Research Square

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Evaluation of Different Blood Feeding Frequencies on *Glossina Palpalis Gambiensis* Performance in A Mass Rearing Insectary

Karifa Camara
CIRDES: Centre International de Recherche-Developpement sur l'Elevage en zone Subhumide
Kadidiata Ilboudo
CIRDES: Centre International de Recherche-Developpement sur l'Elevage en zone Subhumide
Ernest Wendemanegde Salou (salouernest@gmail.com)
Centre International de Recherche-Développement sur l'Elevage en zone Subhumide: Centre International de Recherche-Developpement sur l'Elevage en zone Subhumide
Geoffrey Gimonneau
Centre de Coopération Internationale en Recherche Agronomique pour le Développement: CIRAD

Research

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Abstract

Background: In tsetse flies mass rearing insectaries, the sustainable supply of high-quality blood meals is the main challenge, especially in Africa. Because collection of high-quality and quantity of blood may be an important constraint to production, blood feeding frequency can be a lever to lessen this impact. Therefore, this study evaluates three blood feeding frequencies on *G. p. gambiensis* colony performance.

Methods: Three blood feeding treatments, i.e. three, four and six times per week, were evaluated on female's survival, productivity, and progeny emergence rate and flight ability.

Results: Females survival was significantly higher for flies feed four times per week (87%) than three (72%) and six times (78%, $p<0.05$). Productivity was similar between flies feed four and six times per week (457 and 454 larvae) but significantly reduced when fed three times (280 larvae produced; $p<0.05$). Similarly, emergence rate and flight ability rate were both similar between flies feed four (97 and 94%) and six times (96 and 97%) per week but significantly reduced when fed three times (89 and 84% respectively; $p<0.05$).

Conclusions: Blood feeding frequency could be reduced to four times per week without affecting the mass rearing production and progeny quality. We discuss the implications of these results on tsetse mass rearing production.

Background

Tsetse flies (Diptera; Glossinidae) are the cyclical vector of African trypanosomes across sub-Saharan Africa, which cause human African trypanosomosis (HAT) and African animal trypanosomosis (AAT), a debilitating disease of humans (sleeping sickness) and livestock (nagana), respectively [1, 2]. Due to their distribution over 36 countries in sub-Saharan Africa, tsetse flies impair the development of sustainable and productive agricultural systems in over ten million km^2 of sub-Saharan Africa [3, 4] leading to potential losses in livestock and crop production estimated at USD 4.75 billion per year [5]. So far, animal trypanosomosis is mainly controlled through prophylactic and curative drugs but this approach is no longer sustainable, due to drug resistance development [6, 7]. Therefore, the current efficient way to protect people and livestock is to reduce host vector contact through vector control of sibling species [8].

Vector control programs are based as possible on the area-wide integrated pest management (AW-IPM) approach and according to the environmental context could integrate the sterile insect technique (SIT) component [9, 10]. SIT is a species-specific and environment friendly biological-based control tactic to manage populations (suppression or/and elimination) of insect pests and disease vectors [11]. SIT consists in the mass production of sterilized male adults that are released in the field to out-compete wild males and mate with wild virgin females. These mattings are non-productive, and will lead to population reduction or elimination [12]. SIT has been used successfully in the past against the eradication of *Glossina austeni* from Unguja Island, Zanzibar [13] and is currently achieved in the Niayes area of Senegal against *Glossina palpalis gambiensis* [14].

Tsetse flies released in Senegal are provided as chilled irradiated male pupae to the eradication program by the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso [14]. The CIRDES host a mass rearing insectary with the largest colony of *G. p. gambiensis* in Africa. In tsetse flies, both sexes are obligate blood feeders and one of the main challenges in tsetse fly colonies is to maintain high production rate while reducing production costs and produce pupae of good quality. These results could be achieved through the use of high-quality blood to feed tsetse colonies and a regular assessment of colony performance through measurement of colonies biological parameters such as longevity, fecundity and pupae quality. One way to reduce production costs is to optimize the colony feeding frequency. Indeed, the higher the frequency of feeding, higher will be the production cost of flies (staff and blood collection). Moreover, too many manipulations of fly could be detrimental to the colony production such as regular exposition to heating plate or transfer from a room to another and also increased risk of microorganism's transmission due to multiple feeding [15]. Therefore, this study has for objective to evaluate different feeding frequencies on *G. p. gambiensis* performances in the context of mass rearing production. Three feeding frequencies were evaluated: three, four and six times per week; and four biological parameters were measured: i) females' survival, ii) productivity, iii) progeny emergence rate and iv) flight ability. These parameters were used to assess the effect of feeding frequency on flies performance.

Methods

Insectary

The study was carried out at the CIRDES insectary in Bobo-Dioulasso, Burkina Faso. This is a mass rearing insectary for *G. p. gambiensis* set up in 1975 to support national and regional eradication of tsetse flies [16]. More than 250 000 females are reared and produce over 30 000 male pupae per week that are currently shipped to the eradication program in the Niayes of Senegal as chilled irradiated pupae [14]. Tsetse flies are maintained at 24-25°C, 75-80% relative humidity, and a photoperiod of 5:19 h (L:D).

Biological material

Teneral flies of the *G. p. gambiensis* colony were used in this study. This tsetse colony was established in 1972 at Maison- Alfort (France) with pupae collected from Guinguette (Bobo-Dioulasso, Burkina Faso). In 1975 the colony was transferred to the Centre de Recherche sur la Trypanosomiase Animale (CRTA) (renamed later CIRDES) from 5333 pupae [16]. In 1981 the colony was replenished with wild material from the "Marre aux hippopotames" [17]. Flies are maintained on fresh irradiated bovine blood collected from the local slaughterhouse using in vitro silicon membrane system [18]. The feeding frequency is six times per week, from Monday to Saturday.

Blood collection and conditioning

Bovine blood from different animals (cattle and pigs) were collected at the Bobo-Dioulasso slaughterhouse using 10 liters sterile containers and were defibrinated using a custom-made stain-less

steel electric paddle stirrer. Upon arrival to the insectary, glucose was added to blood as a phagostimulant at the dose of 1g/L and was previously diluted in 10ml of distilled water [19]. Blood was irradiated with 500 Gy in a ^{137}Cs source for 1 hour and 40 minutes and stored in 2 liters containers at 4°C. After irradiation, blood was tested for bacterial contamination. One milliliter of blood samples was poured with a syringe on agar petri dishes and incubated for 72 hours. After incubation, if the irradiated blood contained no more than 10 bacterial colonies, the blood is conserved for feeding, and discarded if more than 10 colonies are found. All blood manipulations were performed under a laminar flow hood to avoid bacterial contamination.

Experimental procedure

The effect of blood feeding frequency was evaluated through the measurement of several biological parameters routinely check in tsetse colonies for performance evaluation. Female's survival (percentage alive on day 30), productivity (number of pupae produced at day 30), fecundity, first larviposition date, pupal size, pupal emergence rate and flight ability of newly emerged flies were assessed for each blood feeding treatment. Among these, female survival, fecundity and pupal size were used in a formula to calculate a Quality Factor (QF) of the blood feeding frequency as a comprehensive indicator for colony production [20,21]. The detailed formula is described in De Beer et al. [22]. A QF factor above 1 indicates that blood is suitable for colony maintenance.

To evaluate the effect of blood feeding frequency, three experimental treatments were performed. Flies were fed three times per week (F3) on Monday, Wednesday and Friday, four times per week (F4) on Monday, Tuesdays, Thursday and Friday, and finally six times per week (F6) from Monday to Saturday. Newly emerged flies were blood fed using an in vitro silicon membrane feeding system with the appropriate blood treatment during 30 days. Bioassays were conducted using 10 males and 30 females per cages of six and three days old respectively. Females were monitored daily for survival and productivity (pupal production and abortion). Pupae produced were sorted in five class sizes (A to E) calibrated for *G. p. gambiensis* according to their weight (mg): A (<22), B (22 <28), C (28<32), D (32<36), E (>36) [21]. After 30 days, all surviving females were dissected to determine their reproductive status (presence/absence of egg/larvae in the uterus and insemination status). For each feeding frequency, pupae produced were put in petri dishes under ~1cm of sand and covered by a flight cylinder (see Seck et al. 2015 for details). The inner wall of the cylinder was coated with unscented talcum powder to prevent the flies from crawling out. This method was used to assess the number of flies able to fly out and thus "available for the SIT". After emergence, the number of pupae that did not emerge was counted. This study was performed between January and June 2018, and four cohorts of flies were studied for all treatments. For each cohort, all bioassays were replicated three times, leading to an overall of twelve replicates per treatment.

Statistical analyses

The survival of flies fed with different feeding frequencies was analyzed using Kaplan-Meier survival curves. Survival curves were compared using the “coxme” function where the blood treatment was used as explanatory variable, the survival as the response variable and cohorts and replicates were used as random effects.

Female’s productivity, first larviposition date and pupal classes were analyzed using a generalized linear mixed effects model with a “poisson” family. Treatments were used as fixed effect and cohorts were used as random effect.

Quality factors were tested using linear mixed effects models. The treatments were used as fixed effect and cohorts as random effect.

The adult emergence rate and percentage of flies able to fly were analyzed using binomial generalized mixed effects models. The treatments were used as fixed effect and cohorts were used as random effect. For each model, the best model was selected on the basis of the lowest corrected Akaike information criterion, and the significance of fixed effect was tested using the likelihood test ratio [23,24]. The R software (version 3.5.0) was used for data analysis [25].

Results

Survival

On day 30, survival was the highest for females fed four times per weeks (87.2%) and the lowest for flies fed three times (72.5%, Table 1, Figure 1). Model analysis showed that blood feeding frequency had a significant effect on female’s survival (Likelihood-ratio test: $\chi^2 = 54.171$, df = 2, $P > 0.001$). Survival for treatment F3 was significantly lower than treatment F4 ($P < 0.001$) and F6 ($P = 0.047$). However, survival in treatment F4 was significantly higher than F6 ($P = 0.003$).

Female’s productivity

A total of 360 females were blood-fed for 30 days for each feeding frequency: 3 days per week (F3), 4 days per week (F4) and six days per week (F6). The first larva was deposited on average on day 15.75 for treatment F6, which was statistically similar to treatment F3 (17.75 days) and F4 (16 days; likelihood-ratio test: $\chi^2 = 0.575$, df = 2, $P = 0.750$).

Female’s productivity and fecundity were the highest for treatment F4 followed by treatment F6 and F3 (Table 1). Model results showed that female’s productivity for treatments F6 and F4 were similar (Tukey post-hoc test value: Z = 0.1, $P = 0.995$) but both were significantly higher from F3 (Tukey post-hoc test value: Z = 6.363, $P < 0.001$).

Pupae produced by the three blood treatments were concentrated in classes A and B with 95% of the overall productivity and 5% in class C and no pupae in class D and E. No difference in productivity was observed between blood feeding treatments for class A. For class B, treatment F6 and F4 produced

significantly more pupae than treatment F3 (Tukey post-hoc test value: Z = 7.020, *P* <0.001) and for class C, treatment F6 produced significantly more pupae than treatments F3 and F4 (Tukey post-hoc test value: Z = 3.072, *P* = 0.005).

All females surviving at day 30 were inseminated (Table 1).

The quality factors obtained for all blood feeding frequency were above 1, with the highest value for treatment F6 (1.59± 0.10) and the lowest for treatment F3 (1.18± 0.10; Table 1). Mixed model analyses showed that QF obtained for treatment F3 was significantly lower than F6 and F4 (Tukey post-hoc test value: Z = 4.283, *P* < 0.001). No difference was observed between F4 and F6 (Tukey post-hoc test value: Z = 1.532, *P* = 0.276).

Progeny emergence rate and flight ability

The emergence rates of the adult flies were between 92 and 96% for all blood feeding frequencies (Table 1). The best model retained the blood feeding frequencies as a significant explanatory variable (Likelihood-ratio test: 2 = 22.951, df = 2, P < 0.001). Emergence rates were significantly higher in treatment F4 and F6 than F3 (Tukey post-hoc test value: Z = 3.597, *P* < 0.001). No difference was observed between treatments F4 and F6 (Tukey post-hoc test value: Z = 0.896, *P* = 0.370).

The flight ability rate (i.e. the number of operational flies) was the lowest for treatment F3 84 and highest for treatment F4 with 96.7% (Table 1). The best model retained the blood feeding frequencies as a significant explanatory variable (Likelihood-ratio test: 2 = 36.914, df = 2, P < 0.001). Flight ability rates were significantly higher in treatment F4 and F6 than F3 (Tukey post-hoc test value: Z = 4.338, *P* < 0.001). No difference was observed between treatments F4 and F6 (Tukey post-hoc test value: Z = 1.824, *P* = 0.068).

Discussion

In the application of the sterile insect technique, one of the main constraints is the production of sufficient good-quality male flies for sterilization and release. Tsetse flies are obligatory haematophagous insects and therefore the blood diet quality and blood feeding frequency are one of the most important parameters to produce flies of good quality. Blood diet quality depends on external factors such as the nutritional status of animals or blood contamination (bacteria or chemicals) that are controlled before use in insectary (i.e. blood quality control). In the same way, blood feeding frequency could be optimized according to tsetse species requirement and production parameters. In this study, we assessed the effect of different feeding frequencies on tsetse fly's production parameters in a mass rearing insectary.

Female fly survival, fecundity and pupal size are three essentials comprehensive parameters routinely used for assessing colony performance. In this study, the best results for all these parameters were obtained for treatment F4. Although not significant, values parameters obtained for treatment F6 were lower than F4. These results highlight that feeding flies four times per week will have no adverse effect on

colony production parameters instead of six times per week. However, it was not the case for treatment F3 that led to significant negative effect on female's survival and production parameters although they remain acceptable to maintain flies. Colony parameters increase between treatment F6 and F4 could be explained by the reduction in handling time for routine colony maintenance. Indeed, transfer of fly cages to feeding room involves some small and rapid changes in environmental conditions. The daily exposition to 37 °C feeding membrane could be detrimental as temperature upon 30 °C may affect survival and fecundity [1, 26]. Although a feeding frequency of six times per week optimize the number of blood fed flies, it is important to note that blood meal digestion needs on average two days to be completed [27]. Therefore, a daily feeding frequency seems to be suboptimal from a physiological point of view and not necessary. This was supported by results of the QF values obtained for treatment F4 and F6. Both values were statistically similar highlighting that reducing the feeding frequency from six days per week to four has no effect on colony production. It should be noticed that treatment F6 produced more bigger pupae than treatment F3 and F4. While pupae of class C represents only 9% of the F6 treatment production, it explains why the QF value for treatment F6 is slightly higher than F4 although all production parameters were higher for treatment F4. Regarding results of the progeny emergence rate and flight ability, reducing the feeding frequency to four days per week has no effect on progeny quality. However, a feeding frequency of three days per week lead to a significant reduction in pupae emergence rate and flight ability. These results are probably the consequence of a lower acquisition of fat reserve in the larva during the intrauterine phase, that is mainly dependent on the amount of blood acquired by the female during the interlarval period [28]. Although results of treatment F3 was acceptable (89% of emergence and 84% of operational flies), on a colony-wide basis, it makes a huge difference in progeny quality in comparison to treatment F4 and F6. Similar findings were recently reported on *Glossina pallidipes*. Flies feed five times per week showed better production parameters if feed three times per week [29]. However, some species tsetse such as *Glossina morsitans submorsitans* seems to be more adapted to starvation. Indeed, no differences were observed on the production parameters of flies feed three, four or six times per week [30].

Females dissection showed that all flies were inseminated. Although, the filling level of spermathecae in the females was not mentioned, result of this study indicates that the feeding frequency did not influence the mating performance of the males.

From an economic standpoint, feeding frequency has an important impact on the insectary running costs. Indeed, a high feeding frequency leads to higher cost in personal and infrastructure (water and electricity, especially expensive in Africa). As a result, such costs need to be balance by an increase in pupae production. However, treatment F6 produce no more pupae than treatment F4 during the 30 days survey. Although such difference was not significant, it could be assumed that reducing the feeding frequency from 6 days per week to 4 days will lead to significant cost saving while increasing or at least maintaining the same colony performance. In the same time, it will reduce the amount of blood used per week and as therefore the frequency of blood collection at the slaughterhouse. It could be assumed that such reduction in feeding frequency will lead to approximately 30% saving per year. Reducing the feeding

to three days per week will be also possible. However, results highlight that it will have a significant effect on colony performance and therefore it seems not to be recommended for a mass rearing insectary.

Conclusions

In conclusion, this study demonstrates that the feeding frequency of the CIRDES mass rearing colony of *G. p. gambiensis* could be reduced to four times per week without affecting the mass rearing production and progeny quality. Moreover, it will have a positive economic impact that could be reinjected in the insectary.

Declarations

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Authors' contributions

GG, KC, KI and EWS drafted the proposal. KC performed the laboratory analyses. GG performed the statistical analyses. PS, GG, KC, KI and EWS drafted the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The data that support the findings of this study are openly available in Dataverse at http://dx.doi.org/10.18167/DVN1/1SPLYD

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All individual authors declare that they have no competing interests.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

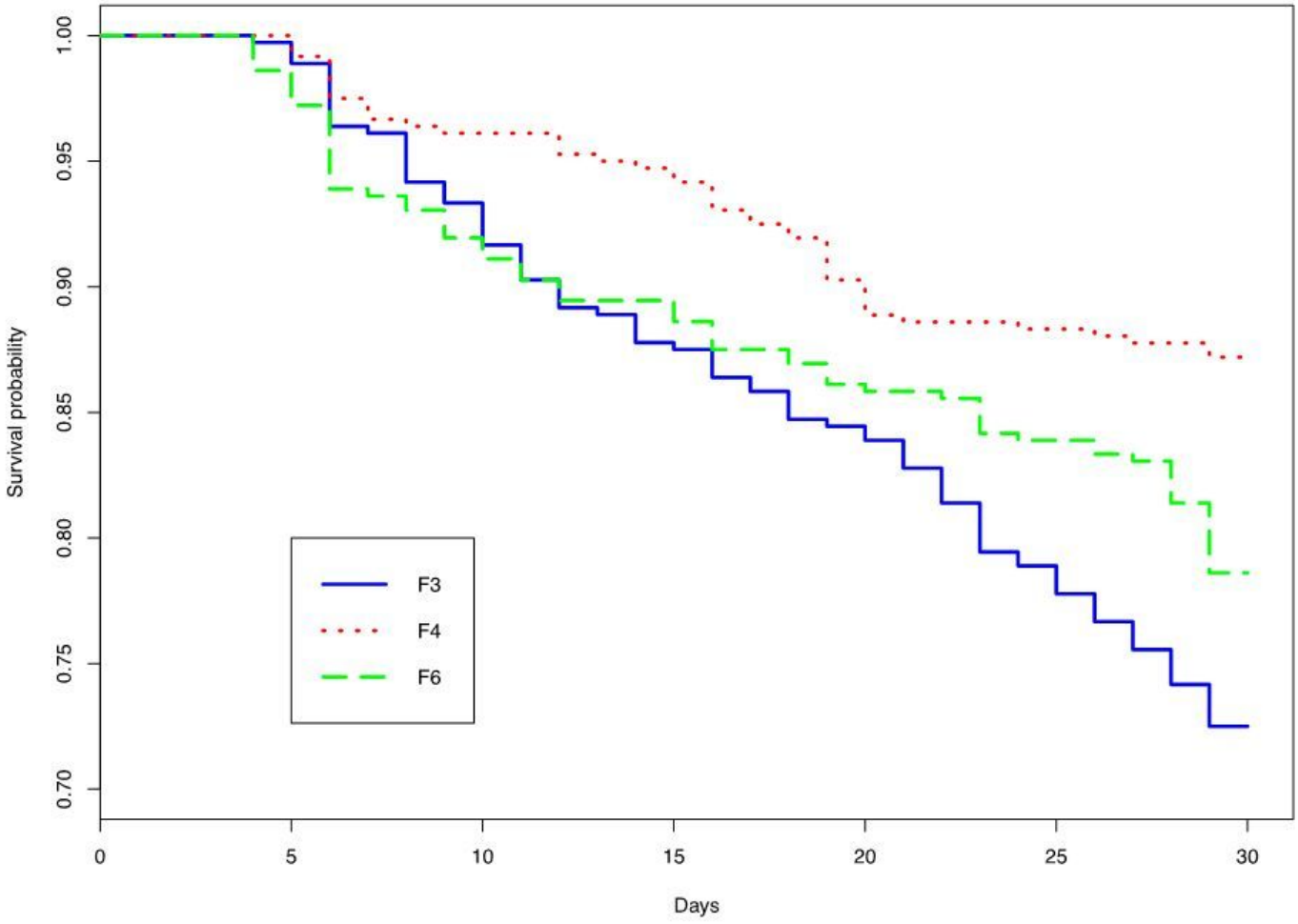

Figure 1

Survival curves of flies feed at different frequencies per week. F3= three blood meals per week; F4= four blood meals per week; F6= six blood meals per week.

Supplementary Files

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- Table1.JPG