ESJ Natural/Life/Medical Sciences

Reproductive Performance of *Glossina palpalis gambiensis* (Diptera: Glossinidae) when fed frozen or fresh bovine blood meals

Soudah Boma
Centre International de Recherche
Développement sur l'Elevage en zone subhumide (CIRDES), Burkina Faso
Ernest Wendemanegde Salou
Centre International de Recherche
Développement sur l'Elevage en zone subhumide (CIRDES), Burkina Faso
Département de Sciences biologiques/UFR-ST,
Université Nazi Boni (UNB), Burkina Faso
Prudenciène Agboho
Cene Bila
Simon Kabore
Abdoul Rahim Sabo
Zakaria Bengaly
Charles Guiguigbaza Kossigan Dayo
Centre International de Recherche
Développement sur l'Elevage en zone subhumide (CIRDES), Burkina Faso

Doi:10.19044/esj.2022.v18n21p138

Submitted: 01 November 2021
Accepted: 21 June 2022
Published: 30 June 2022

Cite As:
Boma S., Salou E.W., Agboho P., Bila C., Kabore S., Sabo A.R., Bengaly Z. & Kossigan Dayo C.G. (2022). *Reproductive Performance of Glossina palpalis gambiensis (Diptera: Glossinidae) when fed frozen or fresh bovine blood meals*. European Scientific Journal, ESJ, 18 (21), 138. https://doi.org/10.19044/esj.2022.v18n21p138

Abstract

The tsetse and trypanosomoses control by sterile insect technique (SIT), requires mass production of quality tsetse pupae and consequently high-quality blood meal for the fly feeding. The reproductive performances of *Glossina palpalis gambiensis* (Diptera : Glossinidae), fed frozen or fresh blood meals has been studied. Three diets of slaughtered bovine blood stored at -18 °C for six months (R6), three months (R3) and one month (R1) were used in the present investigation. Fresh blood samples stored at 4°C and

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

renewed every four days were used as control (R0). The defibrinated blood was packaged in batches of 30 x 20 ml vials. For each blood meal, 30-day feeding tests were conducted at the beginning and end of freezing. All blood meal was irradiated using 137 caesium irradiation before being used. The parameters monitored daily were: the number of dead flies (fed and unfed), the number and weight of pupae and the emergence rate. No difference in survival rates was observed for the three regimes (df: 15; *Chi-square*: 7.53; $p > 0.05$), on average 32.1% (R0), 32.4% (R6), 37.5% (R3) and 38.2% (R1). However, flies fed with the six-month-old frozen blood were less efficient in pupa production (df: 3; F: 6. $p < 0.001$) and pupae weight (df: 3, F: 3.24, $p < 0.05$) compared to the control. The mortality rate was affected by the duration of freezing: 26.7% for R6 and 8% for the other diets ($p < 0.05$). The implications of our results in terms of blood meal management for efficient production of insectary pupae were discussed.

Keywords: *Glossina palpalis gambiensis*, frozen blood, fertility, survival

Introduction

1. Background

African Trypanosomiasis is one of the neglected tropical diseases transmitted mainly by tsetse flies (*Glossina spp*.) that affect humans and animals in sub-Saharan Africa. The animal form of the disease also called "Nagana", is caused by the trypanosomes (*Trypanosoma spp*.) pathogens that infect domestic animals in Africa, namely: *Trypanosoma brucei*, *T. congolense* and *T. vivax*. The human form of the disease (HAT) presents in two forms, depending on the species of trypanosome. The chronic form (98% of HAT cases) is caused specifically by *T. brucei gambiense* in West and Central Africa and the acute form by *T. brucei rhodesiense* in East Africa (De Greef et al., 1989; Fèvre et al., 2001; Jackson et al., 2010). The socio-economic impact of tsetse in sub-Saharan Africa remains enormous, despite decades of control (Muhanguzi et al., 2015; 2017). Currently, no vaccine or prophylaxis against HAT is available, so it is a heavy burden for Africa (Fèvre et al., 2008).

Major studies have been undertaken concerning the biology of tsetse. The control of tsetse populations in an area is based on a coordinated, sequential and integrated strategy (Diall et al., 2017), which combines the deployment of appropriate control techniques, depending on the environmental context. The technique of sterile insects (SIT), which is an environmental-friendly technique, is recommended and used in a tsetse elimination or eradication campaign (Feldmann et al., 2001; Kristjanson et al., 1999; Vreysen, 2001). This technique is based on the principle that fertile female tsetse flies are unable to produce viable larvae when they have mated

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

with sexually sterile males (Dyck et al., 2005). To this end, the mass production of target tsetse fly species is an important step in the technical components of SIT (Parker, 2005).

The "Centre International de Recherche-Developpement sur l'Elevage en zones Subhumides" (CIRDES) in Bobo-Dioulasso (Burkina Faso) has been breeding three tsetse species since 1970: *Glossina palpalis gambiensis* (*G.p.g*), *Glossina morsitans submorsitans* (*G.m.s*) and *Glossina tachinoides* (*G.t*). The rearing of these tsetse populations allows, among other things, to make technologies and biological material available to all those involved in the control of trypanosomosis. *Glossina palpalis gambiensis* irradiated pupae were provided for vector control activities in Burkina Faso and Mali (Mutika et al., 2013). The demand for sterile male tsetse flies has recently increased, particularly in the context of Senegal's tsetse eradication project. In Senegal, approximately 1,000 km² are concerned for tsetse fly elimination using SIT (Bouyer et al., 2014; Bouyer et al., 2010). This necessitates a large number of pupae being produced by tsetse insectariums, including the CIRDES insectarium. The availability of adequate quality and quantity of blood from tsetse fly host animals remains a critical issue in the mass production of tsetse fly pupae. There are two methods for supplying blood meals to tsetse insectarium: host feeding and artificial membrane feeding. The first is the breeding of host animals that tsetse flies feed on, such as rabbits and small ruminants. This method is limited by the high cost of animal management and the tedious work involved in feeding the flies (Langley, 1971). The second is a steady supply of blood gathered from slaughterhouses to make up the fresh blood meals. Whole fresh blood has the advantage of containing all of the components required for tsetse flies' biological needs. It is an option in extreme circumstances that is limited by the shelf life of the blood supply, which should not be more than four days and thus necessitates a frequent supply. Furthermore, there is a risk of bacterial and chemical (antibiotic) contamination of the blood at the collection points, compromising the colony survival and performance (Kaaya et al., 1987). Despite irradiation to eliminate any microbes in the collected blood, high proportions of dead larvae have often recorded at the CIRDES insectarium. This necessitates a preliminary control of the sanitary quality of the blood via biological tests (bio-tests) prior to its use in feeding the colony. Freezing, defined as the process of preserving the biological structure and/or functions of living systems through low-temperature conservation, offers an intriguing solution to the problems associated with long-term sanitary quality control and preservation of blood products.

Previous research has shown that feeding tsetse flies blood meal that has been stored for several months reduces reproductive performance and, as a result, colony size (Wetzel & Luger, 1978). It would be interesting to know

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

if using frozen blood meals for a few months has no effect on the reproductive performance of the CIRDES *G. palpalis gambiensis* colony. The use of frozen bovine blood for several months would reduce pupa production. In this context, this study examined the effect of blood meal cold storage duration on the reproductive performance of *G. palpalis gambiensis*, with the goal of increasing the insectarium's productivity.

2. Material and methods

2.1. Biological material

The experiments used 720 teneral females and 240 male *Glossina palpalis gambiensis* flies from the CIRDES insectary colony. This CIRDES laboratory colony has been in existence since 1975 and is fed using an in vitro silicon membrane system. The experiment population was reared under the normal conditions of the insectary. The feeding schedule was six times per week, Monday through Saturday, using defibrinated bovine blood collected at the Bobo-Dioulasso slaughterhouse (Burkina Faso). The flies were kept in the insectary at 24 - 25°C, 75 - 80 percent relative humidity, and a photoperiod of 12:12 h. (L:D).

2.2. Blood collection and processing

The blood was obtained from cattle slaughtered on the line, which had been certified as healthy and of good quality by the slaughterhouse's veterinary staff. The blood was collected in a mini jerry from the cut neck of the bovine and then poured into 10-litre sterile containers for defibrination. The goal of defibrination is to avoid rapid coagulation of the blood. A stainless steel electric paddle shaker was used to perform defibrination for 10 minutes. Blood was collected in two-litre bottles (Figure 1) and stored at -18°C overnight. A 72-hour bacterial test was used to assess blood microbial contamination. In order to perform the bacteriological culture, 1-ml of blood was inoculated into an agar culture medium at 45°C and incubated in an oven at 37°C. The microbiological quality of the incubated blood was evaluated by counting the colonies present with a colony counter (De Beer et al., 2012). After incubation, batches of blood meals with more than 10 colonies were discarded and incinerated. The microbiological test does not provide definitive information about the health history of the slaughtered animals. All blood products retained after bacteriological testing were irradiated for 1 hour and 40 minutes with (total dose of 1500 Gy: from 137 Cs source) before being used for tsetse flies feeding.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

Figure 1. Blood collection in a 2-liter container and labeling for microbial screening

2.3. Storage of blood meals

The blood meals used in this study were stored as follows. Frozen blood meal was kept at -18°C in a freezer. Fresh blood meal batches were stored at 4°C for up to four days before use. The frozen blood batches were formed in November 2020 (R6), February 2021 (R3), and April 2021, respectively (R1). This corresponds to the experimental diets listed below: R6 represents blood stored at -18°C for six months, R3 represents blood stored at -18°C for three months, and R1 represents blood stored at -18°C for one month. The comparison diet (R0 = fresh blood meal) was made up of batches of fresh blood stored at 4°C and renewed every four days.

2.4. Experimental design

Sixteen (16) small (4.5 x 13 x 8 cm) teneral females were mated and fed every day (except Sunday) for 30 days. Flies were mated in a 3:1 sex ratio, which meant that 30 three-day-old females were mated with 10 five-day-old males. A 30-day feeding test was conducted for each diet in a randomized Square Latin arrangement of four cages per line. The cages were individually placed on a single larviposition box.

2.5. Feeding test

At the beginning and end of the experiment period, 30-day feeding tests were performed for each diet. For comparison, fresh blood batches stored at 4°C were used (R0). Before freezing, the first series of three standard 30-

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

day feeding tests were performed to assess the nutritional value of blood meals (De Beer, Venter and Potgieter, 2012). A preliminary phase of 30-day feeding tests was performed on November 2020 (diet R6), February 2021 (diet R3), and March 2021 (diet R1) batches of frozen diets (diet R1). For the batches R1, R3, and R6, each test was followed by a 2x30-day consecutive test one month, three months, and six months later, respectively. A diet under control This second step of experimentation included whole fresh blood meals stored at 4°C and renewed every four days.

2.6. Biological data collection

Daily biological parameters were collected in order to evaluate the blood quality factor (QF). Larviposition date, number of pupae deposited, individual weight of pupae deposited, and number of dead females were all used to calculate QF (separately fed and unfed females). These variables were combined in a standard formula to produce a blood meal nutritional quality (QF) value as an indicator of colony pupae production (De Beer et al., 2012). The parameters were entered into the blood processing database daily. This includes the number of dead females (fed and unfed), the number of larvae deposited (both living and dead larvae), and the weight of pupae, which was divided into three categories (A: 22 mg, B: 22 to 28 mg, C: 28 to 32 mg, D: 32 to 36 mg, and E: > 36 mg). For the purposes of the study, QF of 1.5 or above this value was considered acceptable and indicates that the blood regime is efficient and suitable for the production of quality pupae. The nutritional quality of each diet was established on the basis of information collected over 30 days period.

2.7. Data analysis

Kaplan-Meier survival curves (Cox, 1972; Tarone, 1975; Tarone and Ware, 1977) were calculated by taking into account each treatment (consisting of a cohort of 120 flies), the day of occurrence of each mortality. Each flies' mortality was recorded separately in an Excel database as an event (death). Then, coding in the database was performed considering "one" as the occurrence of mortality recorded in a fly at a given date. For each frozen blood treatment, QF before and after freezing was compared using the T-student test. A multivariate analysis of variance model was used to assess the cross-effect of treatments and flies' feeding status (fed and unfed) on average daily mortality. Post hoc multiple comparison tests were used to test differences in the number of pupae per day per initial female (APPFiD) and pupae weight means (F tests). The Tukey test was used to perform pairwise multiple comparisons. These tests are based on pairwise linearly independent comparisons of estimated marginal means. For data analysis, IBM SPSS V20 software was used.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

3. Results

3.1. Survival rate

Flies were fed frozen blood treatments on artificial membranes for one month, three months, and six months, with fresh blood control renewed every four days. During the 60-day monitoring period, there was no significant difference in fly survival rates between treatments (df: 15; Chi-Square: 7.53; p-value: 0.941; Figure 2). Thus, mortality rates for the control diet (R0) were relatively low, at 32.1%, compared to 32.4%, 37.5%, and 38.2% for R3, R6, and R1. Alternatively, the daily mortality of females before the first larviposition was not similar (df: 3; F: 68.62; *p*-value = 0.0001), ranging from 0.6 ± 0.3% (R3) to 2.71 ± 0.9% (R6), with 1.7 ± 0.7% for the control (R0). Furthermore, the analysis revealed that the proportion of dead fed flies in six-month frozen blood was relatively high (0.27) when compared to whole fresh blood (0.08) and one-month frozen blood (0.07) (df: 3; F: 3.66; p = 0.013).

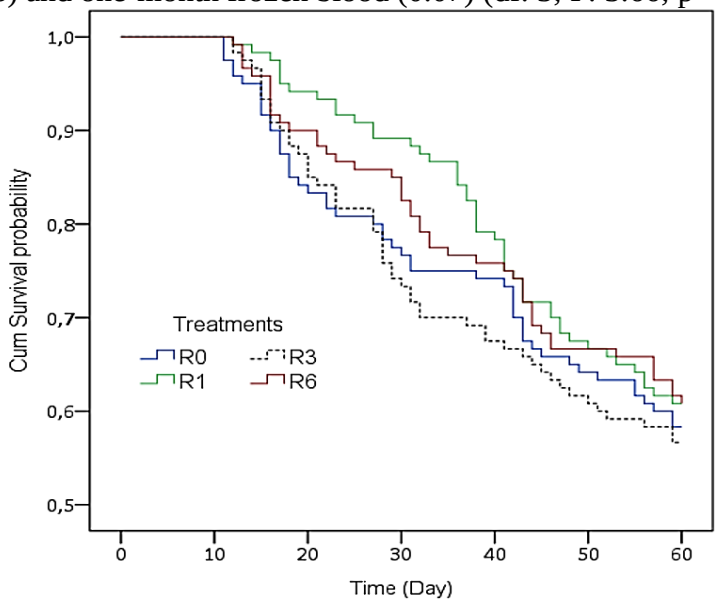

Figure 2. Survival curves of female flies fed with blood meals at different cold storage times (1: fresh blood 4°C, 2: frozen blood one month, 3: frozen blood three months, 4: frozen blood six months)

3.2. Nutritional value of blood

Table 1 summarizes the effect of freeze storage duration on diet QF. A comparison of the reproductive performance analysis model values (QF) revealed a decrease in nutritional quality of the frozen blood that correlated with storage duration (df: 3; T-student: 5.76; S: 0.01 0.05; Table 1).

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

Table 1. Nutritional value (QF) of blood meal treatments tested on tsetse flies before and after freezing

QFs per Test	R 1	R 3	R 6
QF Test 1	1.65 ± 0.1	2.11 ± 0.14	1.29 ± 0.08
QF Test 2	0.94 ± 0.12	1.43 ± 0.27	1.05 ± 0.11
T-test (df: 3)	*T-student*: 5.76; *S* : 0.01 < 0.05	*T-student*: 2.45 *S*: 0.092 > 0.05	*T-student*: 2.93; *S*: 0.061 > 0.05

Treatment 1 to 3: frozen bovine blood at - 18 °C for 6 months, 3 months and 1 month, respectively.
QF: an indicator of the nutritional value of the blood meal in tsetse flies colony.

3.3. Female productivity

The flies fed fresh blood deposited their larvae on average 16.25 days earlier than the other treatments, where the first larvae were deposited between days 17 (R1 and R3) and day 18. (R6). After 60 days of rearing, the number of pupae per initial female was 2.64 (R0), 1.56 (R1), 1.5 (R3), and 1.02 (R6) (Figure 3). As a result of the decreased nutritional quality of the blood meal, flies performed poorly on the six-month-old frozen blood meal when compared to fresh blood meal (df: 3; F: 6.68; $p < 0.001$; Table 1). The average pupae weight varied between 22.09 0.3 mg (R3) and 23.37 0.28 g (R0), with a statistically significant difference (Figure 3; df: 3, F: 3.24, p 0.05). Tukey's pairwise comparison, on the other hand, revealed comparable mean pupae weights for R6, R3, and R1 (Figure 4). Fly emergence was relatively low, at 93% for the R6 treatment compared to 96 percent for the R3, 96% for the R1, and 97% for the R2 (R0).

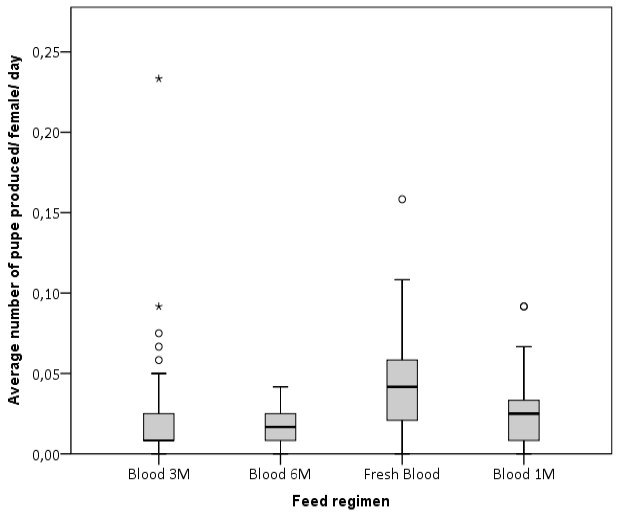

Figure 3. The average number of pupae per day per initial female (APPFiD) according to blood meal type after 60 days of feeding (M: month)

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

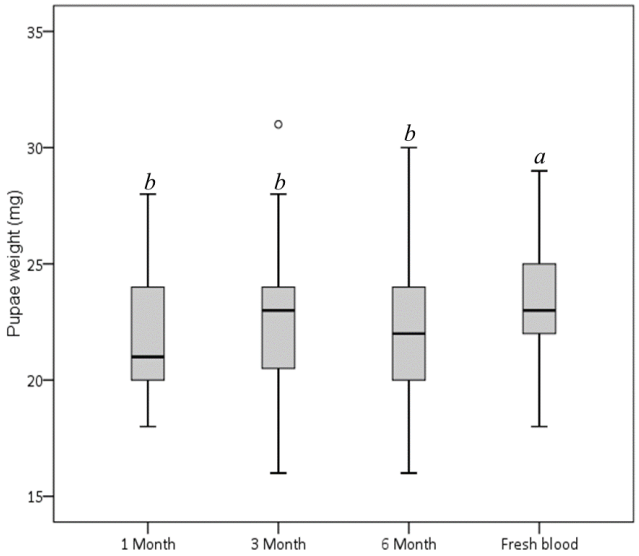

Figure 4. Average weight (mg) of pupae collected according to the duration of the blood storage. Data with different letters are significantly different ($p < 0.05$) (Tukey univariate test)

Discussions

The tsetse species reared at the insectary are fed with frozen blood, which provides consistent and adequate reserve treatment. Apart from the environmental breeding parameters that are more or less controllable in the breeding of these tsetse flies, biological parameters such as survival, fecundity, pupae size, etc., could be influenced by the nutritional value of the blood meal.

The survival rate recorded for all experimental blood diets is lower than the average value of 75.9% (for 50-days period) recorded by (Bauer & Aigner, 1978) in *G. palpalis palpalis* colony fed on the membrane with pig blood. The survival rates recorded for all frozen blood diets were comparable to those recorded by Camara et al., (2021) in the same insectary and by Pagabeleguem et al (2021). The survival rate of the control diet constituted with fresh blood was comparable to the proportion (56%) recorded by Wetzel & Luger (1978) with G. palpalis palpalis fed on fresh blood. The nutritional value of the blood meal was lost after a long period of freezing, according to QF analysis (Wetzel & Luger, 1978). The tsetse fecundity in the control treatment was higher than the previous results (1.35 and 1.25) obtained with the same CIRDES insectary (Percoma, 2006). According to Byamungu et al., (2011), a poor fecundity of 0.94 pupae per female was recorded after 30-days test with *G. austeni* when feeding with 5-year-old frozen blood. As an alternative to decreasing the nutritional value of frozen blood with negative consequences on tsetse fly reproduction, the study conducted by Wetzel &

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

Luger, (1978) shows that an addition of 10-3 Moles of ATP to the blood meal improves tsetse fly fecundity. In the absence of ATP, which is relatively expensive, our results suggest that in terms of blood bank management and for better colony productivity, the stored blood should be used rapidly once the results of the 30-day feeding test are available.

Furthermore, our findings show that fresh blood is nutritionally superior to frozen blood because I flies fed with fresh blood larviposited earlier, ii) these batches of flies produced an average of 25% more pupaes than the flies in the frozen blood groups, and iii) their pupae weight was greater. Thus, the study showed a significant decrease in pupae production for the six-month blood treatment, with high mortality.However, although the control treatment showed better survival than the other treatments, overall daily losses of females were abnormally high, i.e. well above the acceptable value of 1.2% (Gooding et al., 1997). The poor performance of a colony fed with a whole fresh blood meal be explained in this way if we consider the health problems concerning bacterial contamination (Bauer & Aigner, 1978; Kaaya et al., 1987; Wetzel & Luger, 1978) and/or the presence of antibiotic residues at low doses in the collected blood (Kaaya et al., 1987; Pooda et al., 2013). Using fresh blood for colony rearing may therefore expose the flies to health risks. In view of the above-mentioned health issues not elucidated in this study, research efforts in the insectary colony should focus on the detection of pathogens and chemical residues that may be present in blood meals before feeding.

Conclusion

The study provides a template for the possible creation of a frozen blood bank that could conceivably reduce a continuous supply of slaughter blood for feeding the tsetse fly colony. To ensure mass production of pupae, the blood bank must be renewed within a period not exceeding one month after the feeding test. Nevertheless, additional studies aimed at determining the maximum time of freezing of the blood meal and at developing technologies allowing the detection of traces of chemical residues present in fresh blood meals are necessary for optimal management of the blood meal bank of the CIRDES insectarium.

Acknowledgements

The work was supported by the CIRDES Bobo-Dioulasso (Burkina Faso).
We thank Dr YAPI-GNAORE C. Valentine who agreed for data collection.

Conflic of interest

The authors declare that they have no competing interests.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

References:

1. Bauer, B., Aigner, H., (1978). In vitro maintenance of Glossina palpalis palpalis (Robineau-Desvoidy) (Diptera: Glossinidae). Bull. Entomol. Res. 68, 393–400. https://doi.org/10.1017/S0007485300009378.
2. Bouyer, F., Seck, M.T., Dicko, A.H., Sall, B., Lo, M., Vreysen, M.J.B., Chia, E., Bouyer, J., Wane, A. (2014). Ex-ante Benefit-Cost Analysis of the Elimination of a Glossina palpalis gambiensis Population in the Niayes of Senegal. PLoS Negl. Trop. Dis. 8, e3112. https://doi.org/10.1371/journal.pntd.0003112.
3. Bouyer, J., Seck, M.T., Sall, B., Ndiaye, E.Y., Guerrini, L., Vreysen, M.J.B. (2010). Stratified Entomological Sampling in Preparation for an Area-Wide Integrated Pest Management Program: The Example of Glossina palpalis gambiensis (Diptera: Glossinidae) in the Niayes of Senegal. J. Med. Entomol. 47, 543–552. https://doi.org/10.1093/jmedent/47.4.543.
4. Byamungu, M., Matembo, S., Benedic, K., Mashenga, G. (2011). Storage time effect on blood diet for tsetse mass production in sterile insect technique. J. Vector Borne Dis. 48, 93–95.
5. Camara, K., Ilboudo, K., Salou, E., Gimonneau, G. (2021). Evaluation of different blood feeding frequencies on Glossina palpalis gambiensis performance in a mass rearing insectary. https://doi.org/10.18167/DVN1/1SPLYD.
6. Cox, D. R. (1972) Regression models and life tables (with discussion). Journal of the Royal Statistical Society, Series B, 34, 187-220.
7. De Beer, C.J., Venter, G.J., Potgieter, F.T. (2012). Developing Quality Control Procedures to Sustain a Supply of High Quality Blood for Mass Rearing Tsetse Flies.
8. De Greef, C., Imberechts, H., Matthyssens, G., Van Meirvenne, N., Hamers, R. (1989). A gene expressed only in serum-resistant variants of Trypanosoma brucei rhodesiense. Mol. Biochem. Parasitol. 36, 169–176. https://doi.org/10.1016/0166-6851(89)90189-8.
9. Diall, O., Cecchi, G., Wanda, G., Argilés-Herrero, R., Vreysen, M.J.B., Cattoli, G., Viljoen, G.J., Mattioli, R., Bouyer, J. (2017). Developing a Progressive Control Pathway for African Animal Trypanosomosis. Trends Parasitol. 33, 499–509. https://doi.org/10.1016/j.pt.2017.02.005.
10. Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.) (2005). Sterile Insect Technique. Springer-Verlag, Berlin/Heidelberg. https://doi.org/10.1007/1-4020-4051-2.
11. Feldmann, U., Hendrichs, J., Nations, F. and A.O. of the U. (2001). Integrating the Sterile Insect Technique as a Key Component of Area-

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

wide Tsetse and Trypanosomiasis Intervention. Food & Agriculture Org.

12. Fèvre, E., Coleman, P., Odiit, M., Magona, J., Welburn, S., Woolhouse, M. (2001). The origins of a new Trypanosoma brucei rhodesiense sleeping sickness outbreak in eastern Uganda. The Lancet 358, 625–628. https://doi.org/10.1016/S0140-6736(01)05778-6.
13. Fèvre, E.M., Wissmann, B. v., Welburn, S.C., Lutumba, P. (2008). The Burden of Human African Trypanosomiasis. PLoS Negl. Trop. Dis. 2, e333. https://doi.org/10.1371/journal.pntd.0000333.
14. Gooding, R.H., Feldmann, U., Robinson, A.S. (1997). Care and maintenance of tsetse colonies, in: Crampton, J.M., Beard, C.B., Louis, C. (Eds.), The Molecular Biology of Insect Disease Vectors. Springer Netherlands, Dordrecht, pp. 41–55. https://doi.org/10.1007/978-94-009-1535-0_5.
15. Jackson, A.P., Sanders, M., Berry, A., McQuillan, J., Aslett, M.A., Quail, M.A., Chukualim, B., Capewell, P., MacLeod, A., Melville, S.E., Gibson, W., Barry, J.D., Berriman, M., Hertz-Fowler, C. (2010). The Genome Sequence of Trypanosoma brucei gambiense, Causative Agent of Chronic Human African Trypanosomiasis. PLoS Negl. Trop. Dis. 4, e658. https://doi.org/10.1371/journal.pntd.0000658.
16. Kaaya, G.P., Darji, N., Otieno, L.H. (1987). Effects of bacteria, antibacterial compounds and trypanosomes on tsetse reproduction and longevity. Int. J. Trop. Insect Sci. 8, 217–220. https://doi.org/10.1017/S1742758400007232.
17. Kristjanson, P.M., Swallow, B.M., Rowlands, G.J., Kruska, R.L., de Leeuw, P.N. (1999). Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. Agric. Syst. 59, 79–98. https://doi.org/10.1016/S0308-521X(98)00086-9.
18. Langley, P.A. (1971). Rearing of tsetse flies in the absence of a living host. Trans. R. Soc. Trop. Med. Hyg. 65, 220–221. https://doi.org/10.1016/0035-9203(71) 90224-0.
19. Muhanguzi, D., Mugenyi, A., Bigirwa, G., Kamusiime, M., Kitibwa, A., Akurut, G.G., Ochwo, S., Amanyire, W., Okech, S.G., Hattendorf, J., Tweyongyere, R. (2017). African animal trypanosomiasis as a constraint to livestock health and production in Karamoja region: a detailed qualitative and quantitative assessment. BMC Vet. Res. 13, 355. https://doi.org/10.1186/s12917-017-1285-z.
20. Muhanguzi, D., Okello, W.O., Kabasa, J.D., Waiswa, C., Welburn, S.C., Shaw, A.P. (2015). Cost analysis of options for management of African Animal Trypanosomiasis using interventions targeted at cattle in Tororo District; south-eastern Uganda. Parasit. Vectors 8, 1–9.

 ISSN: 1857-7881 (Print) e - ISSN 1857-7431

21. Mutika, G.N., Kabore, I., Seck, M.T., Sall, B., Bouyer, J., Parker, A.G., Vreysen, M.J.B. (2013). Mating performance of Glossina palpalis gambiensis strains from Burkina Faso, Mali, and Senegal. Entomol. Exp. Appl. 146, 177–185. https://doi.org/10.1111/j.1570-7458.2012.01305.x.
22. Pagabeleguem, S., Toé, A.I., Pooda, S.H., Dera, K.M., Belem, A.S., Belem, A.M.G., Ouedraogo/Sanou, G.M.S., Ira, M., Kaboré, B.A., Percoma, L., Sidibé, I. (2021). Optimizing the feeding frequency to maximize the production of sterile males in tsetse mass-rearing colonies. PLOS ONE 16, e0245503. https://doi.org/10.1371/journal.pone.0245503.
23. Parker, A.G. (2005). Mass-Rearing for Sterile Insect Release, in: Dyck, V.A., Hendrichs, J., Robinson, A.S. (Eds.), Sterile Insect Technique. Springer-Verlag, Berlin/Heidelberg, pp. 209–232. https://doi.org/10.1007/1-4020-4051-2_8.
24. Percoma, L. (2006). Memoire Online - Production de masse de glossines de qualité. Contribution à la campagne panafricaine d'éradication de la mouche tsé-tsé et des trypanosomoses (pattec). - Lassan PERCOMA [WWW Document]. Memoire Online. URL https://www.memoireonline.com/03/20/11636/m_Production-de-masse-de-glossines-de-qualite-Contribution--la-campagne-panafricaine-d-eradicatio4.html (accessed 8.22.21).
25. Pooda, S.H., Mouline, K., De Meeûs, T., Bengaly, Z., Solano, P. (2013). Decrease in survival and fecundity of Glossina palpalis gambiensis vanderplank 1949 (Diptera: Glossinidae) fed on cattle treated with single doses of ivermectin. Parasit. Vectors 6, 165. https://doi.org/10.1186/1756-3305-6-165.
26. Tarone, R., and Ware J. (1977). On distribution free tests for equality of survival distributions. Biometrika, 64, 156-160.
27. Tarone, R. (1975). Tests for trend in life table analysis. Biometrika, 62, 679-682.
28. Vreysen, M.J. (2001). Principles of area-wide integrated tsetse fly control using the sterile insect technique. Med. Trop. Rev. Corps Sante Colon. 61, 397–411.
29. Wetzel, H., Luger, D. (1978). The use of deep frozen, stored bovine blood for in vitro feeding of tsetse flies. Z. Fr Parasitenkd. Parasitol. Res. 57, 163–168. https://doi.org/10.1007/BF00927157.