruche membrane [8, 18, 19] while others used Parafilm membrane [14, 20]. Interestingly, a study done by Coulibaly and colleagues showed that there was no significant difference between the feeding rates, survival and infection rates from feeding experiments with either Baudruche or Parafilm membranes, for *Anopheles coluzzii* mosquitoes [10].

Mosquitoes 2–8 days post-emergence have been used in different studies [6, 7, 10, 12, 14, 15, 21–23]. The main consideration in this is that mosquitoes are fed at an early age so that they survive for the required duration for either oocysts [8, 9, 14, 24, 25] or sporozoites [8, 24, 25] to develop. Coulibaly and colleagues compared the feeding rate for *An. coluzzii* mosquitoes between 3 days and 9 days post emergence and found that 3 day old mosquitoes had a significantly higher feeding rate compared to 6 and 9 day old mosquitoes [10].

Mosquito density is another factor that may influence the mosquito feeding rate. Rutledge and colleagues observed that having more mosquitoes per cage can result in lower feeding rates [3] and crowding, making handling, especially removing of unfed mosquitoes, difficult. Vallejo and colleagues observed that 100 *An. coluzzii* mosquitoes per cage (or 1 mosquito per 5 cm^2) resulted in the highest *P.*

vivax infection prevalence after DMFA [25]. However, the study did not report on the feeding rate of the different mosquito densities in relation to infection success.

Not much has been reported also with respect to the impact of the other parameters listed above on the feeding rates. Much of the focus is on the infection rates. As such the focus of this study was to determine the optimal feeding conditions for *Anopheles farauti s.s* colony mosquitoes in order to maximise the feeding rate during the DMFA.

Methods

Mosquito colony maintenance

The *An. farauti* mosquito colony were taken from a colony established in 1968 from Rabaul, East New Britain province. [26] The laboratory colony was maintained at 28 ± 8 ºC and 68 ± 25 % relative humidity. The light cycle is approximately 11 h dark and 12 h light including a 30 min dusk and 30 min dawn period. The larvae were fed ground fish food (Marine Master, Tropical Fish Flake) while the adults were provided 10 % sucrose (Ramu Sugar) solution available as soaked cotton wool balls placed on top of the mosquito cages. Currently to maintain the colony, uninfected blood (no malaria) was obtained from donors following informed consent.

Direct Membrane Feeding Assays

Water-jacketed glass membrane feeders were connected in series by rubber hoses to a mini aquarium pump placed inside a 37–38 ºC water bath (Fig. 2). Unless otherwise stated, all trials used an average of 5 day old mosquitoes with 50 female *An farauti* were placed in a cup (surface area of ~ 340 cm^2 with a total volume of ~ 476 cm^3) and offered blood meals from water jacketed direct membrane feeders with a diameter of 2.5 cm (a surface area of ~ 5 cm^2 with a maximum blood volume capacity of 1 mL of blood). The duration of overnight starving ranged between 18–21 h.

The experiments were done sequentially with a single parameter being varied and tested, incorporating the optimal conditions of the preceding tests. Following the feeding experiments, unfed mosquitoes were separated from the fully fed mosquitoes and the feeding rate calculated. The Wilcoxon matched-pairs signed rank test tested the significance of the difference observed between the reference and the test arms. A flow chart summarizing the parameters tested is provided in the Supplementary section Table S1.

Starving time

An. farauti were dry starved (no access to sugar or water) for 2 h, 4 h, 6 h, and overnight (~ 21 h) and compared to a control of mosquitoes exposed without starving. Mosquitoes were exposed to ~ 750 µL of blood for 30 min in the dark (with a black piece of blanket draped over the membrane feeder) following starvation. Parafilm membrane (cut into 3 cm x 3 cm and stretched to ~ 5 cm x ~ 5 cm) was used to feed

the mosquitoes. The parafilm was not exposed to human odor prior to feeding. Ten replicates were performed.

Type of starving; access to water versus dry (no access to water)

An. farauti mosquitoes were starved overnight with one cup of mosquitoes having access to cotton soaked in water while the other did not have access to water (dry starved). The mosquitoes were then given access to ~ 750 µL of blood using a Parafilm membrane for 30 min in the dark. Five replicates were performed.

Membrane type

Two membrane types were tested, namely Parafilm and Baudruche membranes. The Parafilm membrane was standardized by cutting it into 3 cm x 3 cm pieces and stretching to ~ 5 cm x ~ 5 cm. Mosquitoes were dry starved overnight. The mosquitoes were given access to ~ 750 µL of blood in a membrane feeder for 30 min in the dark. Eight replicate experiments were performed.

Exposure time

Exposure times of 10 min, 20 min and 30 min were evaluated following overnight starving and the mosquitoes were then exposed to ~ 750 µL of blood for the specified time. Baudruche membrane was used to feed the mosquitoes. Eight replicate experiments were performed.

Mosquito age

Mosquitoes aged 3, 5 and 7 days were tested in nine replicates. The mosquitoes were starved overnight before exposure to ~ 750 µL of blood via a Baudruch membrane for 20 min in the dark.

Illumination level

A total of 2 cups of 5 days old mosquitoes were prepared and starved overnight. One cup of mosquitoes was fed with the net top exposed to ambient room lighting while the second cup had a black blanket placed over it while they were exposed to ~ 750 µL of blood via Baudruche membrane for 20 min. Seven replicate experiments were performed.

Volume of blood

The following blood volumes were tested to determine the minimum blood volume, which could yield high feeding rates; 125 µL, 250 µL and 500 µL in a water-jacketed glass feeder of 1 ml maximum capacity. Three cups of 6 day old (mean age) *An. farauti* were prepared, starved overnight and allowed 20 min to feed in the dark at each blood volume via a Baudruche membrane. Six replicate experiments were performed.

Mosquito Density

Three different mosquito numbers 20, 50 and 100 per cup were tested. A mean of 4 day old mosquitoes were dry-starved overnight. The mosquitoes were allowed to feed on ~ 500 µL of blood for 20 min under illuminated conditions via a Baudruche membrane. Six replicates were performed for this test.

Water Temperature

Four different water bath temperatures were tested 34°C, 38°C, 42°C and 46°C. Mosquitoes were dry-starved overnight before allowed to feed on ~ 500 µL of blood for 20 min under illuminated conditions via a Baudruche membrane. Seven replicates were performed for this test.

Results

The volunteers who donated blood for membrane feeding were adults with a median age of 46 with the range of 28–52 and had a median hemoglobin level of 15.5 g/dl with the range of 11.1–17.7 g/dl. The median room temperature was 28.05 □C with a range of 22.2–30.34 □C with a relative humidity of 78.38 % in the range of 56-89.3 %. The base line parameters to which subsequent tests were compared were: 50 female *An. farauti* per cup, aged between 3–5 days, dry starved between 0–4 h, and fed in the dark using Parafilm as the membrane for ~ 30 min, with 750 µL of blood at a water bath temperature of ~ 38°C.

Table 1 summarizes the results of the analysis for the starving duration, type of starving, membrane type, feeding duration, mosquito age, illumination, volume of blood, mosquito density and water bath temperature.

Table 1
Mosquito feeding rate according the feeding parameters being tested.

Feeding Parameters		Total number of mosquitoes in cups	Total number fed	Total unfed	*Empirical average feeding rate (%)	Range (%)	**Adjusted mixed model feeding P value
Starving time	0 h	916	240	676	27	4–45	< 0.01
	2 h	864	288	576	34	15–56	< 0.01
	4 h	933	368	565	40	20–54	< 0.05
	6 h	954	405	549	45	23–82	< 0.01
	Over Night (~ 21 h)	841	492	349	60	19–97	Ref
Type of starving	Access to water	217	134	83	62	43–89	0.44
	Dry starving	234	167	67	71	31–92	Ref
Membrane type	Baudruche	476	389	87	85	70–100	< 0.01
	Parafilm	457	268	189	53	42–76	Ref
Exposure time	10 min	426	326	100	77	63–98	0.44
	20 min	469	368	101	80	43–100	0.53
	30 min	386	314	72	81	72–89	Ref
Mosquito Age	3 days old	631	447	184	75	39–96	0.41

Ref: reference group for the calculation of the *P* values.

*Empirical averages were calculated as the average feeding rate of all replicates obtained for a specific condition.

**Significantly different from the reference when $p < 0.05$ by Wilcoxon matched-pairs signed rank test.

Significant differences among starvation duration were observed compared to overnight starving (21 h, $p < 0.05$) (Fig. 3) with a 33 % increase in feeding rate from 0 h (27 %) to ~ 21 h (60 %). There was no significant difference observed between the type of starving, whether the mosquitoes were dry starved or allowed to feed on water during the starving period ($p = 0.44$).

Feeding Parameters		Total number of mosquitoes in cups	Total number fed	Total unfed	*Empirical average feeding rate (%)	Range (%)	**Adjusted mixed model feeding P value
	5 days old	606	473	133	81	50–93	Ref
	7 days old	613	449	164	75	55–90	0.14
Illumination	Light	317	274	43	85	64–96	0.88
	Dark	326	276	50	84	63–96	Ref
Volume of blood	125 uL	289	190	99	65	50–88	< 0.05
	250 uL	272	229	43	84	67–96	0.69
	500 uL	295	256	39	87	77–98	Ref
Mosquito density	20 mosquitoes	138	99	39	72	53–90	< 0.05
	50 mosquitoes	335	289	46	86	79–91	Ref
	100 mosquitoes	635	460	175	72	61–81	< 0.05
Water bath temperature	34°C	330	271	59	82	51–95	0.23
	38°C	335	299	36	89	81–100	Ref
	42°C	320	277	43	86	72–98	0.61

Ref: reference group for the calculation of the *P* values.

*Empirical averages were calculated as the average feeding rate of all replicates obtained for a specific condition.

**Significantly different from the reference when $p < 0.05$ by Wilcoxon matched-pairs signed rank test.

Significant differences among starvation duration were observed compared to overnight starving (21 h, $p < 0.05$) (Fig. 3) with a 33 % increase in feeding rate from 0 h (27 %) to ~ 21 h (60 %). There was no significant difference observed between the type of starving, whether the mosquitoes were dry starved or allowed to feed on water during the starving period ($p = 0.44$).

Feeding Parameters	Total number of mosquitoes in cups	Total number fed	Total unfed	*Empirical average feeding rate (%)	Range (%)	**Adjusted mixed model feeding P value
46°C	305	244	61	79	58–96	0.09
Ref: reference group for the calculation of the *P* values.						
*Empirical averages were calculated as the average feeding rate of all replicates obtained for a specific condition.						
**Significantly different from the reference when $p < 0.05$ by Wilcoxon matched-pairs signed rank test.						
Significant differences among starvation duration were observed compared to overnight starving (21 h, $p < 0.05$) (Fig. 3) with a 33 % increase in feeding rate from 0 h (27 %) to ~ 21 h (60 %). There was no significant difference observed between the type of starving, whether the mosquitoes were dry starved or allowed to feed on water during the starving period ($p = 0.44$).						

There was a statistically significant difference between the feeding rates of the two types of membranes tested, Baudruche and Parafilm ($p < 0.01$) (Fig. 4). Feeding rate increased to 85 % when the Baudruche membrane was used.

Exposure times, mosquito age or illumination were not observed to significantly influence membrane feeding rates. However, feeding rates statistically significantly increased when the blood volume was increased from 125 µL to 500 µL ($p < 0.05$) (Fig. 5A). Also, there was a significant difference between the mosquito density of 50 mosquitoes per cup and 20 and 100 mosquitoes per cup ($p < 0.05$) (Fig. 5B) indicating that 50 mosquitoes per cup was closer to the optimum mosquito density. The feeding rate was not significantly different between the water-bath temperatures 34°C ($p = 0.23$), 42°C ($p = 0.61$), 46°C ($p = 0.09$) and 38°C.

The feeding rate increased to 85 % when mosquitoes were dry starved overnight and fed through a Baudruche membrane (Figs. 3 and 4). No further significant increase was noted when the other feeding parameters were tested. Figure 6 illustrates this increase in feeding rate when the optimal feeding conditions were selected. The other selected feeding parameters maintained a high feeding rate with an average of 84 %.

Discussion

In this study, parameters affecting the feeding rate of *An. farauti* colony mosquitoes in DMFAs were investigated to identify the critical parameters to enable high feeding rates. By systematically varying individual parameters sequentially, the baseline feeding rate of ~ 50 % was increased to ~ 85 % ($p < 0.01$).

Two parameters in particular were associated with improved feeding rates: the starving duration prior to membrane feeding and the membrane type used during the feed. Increasing the starving duration to

overnight (~ 21 h) resulted in a statistically significant increase in the feeding rate to 60 %. Overnight starving was used in several previous studies [10, 14, 20, 25, 27] while some studies used a minimum of 5 h of starvation with *Anopheles* mosquito species. [9, 18] Use of Baudruche membrane increased the feeding rate to ~ 85 %. Previous studies have used either Baudruche membrane or Parafilm for performing DMFAs [8, 14, 18–20] with different mosquito species. A study by Coulibaly and colleagues comparing the two membrane types using *An. coluzzii* mosquitoes showed that there was no significant difference between the Baudruche and Parafilm membranes when adjusting for other covariates. [10] The superior performance of Baudruche membrane over Parafilm observed here may be particularly due to the fact that Baudruche membrane is made from a natural organic material. Changing other feeding parameters (e.g., illumination, mosquito age, blood volume, feeding duration and water bath temperature) did not significantly increase the feeding rate further.

However, other feeding parameters contributed towards the economical use of resources. It was observed that feeding mosquitoes for 10 min yielded similar feeding rates as feeding for 20–30 min. This is within the range of feeding times that have been used in different studies, starting from 10 min to 30 min [8, 19, 27, 28]. We chose to use 20 min as it worked well with our feeding schedule. The optimal volume of blood used per glass feeder is important especially when working with limited amounts of infected blood. A volume of 350 µL was recommended for the size (2.5 cm in diameter, surface area of ~ 5 cm^2) of the glass feeders used here [8] while another study used a total of 1.5 mL [18] However, volumes as low as 250 µL to 500 µL yielded similar high feeding rates. We chose to use a volume within the range of 350 µL – 500 µL to make sure that the total membrane area is covered with blood so that we ensure that there is less crowding during feeding.

With respect to mosquito density, it was observed that according to the cup size (surface area of ~ 340 cm^2 with a volume of ~ 476 cm^2) 50 mosquitoes (1 mosquito/6.8 cm^2) feeding on a ~ 5cm^2 membrane surface area yielded high feeding rates while 20 and 100 mosquitoes per cup resulted in significantly lower feeding rates. Using 50 mosquitoes for a similar sized cup has been corroborated by two other published protocols that suggested a mosquito density of 50–100 mosquitoes but recommended that considerations be made to overcrowding with respect to the size of the feeder and the feeding rate when choosing the ideal mosquito numbers per cup. [8, 9]

Although various groups preferred using either of the two types of starving conditions dry or exposing the mosquitoes to water prior to feeding [11–16], we did not observe any significant difference between the two. It may be that *An. farauti* mosquito efficiently excretes the water from its digestive tract when it feeds on blood or that the mosquitoes do not feed on water as efficiently as they would on sugar solution. We opted for dry starving.

We also did not observe any significant difference between feeding the mosquitoes under illuminated conditions or in the dark. However, other studies revealed that the membrane feeding experiments were performed in the dark. [18, 20] Also a protocol by Ouedraogo and colleagues indicated that DMFA should be performed in the dark to mimic the natural feeding conditions which occurs during the night while

membrane feeding experiments are commonly undertaken during the day. [9] Our contrasting observation may be because this mosquito species has been colonized for over 50 years and feeds well regardless of the light condition. Usually in the wild the *An. farauti* mosquito would feed in the evening starting at 6 pm and peak between 10 and 11 pm when it is dark.[29] Here we chose to feed the mosquitoes under illuminated conditions as it is easier to monitor the progress of the mosquitoes feeding.

Furthermore, we did not detect any significant difference between the feeding rates of age groups 3, 5 and 7 day old mosquitoes. This may be due to the mosquito species we were using as Coulibaly and colleagues noted contrasting results where they noted a significant difference in the feeding rates between 3 days and 6–9 day old with *An. coluzzi.* Collectively, studies reported using mosquitoes of ages 2–8 day olds for various mosquito species. [6, 7, 10, 12, 14, 15, 21–23]. Here we chose to work with 2–5 day old mosquitoes to ensure that we achieve high survival rates on day 7 for the dissection for oocysts and day 14 for the dissection for sporozoites. Finally we did not observe any significant difference between the water bath temperatures of 34°C, 38°C, 42°C and 46°C. While studies have indicated using a water bath at 37°C [16, 27], this parameter has not been investigated before. Our results show that mosquitoes are able to feed efficiently regardless of fluctuations in the water bath temperatures. We chose to use a water bath temperature of 37–38°C as it closely resembles the human body temperature and will be most conducive for parasite survival.

Conclusion

By sequentially and systematically varying individual membrane feeding parameters, the blood feeding rate was increased significantly to 85%. This highlights the importance of parameter selection and optimization in direct membrane feeding assays. Further work will need to be performed with infected blood to ensure that these parameters result in high infection rates and survival for *An. farauti* colony mosquitoes using DMFA.

Abbreviations

PNG
Papua New Guinea
DMFA
Direct Membrane Feeding Assay
ON
over night
An. farauit
Anopheles farauti
An. coluzzii
Anopheles coluzzii

Declarations

Ethics approval and consent to participate

This study was approved by the Papua New Guinea Medical Research Advisory Council (MRAC 16.26), and the PNGIMR Institutional Review Board (1516).

Consent for publication

“Not applicable”

Availability of data and material

The raw data pertaining to this manuscript can be obtained from the corresponding author upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding was received.

Authors' contributions

Designed the study: LT, SK; Conducted the laboratory work: LT; Secured funding: SK; Drafting and preparation of the manuscript: LT, SK; Critically revising the manuscript: SK, RV, MK, TB, LS.

Acknowledgements

We would like to thank the following hardworking staff at the Papua New Guinea Institute of Medical Research, Entomology Laboratory who helped in rearing the *An. farauti* colony mosquitoes, particularly Hega Sekel, Siub Yabu and Susie Ibam. We would like to thank Dr. Jetsumon Sattabongkot Prachumsri at Mahidol University, Faculty of Tropical Medicine for providing the glass membrane feeders.

References

1. Smith RC, Vega-Rodríguez J, Jacobs-Lorena M. The Plasmodium bottleneck: malaria parasite losses in the mosquito vector. Memorias Do Instituto Oswaldo Cruz. 2014;109:644–61.

2. Sauerwein RW, Bousema T. Transmission blocking malaria vaccines: Assays and candidates in clinical development. Vaccine. 2015;33:7476–82.
3. Rutledge LC, Ward RA, Gould DJ. Sutdies on the feeding response of mosquitoes to nutritive solution in a new membrane feeder. Mosquito News. 1964;24:407–19.
4. Graves PM, Burkot TR, Carter R, Cattani JA, Lagog M, Parker J, Brabin BJ, Gibson FD, Bradley DJ, Alpers MP. Measurement of malarial infectivity of human populations to mosquitoes in the Madang area, Papua, New Guinea. Parasitology. 1988;96(Pt 2):251–63.
5. Hisaeda H, Yasutomo K. Development of malaria vaccines that block transmission of parasites by mosquito vectors. J Med Invest. 2002;49:118–23.
6. Ubalee R, Kim H-C, Schuster AL, McCardle PW, Phasomkusolsil S, Takhampunya R, Davidson SA, Lee W-J, Klein TA. Vector Competence of Anopheles kleini and Anopheles sinensis (Diptera: Culicidae) From the Republic of Korea to Vivax Malaria-Infected Blood From Patients From Thailand. J Med Entomol. 2016;53:1425–32.
7. Kiattibutr K, Roobsoong W, Sriwichai P, Saeseu T, Rachaphaew N, Suansomjit C, Buates S, Obadia T, Mueller I, Cui L, et al. Infectivity of symptomatic and asymptomatic Plasmodium vivax infections to a Southeast Asian vector, Anopheles dirus. Int J Parasitol. 2017;47:163–70.
8. Sattabongkot J, Kumpitak C, Kiattibutr K. Membrane Feeding Assay to Determine the Infectiousness of Plasmodium vivax Gametocytes. Methods Mol Biol. 2015;1325:93–9.
9. Ouedraogo AL, Guelbeogo WM, Cohuet A, Morlais I, King JG, Goncalves BP, Bastiaens GJH, Vaanhold M, Sattabongkot J, Wu Y, et al. **A protocol for membrane feeding assays to determine the infectiousness of** *P. falciparum* **naturally infected individuals to** *Anopheles gambiae*. Malaria World Journal. 2013;4:4.
10. Coulibaly MB, Gabriel EE, Sinaba Y, Sylla D, Sacko A, Sylla L, Coulibaly B, Hume JCC, Baber I, Assadou MH, et al. Optimizing Direct Membrane and Direct Skin Feeding Assays for Plasmodium falciparum Transmission-Blocking Vaccine Trials in Bancoumana, Mali. Am J Trop Med Hyg. 2017;97:719–25.
11. Abduselam N, Zeynudin A, Berens-Riha N, Seyoum D, Pritsch M, Tibebu H, Eba K, Hoelscher M, Wieser A, Yewhalaw D. Similar trends of susceptibility in Anopheles arabiensis and Anopheles pharoensis to Plasmodium vivax infection in Ethiopia. Parasites Vectors. 2016;9:552.
12. Ouédraogo AL, Guelbéogo WM, Cohuet A, Morlais I, King JG, Gonçalves, Bastiaens GJH, Vaanhold M, Sattabongkot J, Wu Y, et al: **A protocol for membrane feeding assays to determine the infectiousness of** *P. falciparum* **naturally infected individuals to Anopheles gambiae**. *Malaria World Journal* 2013, 4.
13. Da DF, Churcher TS, Yerbanga RS, Yameogo B, Sangare I, Ouedraogo JB, Sinden RE, Blagborough AM, Cohuet A. Experimental study of the relationship between Plasmodium gametocyte density and infection success in mosquitoes; implications for the evaluation of malaria transmission-reducing interventions. Exp Parasitol. 2015;149:74–83.
14. Rios-Velásquez CM, Martins-Campos KM, Simões RC, Izzo T, dos Santos EV, Pessoa FA, Lima JB, Monteiro WM, Secundino NF, Lacerda MV, et al. Experimental Plasmodium vivax infection of key

Anopheles species from the Brazilian Amazon. Malar J. 2013;12:460.

15. Zhu G, Xia H, Zhou H, Li J, Lu F, Liu Y, Cao J, Gao Q, Sattabongkot J. Susceptibility of Anopheles sinensis to Plasmodium vivax in malarial outbreak areas of central China. Parasit Vectors. 2013;6:176.
16. Sattabongkot J, Maneechai N, Phunkitchar V, Eikarat N, Khuntirat B, Sirichaisinthop J, Burge R, Coleman RE. Comparison of artificial membrane feeding with direct skin feeding to estimate the infectiousness of Plasmodium vivax gametocyte carriers to mosquitoes. Am J Trop Med Hyg. 2003;69:529–35.
17. Novak MG, Berry WJ, Rowley WA. Comparison of four membranes for artificially bloodfeeding mosquitoes. J Am Mosq Control Assoc. 1991;7:327–9.
18. Awono-Ambene HP, Diawara L, Robert V. Comparison of direct and membrane feeding methods to infect Anopheles arabiensis with Plasmodium falciparum. Am J Trop Med Hyg. 2001;64:32–4.
19. Kiattibutr K, Roobsoong W, Sriwichai P, Saeseu T, Rachaphaew N, Suansomjit C, Buates S, Obadia T, Mueller I, Cui L, et al. Infectivity of symptomatic and asymptomatic Plasmodium vivax infections to a Southeast Asian vector, Anopheles dirus. Int J Parasitol. 2017;47:163–70.
20. Bonnet S, Gouagna C, Safeukui I, Meunier JY, Boudin C. Comparison of artificial membrane feeding with direct skin feeding to estimate infectiousness of Plasmodium falciparum gametocyte carriers to mosquitoes. Trans R Soc Trop Med Hyg. 2000;94:103–6.
21. Vallejo AF, Rubiano K, Amado A, Krystosik AR, Herrera S, Arévalo-Herrera M. **Optimization of a Membrane Feeding Assay for Plasmodium vivax Infection in Anopheles albimanus**. *PLoS Neglected Tropical Diseases* 2016, 10.
22. Witmer K, Sherrard-Smith E, Straschil U, Tunnicliff M, Baum J, Delves M. An inexpensive open source 3D-printed membrane feeder for human malaria transmission studies. Malar J. 2018;17:282.
23. Bousema T, Dinglasan RR, Morlais I, Gouagna LC, van Warmerdam T, Awono-Ambene PH, Bonnet S, Diallo M, Coulibaly M, Tchuinkam T, et al. Mosquito feeding assays to determine the infectiousness of naturally infected Plasmodium falciparum gametocyte carriers. PLoS One. 2012;7:e42821.
24. Stone WJ, Eldering M, van Gemert GJ, Lanke KH, Grignard L, van de Vegte-Bolmer MG, Siebelink-Stoter R, Graumans W, Roeffen WF, Drakeley CJ, et al. The relevance and applicability of oocyst prevalence as a read-out for mosquito feeding assays. Sci Rep. 2013;3:3418.
25. Vallejo AF, Rubiano K, Amado A, Krystosik AR, Herrera S, Arevalo-Herrera M. Optimization of a Membrane Feeding Assay for Plasmodium vivax Infection in Anopheles albimanus. PLoS Negl Trop Dis. 2016;10:e0004807.
26. Sweeney AW. Larval salinity tolerances of the sibling species of Anopheles farauti. J Am Mosq Control Assoc. 1987;3:589–92.
27. Diallo M, Toure AM, Traore SF, Niare O, Kassambara L, Konare A, Coulibaly M, Bagayogo M, Beier JC, Sakai RK, et al. Evaluation and optimization of membrane feeding compared to direct feeding as an assay for infectivity. Malar J. 2008;7:248.

28. Damiens D, Soliban SM, Balestrino F, Alsir R, Vreysen MJ, Gilles JR. Different blood and sugar feeding regimes affect the productivity of Anopheles arabiensis colonies (Diptera: Culicidae). J Med Entomol. 2013;50:336–43.
29. Reimer LJ, Thomsen EK, Koimbu G, Keven JB, Mueller I, Siba PM, Kazura JW, Hetzel MW, Zimmerman PA. Malaria transmission dynamics surrounding the first nationwide long-lasting insecticidal net distribution in Papua New Guinea. Malar J. 2016;15:25.

Figures

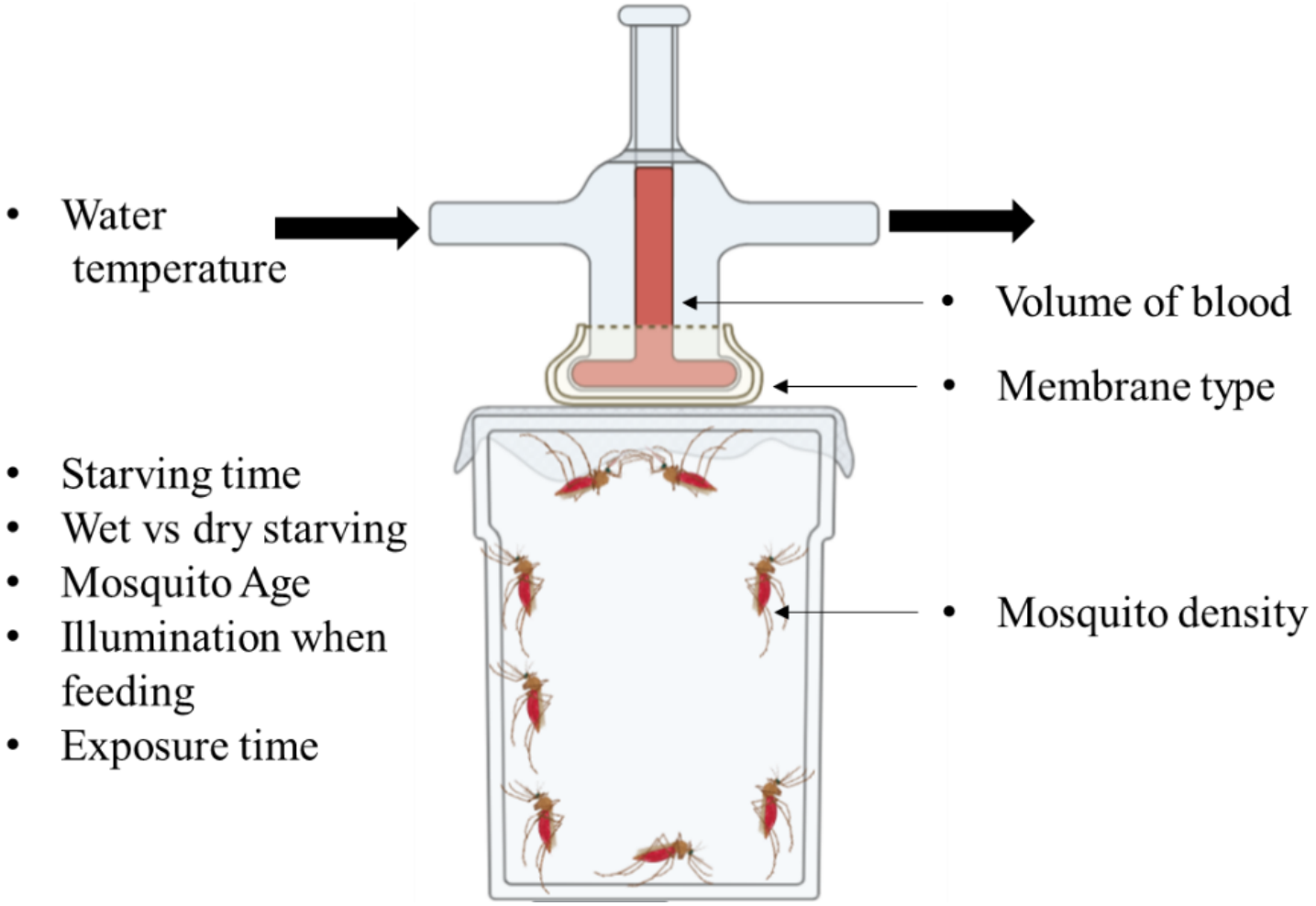

Figure 1

Membrane feeding assay set up with the parameters impacting the feeding success of mosquitoes on the direct membrane feeders (figure created using BioRender.com).

Figure 2

Direct membrane feeding assay (DMFA) set up. Cups connected in a series by tubes to a mini aquarium pump within the water bath which is maintained at ~38 ºC.

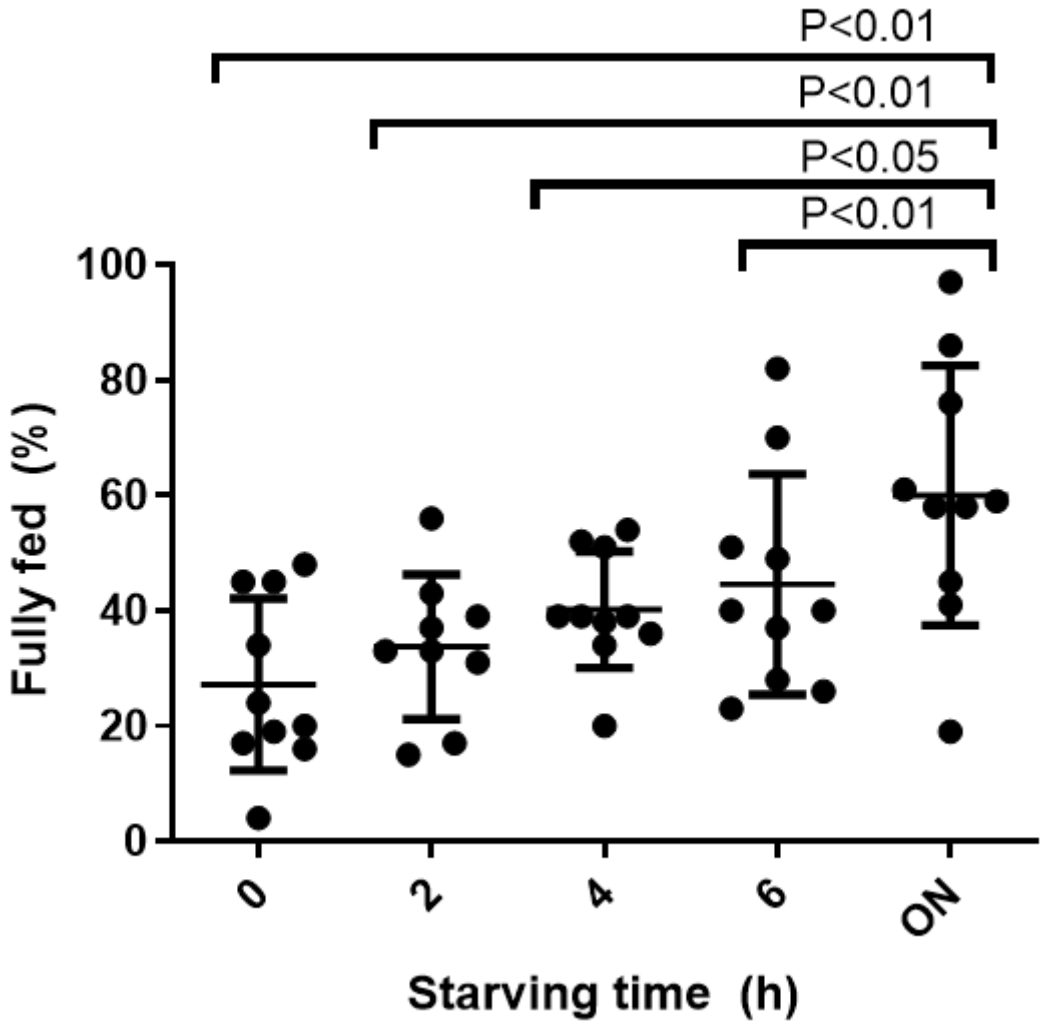

Figure 3

Proportion of mosquitoes that fed following the different starving times. The difference between the starving times of 0-4 h and overnight (ON) starving was statistically significant. The bars are the means with the standard deviations.

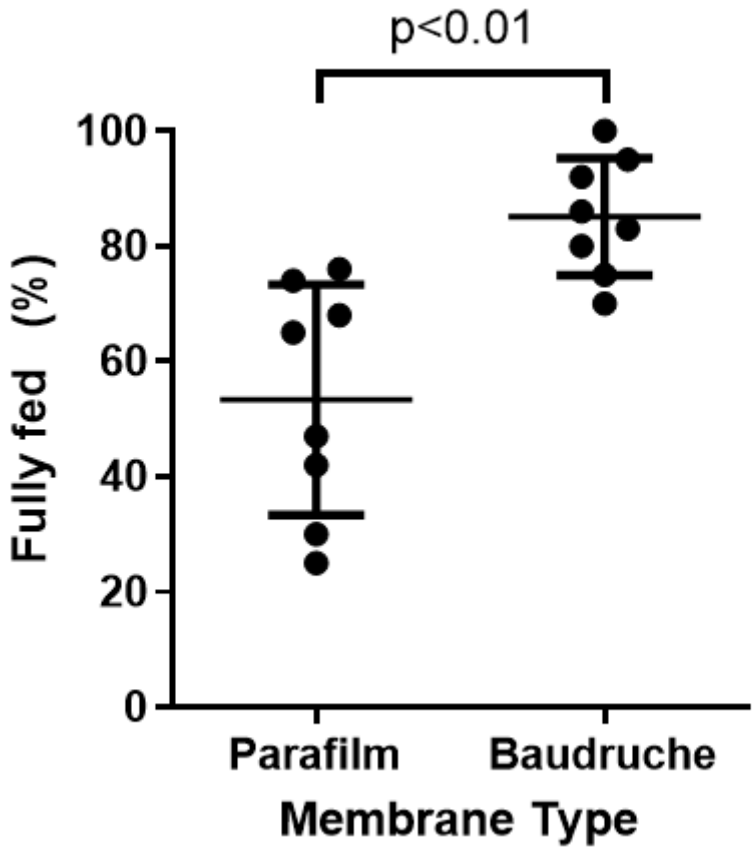

Figure 4

Proportion of mosquitoes that fed when using Parafilm and Baudruche membrane types. The observed difference between the performance of Parafilm and Baudruche membrane is statistically significant ($p<0.01$). The bars are the means with the standard deviations.

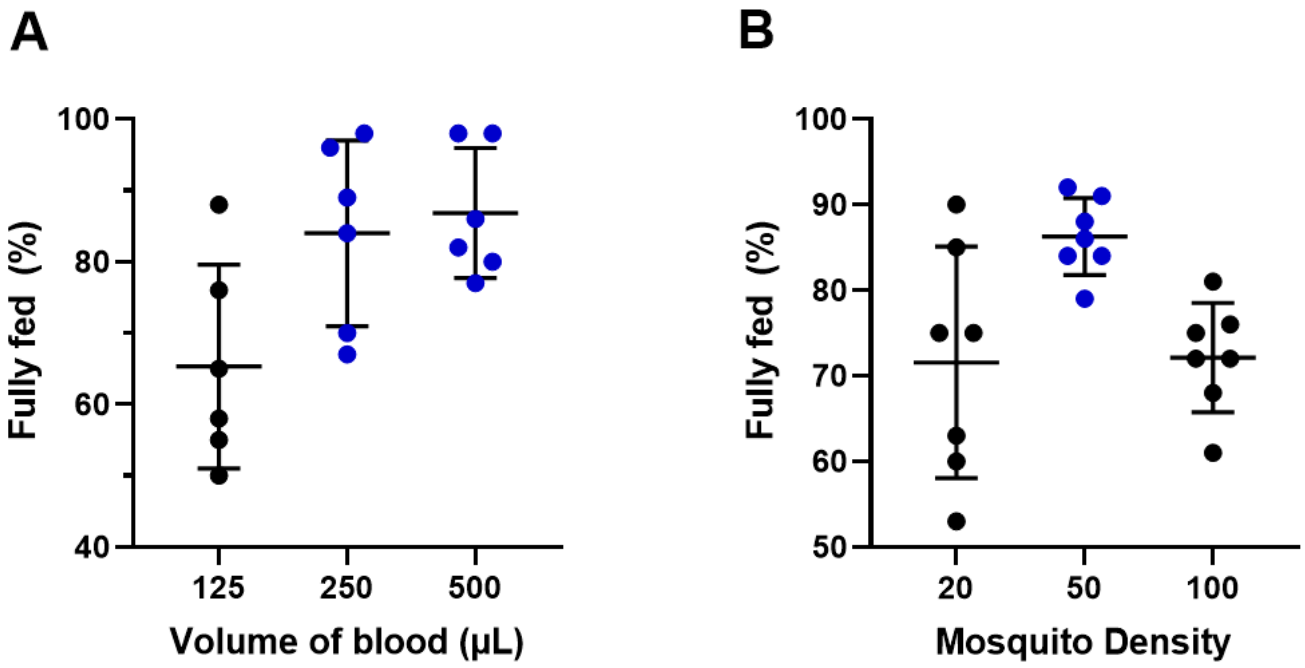

Figure 5

A) Feeding rate on varying blood volume. A significantly higher proportion of mosquitoes fed on 250 µL and 500 µL of blood compared to the feeding rate of 125 µL of blood ($p<0.05$) B) Feeding rate at different mosquito densities. The feeding rate of 50 mosquitoes per cup was significantly higher than both 20 and 100 mosquitoes per cup ($p<0.05$). The bars are the means with the standard deviations while the blue dots represent the variations within parameters that were significantly different from the black dots.

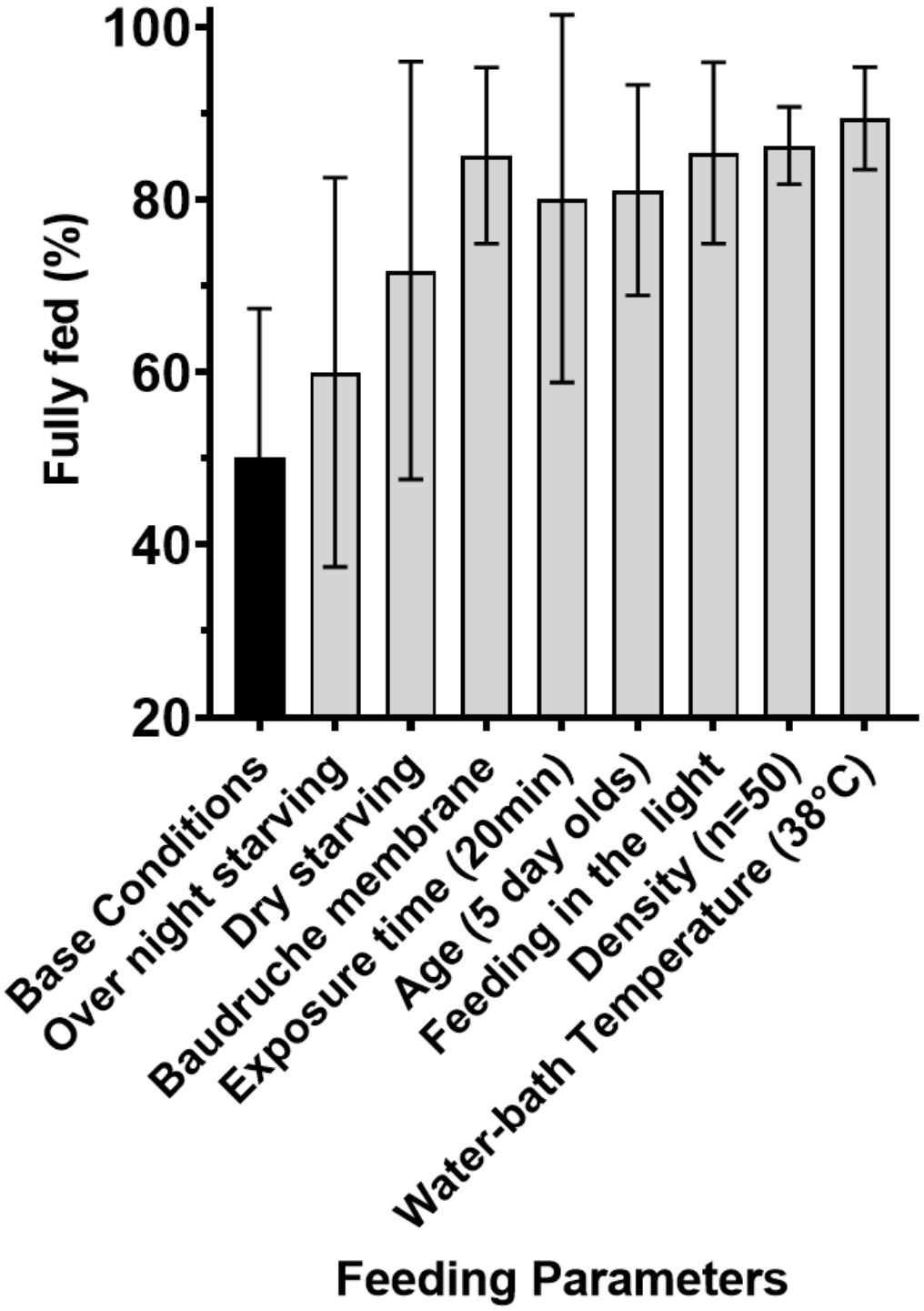

Figure 6

Optimal feeding conditions that were chosen compared to the base conditions prior to the optimization of the feeding parameters. The mean proportions are presented with the error bars as standard deviations.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.docx