

Feeding frequency and its associated effects on the production and survival rate of *Glossina fuscipes fuscipes*

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Abstract

Tsetse flies are large blood-sucking flies of the genus *Glossina*, which are exclusively found in Africa. They are important vectors of *Trypanosomes*, which cause sleeping sickness in humans and African Animal Trypanosomosis (AAT) in livestock. Due to the lack of effective vaccines to control this disease, vector control has been an ideal strategy. There are many vector control methods developed so far. Sterile Insect Technique (SIT) is one of such method which depends on the mass production of male flies in the laboratory, sterilization, and release of these flies in the target area. The study was conducted to evaluate the effect of different feeding frequencies on the production and survival rate of *Glossina fuscipes fuscipes* to identify the best feeding frequency that could optimize the production and minimize the cost of production in a mass-rearing insectary facility at Kaliti Tsetse Fly Mass Rearing and Irradiation Centre, Addis Ababa, Ethiopia. Three experimental groups were established based on feeding frequency regimes. The first group was fed three times per week, the second four times per week, and the third group five times per week. Parameters that are routinely used for assessing colony performance such as; survival rate, fecundity, pupae per initial females (PPIF), and pupal quality were evaluated for each treatment group and statistical comparisons were made between the groups. The results revealed that feeding frequency had a crucial effect on fly production and survival. The lowest results for all parameters were obtained in flies fed three times per week and relatively the best results were obtained in the four-times-per-week feeding regime. Therefore, we recommend a further study on aspects that are not addressed in this study, such as emergency rate and progeny quality, to apply the four times per week feeding regime as it will have a positive economic impact that could enable

more resources to be re-injected into the insectary compared to five or more feeding regimes which are currently in use in the study site.

Keywords: Feeding regime; *G. f. fuscipes*; Mass-Rearing Productivity; Survival Rate.

Introduction

Tsetse flies are blood-sucking flies of the genus *Glossina* that belong to the family Glossinidae (Radostitis *et al.*, 2007). They are found exclusively on the African continent, between 5°N to 20°S latitudes (Warnes *et al.*, 1999). In Ethiopia, the vector distribution is mainly confined to the southern and western regions between longitudes of 33° and 38 °E and latitudes of 5° and 12 °N (Kotye, 2006). Among 31 species of tsetse flies found in Africa, five species, namely, *Glossina pallidipes*, *G. morsitans*, *G.f. fuscipes*, *G. tachinoides*, and *G. longipennis* are known in different regions of Ethiopia (Amhara, Benishangul Gumuz, Gambella, Oromia, Sidama, and Southern and Southwestern regions) (Langridge, 1976).

Tsetse flies can be ranked among the most destructive pests and are the vectors of the causative agents for sleeping sickness in humans and African Animal Trypanosomosis (AAT) or Nagana in livestock (Vreysen, 2001). Due to the lack of effective vaccines and high costs of disease treatment, disease control via control of the tsetse vector is the most effective strategy. So far, many chemical and biological methods of tsetse fly control have been developed, each of which has its advantages and limitations. Currently, the vector control interventions involve the use of insecticides either through sequential aerosol spraying technique (SAT); ground spraying; insecticide-treated targets or insecticide-treated animals – live baits; the use of traps, and the Sterile Insect Technique (SIT) (WHO, 2011).

Sterile Insect Technique is a biological insect control method based on genetics. It involves the mass production of male flies in a laboratory, sterilization, and release of these flies onto the target area. As compared to other techniques, the SIT is non-intrusive to the environment, has no adverse effects on non-target organisms, is species-specific, and can easily be integrated with biological control methods such as parasitoids, predators, and pathogens (Leak, 1999). There is no threat of resistance development to the effects of sterile

males if adequate quality assurance is assured during the production process and the sterile insects cannot be established in released areas, as is the case with other biological control programs (Vreysen, 2001). On the other hand, the SIT necessitates efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen, 2005). Sterile Insect Technique is an ideal mechanism to be combined with other methods of control and it fits well with the concept of integrated pest management (IPM). The control of diseases within an area-wide integrated pest management approach (AW-IPM) using the SIT has shown its effectiveness in some Sub-Saharan African countries for example, in Zanzibar (Vreysen *et al.*, 2000). Motivated by these encouraging results, the SIT project in Ethiopia was initiated and designed in 1997 with the support of the International Atomic Energy Agency (IAEA) in the southern rift valley area. Its ultimate objective was to create a tsetse-free zone in a 25,000 square kilometers area suitable for agricultural development. To achieve this objective, Kaliti Tsetse Fly Mass Rearing and Irradiation center was inaugurated on 3 February 2007 with the mission of creating a zone free of the tsetse fly in the Southern Rift Valley of Ethiopia. When completely equipped and operational, the new facility will have a colony capacity of approximately 7 million female flies and produces over 700,000 sterile male flies per week, enough to treat approximately 7000 km² at a time (IAEA, 2007).

Currently, two species of flies namely, *G. pallidipes* and *G. f. fuscipes* are mass-reared and efforts have been made to satisfy the needs of sterile males for the program of the country, Ethiopia and other countries infected by the same tsetse species both in quantity and in quality. Despite the coordinated effort to meet the objectives, the demand is not satisfied yet indicating the need to assess and solve the problems that are hindering the effort. The quality of the insects can be impaired by many factors in the rearing facility including crowding, rearing procedures, diets, insect pathogen load, deterioration of the strain, laboratory adaptation, genetic drift, and others or through handling, irradiation, packaging, release methods, etc. (Simmons *et al.*, 2010).

Feed and feeding strategy are among the vital factors that determine the success of mass production in tsetse fly insectary facilities. The amount of blood imbibed during the inter-larval period is the major factor determining the productivity and survival rate of tsetse, *Glossina* species. Therefore, a qualitative and quantitative amount of blood is needed for the maintenance of fly colonies (IAEA, 2007) *in vitro*. This in turn requires more resources to be invested to

maintain production at a possible optimum level with affordable cost. As a result, optimized feeding strategies which are both efficient and cost-effective are mandatory in any given insectary facility. With the above background, the objectives of the present study were to test various feeding regimes and recommend the best, both in cost-effectiveness as well as to optimize the production of sterile male flies of *G. f. fuscipes* in tsetse mass-rearing facilities.

Materials and methods

Study area

This experimental study was conducted at Kaliti Tsetse Fly Mass Rearing and Irradiation Centre in Addis Ababa. Addis Ababa is the capital city of Ethiopia with geographical locations of 9.03° North; 38.74° East, latitude and longitude, respectively. The center was inaugurated on 3 February 2007 with a mission of creating a zone free of tsetse flies in the Southern Rift Valley of Ethiopia and, when completely equipped and operational, the facility was supposed to have a colony capacity of approximately 7 million female flies and will be able to produce over 700,000 sterile male flies per week enough to treat approximately 7,000 Km² at a time (IAEA, 2007).

Study population and study period

The study was conducted from March to May 2022 to evaluate the effects of feeding frequency on *G. f. fuscipes*. The experimental flies used for this study, 450 (360 females and 90 males flies, were obtained from the same production batch of pupae that originated from the main colonies of the insectary (Kaliti Tsetse Fly Mass Rearing and Irradiation Centre). Immediately after they had emerged from incubated pupae flies were rendered to immobility by using a chiller (+4 - sex-sorted and placed separately in colony cages with 50 (40 females per 10 males) flies per cage called colonies, following the predefined sex ratio (4:1) and maintained in a replicate of cages diameter 20 cm and width of 5 cm netting on top and bottom for feeding and collection of the hatched larvae (FAO/IAEA, 2006) within the rearing section (room) as the main colony throughout the experimental period.

Study design

An experimental study was conducted on feeding frequency and its associated effects on *G. f. fuscipes*, which is one of the *Glossina* species reared at Kaliti

Tsetse Fly Mass Rearing and Irradiation Centre. Three experimental groups with a total of 450 tsetse flies were established based on feeding frequency regimes in a week. These groups were coded as F3, F4, and F5 based on a feeding frequency regime representing a feeding frequency group of three, four, and five times per week, respectively. Each group had 150 flies and a replicate of 3 mating cages with 50 (40 female and 10 male) flies per cage. All groups were maintained in the same controlled and optimum environmental conditions of an insectarium similar to the general colony of the center, at a temperature of 23 – 25 °C and a relative humidity (RH) of 75 – 80% (FAO/ IAEA, 2006). Then, they were subjected to this experimental study, and the experimental parameters such as the average productivity, parameters measured as the number of Pupae Per Initial Female (PPIF), fecundity, survival rate (mortality), and the number of pupae production, were measured and analyzed for all the tested groups accordingly.

Feeding frequency test: The first group (F3) was fed three times (Monday, Wednesday, and Friday), the second group (F4) four times (Monday, Tuesday, Thursday, and Saturday), and the third group (F5) five times (Monday, Tuesday, Thursday, Friday and Saturday). All experimental groups of flies were fed with a similar quality of blood factor from the same batch of defibrinated gamma irradiated bovine blood meal using an in vitro silicon membrane system which was previously collected aseptically and frozen at -20 °C (IAEA, 2007). The feeding system was installed in a climatic room that maintained a similar environmental condition of 25 ± 1 °C and $50 \pm 5\%$ humidity for all treatments. The flies remained in the feeding room for less than 30 minutes and were placed under similar and optimum environmental conditions (FAO/ IAEA, 2006). The holding room temperature and humidity were adjusted and monitored daily from the data logger set in the rearing room. Pupae were collected daily and a mortality check was performed weekly. The weekly datasets of the colonies, in different feeding regimes, were measured by testing tsetse production and survival parameters including fecundity, Pupae per Initial Female (PPIF), mortality rate and pupae production, weight, and pupal size (classes are graded based on their size).

Fecundity test: Fecundity was expressed as the number of pupae produced per female per 10 days, by considering day 18 immediately after they emerged from the pupae stage as the first larviposition day (FAO/IAEA, 2006).

Pupae sorting and grading test: The collected Pupae from larviposition cups on daily bases throughout the experimental period and sorted into normal and aborted L3 by visual observation. Then the normal pupae were categorized into five size classes by a sorting machine. The standard system has five collecting shuts labeled, A (smallest) to E (largest); the length of the collection area has been adjusted to correspond to the five weight classes that previously have been defined to tsetse pupae of *G. fuscipes* (Zelger and Russ, 1976).

Mortality test: Mortality was recorded daily for each test group throughout the experimental period. Dead flies were sorted into blood-fed and starved fly mortalities. The mortality rate was calculated according to Standard Operating Procedures (SOP) for Mass-Rearing Tsetse flies (FAO/ IAEA, 2006).

Data analysis

The data from this experimental study results were organized, coded, and analyzed by Stata version 12.0 software. One-way analysis of variance (ANOVA) was used to investigate the link between parameters and feeding frequency. After a significant ANOVA, paired mean comparison tests were used to examine differences between pairs of feeding regimes. Statistical significance was set at the conventional level of “less than 5%” for all analyses.

Results

Pupae production and pupal size class distribution

The first larviposition date for all blood-feeding treatment groups (3 times per week (F3), 4 times per week (F4), and 5 times per week (F5)) was the same as on day 18. The highest number of pupae was produced by the flies fed 5 times per week (n = 390) followed by flies fed 4 times per week (n = 386) and 3 times per week (n = 271) respectively (Table 1).

Table 1. The effect of feeding frequency on pupal production, pupal size, and PPIF among the test groups – F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

Treatment groups	Total pupae produced	Pupal size classes distribution percentage within the group					PPIF
		A	B	C	D	E	
F3	271	49 (18.08%)	78 (27.80%)	105 (38.70%)	36 (13.30%)	3 (1.12%)	2.26
F4	386	40 (10.36%)	87 (27.54%)	137 (35.50%)	115 (29.80%)	7 (1.80%)	3.22
F5	390	38 (9.70%)	86 (22.00%)	112 (28.70%)	127 (32.60%)	27 (6.90%)	3.25

The results showed that the size-wise distribution of the pupae was not the same both within and among the test groups. The highest percentage of the total pupae produced was concentrated in class A – C sized pupae in the F3 feeding treatment group covering about 84.58% of the total pupae produced by the group, while class C – E sized pupae constituted the highest percentage in F4 and F5 feeding treatment groups contributing about 67.10% and 68.20% of their overall production respectively. Additionally, the highest number of the smallest size class pupae (class A) were produced by F3 (18.0%) followed by F4 (10.36%), and the least was produced by F5 (9.70%) and the reverse was true for the largest pupal class (class E) covering 6.90%, 1.80% and 1.20% of the total pupal production of their respective group, F5, F4, and F3 respectively. The pupal size classes' distribution for each test group is shown in figure 1.

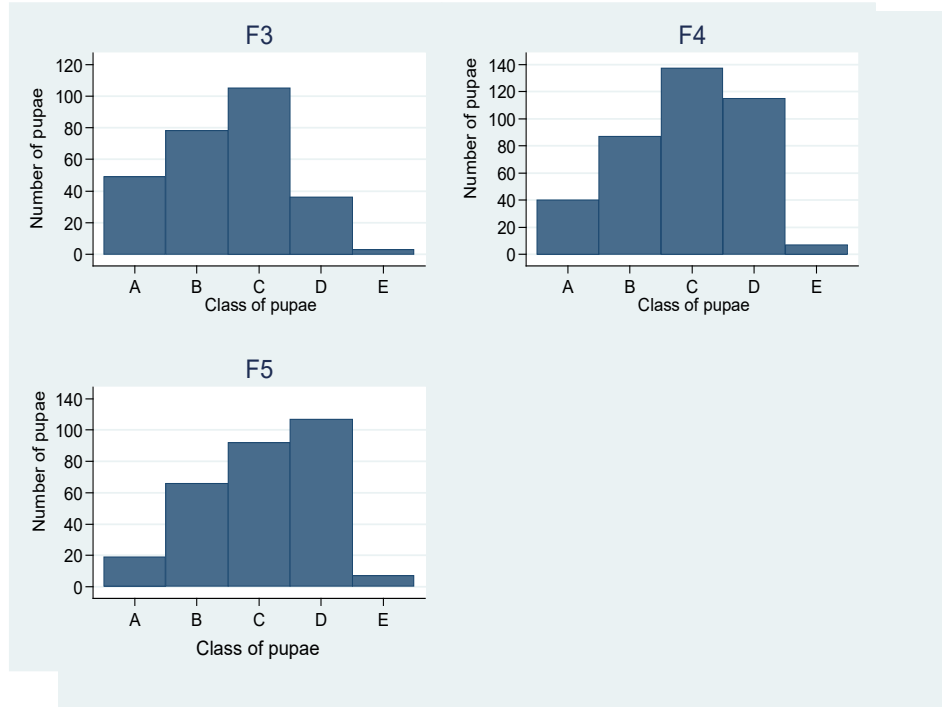


Figure 1. Pupal size classes' distribution of test group, F3 (three blood meals per week), F4 (four blood meals per week), F5 (five blood meals per week).

Pupae per initial females (PPIF) and fecundity

Pupae per initial females (PPIF), which is expressed by the total pupae produced by initial females at a given time was evaluated for each test group. The result revealed that the highest overall PPIF was produced by group F5 (3.25 PPIF) followed by group F4 (3.22 PPIF) and the least was by group F3 (2.26). Similarly, fecundity, which is defined as pupae per female per 10 days (P/F/10 days), was assessed for each group. The lowest fecundity was observed for the feeding treatment group F3 with a mean fecundity of 0.559 ± 0.15 pupae/females/10 days compared to both F4 and F5 with a mean fecundity of 0.721 ± 0.15 and 0.697 ± 0.01 pupae/females/10 days in that order. A statistically significant link was found between feeding frequency and fecundity (ANOVA, $p > 0.02$ $df = 2$). The mean difference was significant for F3 vs F4 and F3 vs F5, but not for F4 vs F5 (Table 2).

Table 2. Pairwise comparisons of means of fecundity between test groups – F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

Paired groups	Contrast	Std. Err.	t	P>t	[95% Confidence Interval]	
F4 vs F3	0.162	0.059	2.753	0.014	0.035	0.289
F5 vs F3	0.138	0.059	2.345	0.027	0.017	0.259
F5 vs F4	-0.024	0.059	-0.408	0.687	-0.145	0.097

Figure 2 shows the trend of PPIF for the three treatment groups over the study period.

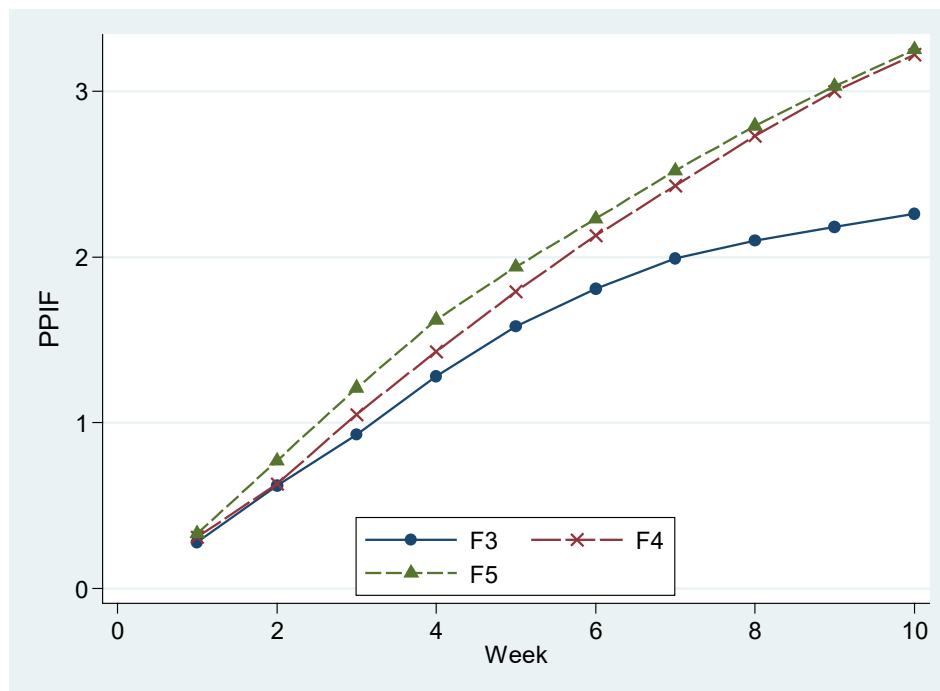


Figure 2. The trend of PPIF for the three treatment groups, F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

Fly mortality and survival rate

The overall female mortality rate was highest for females fed 3 times per week (41.60%) followed by females fed 5 times per week (29.80%) and the lowest was for flies fed 4 times (28.60%). The dissection result of the dead female flies indicated that only 1.80% of the total dead flies had blood in their abdomen (blood mortality), 0.42% died of unknown causes and all the rest died due to starvation. The statistical analysis showed that there was no link between mortality and the causes of mortality ($p > 0.05$). Nevertheless, the frequency of blood feeding had a significant effect on female survival and/or mortality (ANOVA, $p > 0.044$ $df = 2$). Females' mortality was highest for females fed 3 times per week (41.60%) followed by females fed 5 times per week (29.80%) and the lowest was for flies fed 4 times (28.60%) with an average daily mortality rate of 1.15%, 0.79% and 0.82% for F3, F4, and F5 respectively. When a pairwise mean comparison was made between the groups, mortality in F3 was significantly higher than in both F4 and F5. However, there was no statistically significant difference in females' mortality rate of treatment groups F4 and F5 (Table 3).

Table 3. Pairwise comparisons of means for mortalities between test groups – F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week).

Paired groups	Contrast	Std. Err.	t	P>t	[95% Confidence Interval]	
F4 vs F3	-3.000	1.260	-2.381	0.030	-5.704	-0.296
F5 vs F3	-2.727	1.260	-2.164	0.039	-5.301	-0.154
F5 vs F4	0.273	1.260	0.216	0.830	-2.301	2.846

Figure 3 shows the trend of survival rate for the three feeding frequency groups over the study period.

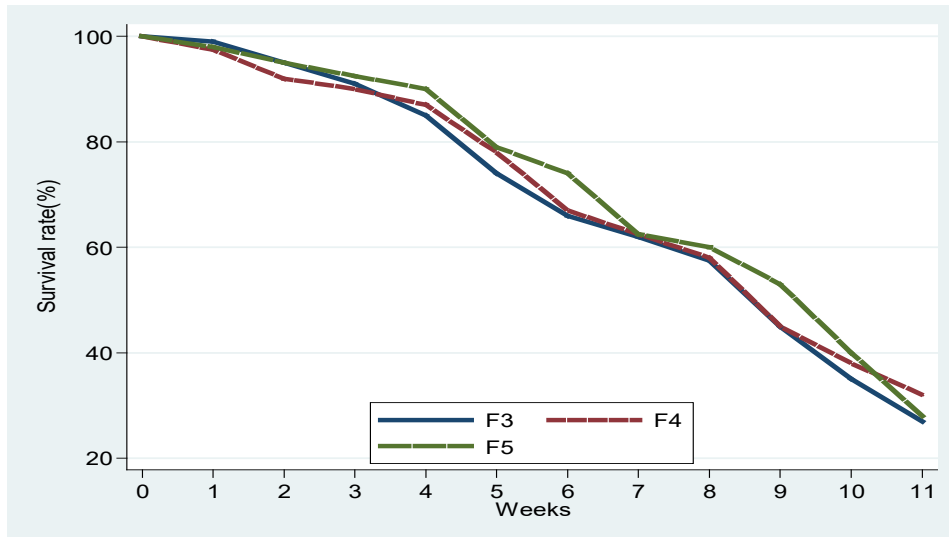


Figure 3. The trend of survival rate for flies fed at different frequencies per week, F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week).

Discussion

In this study, the effect of different feeding frequencies on the survival and production of *G. f. fuscipes* was assessed to identify the best feeding frequency that could be applied in tsetse fly mass-rearing facilities. Survival, fecundity, pupae per initial females (PPIF), and pupal quality are known to be the crucial parameters routinely used for assessing colony performance in all standardized insectary facilities. Hence, these parameters were evaluated for each treatment group and statistical comparisons were made between the groups.

The current results revealed that feeding frequency had a crucial effect on the productivity and survival rate of *G. f. fuscipes* subjected in the study. Flies fed four times per week and five times per week produced significantly more pupae with a total pupal production of 386 and 390 pupae, respectively, compared to those fed three times per week that produced only 271 pupae throughout the study period. This finding is in agreement with the findings of other *Glossina* species, *G. pallidipes*, by Tsegaye *et al.* (2020) who reported low pupal production for flies fed three times per week compared to five times per week.

It is a fact that pupal class is a good overall quality indicator of the effectiveness of colony maintenance where each weight class can be defined using a size-sorting machine. The mean pupal weights should approximate the values developed by Zelger and Russ (1976), and no more than 10% of the puparium should be in weight class A (FAO/IAEA, 2006). Based on this fact, the results of this study indicated that flies fed four and five times per week produced higher quality pupae than flies fed three times per week. Likewise, the highest percentage of pupae, which are considered to be of high-quality classes (class C and D), was highest in F5 followed by F4, although the difference between the two was not significant. These results were in line with the findings of Kettle (1995) who found that the weight of a pupa depends on the amount of blood taken by a female during pregnancy, with a highly significant correlation between puparia weight and quality of blood ingested. The reason for this finding could be due to the nutrient shortfall needed for the required performance in flies fed three times per week as stated by previous findings, which stated pupal quality is an indication of the nutritional status of the fly and is reflected by pupal weight and size (IAEA, 2007).

Regarding PPIF, the overall commutative Pupae per Initial Female (PPIF) for the group of flies subjected to five times per week feeding regime produced 3.25 PPIF which was the highest of all, although the difference was not significant compared to four times per week feeding frequency regime with overall PPIF of 3.22. On the other hand, flies subjected to the three-times-per-week feeding frequency regime produced an overall PPIF of 2.26, which was significantly lower than the four and five-times-per-week regimes. Similarly, the lowest fecundity was observed for the feeding treatment group F3 with a mean fecundity of 0.559 ± 0.15 pupae/females/10 days compared to both F4 and F5 with a mean fecundity of 0.721 ± 0.15 and 0.697 ± 0.01 pupae/females/10 days in that order. The data on PPIF for *G. f. fuscipes* and *G. pallidipes* showed a wide range of PPIF depending on different factors. Results range from 4 – 7 using different food sources obtained by Langley and Pimley (1979) indicating that the PPIF could be influenced not only by the frequency of feeding but also by the quality of the feeding source. In the same way, seven pupae per initial female were reported when flies were fed on cow blood, and 6.8–7.8 when fed on rabbits (Mews *et al.*, 1976). The values of PPIF can vary greatly, from 1.6 to 14 and the PPIF required for the establishment of a colony should be ≥ 3 . Thus, the results for both F4 (3.22) and F5 (3.25) were above the previously established limit and satisfactory, but the values obtained for F3 colonies in our study were lower than the limit required to establish a fly colony in a given insectary facil-

ity. On the other hand, studies on tsetse fly-rearing facilities stated that for a steadily growing tsetse colony, it was important to make sure that the colony's daily mortality was kept below 1% and the fecundity (pupae/female/10 days) was above 0.60 (Jordan, 1980). Thus, the fecundity of the F4 and F5 groups in this study was above the average acceptable level whereas F3 was below the average lower limit.

The results of this study on mortality and survival revealed that the overall female mortality was highest for females fed 3 times per week (41.60%), followed by females fed 5 times per week (29.80%), and the lowest was for flies fed 4 times (28.60%) with an average daily mortality rate of 1.15%, 0.79% and 0.82% for F3, F4, and F5, respectively. The daily mortality rate recorded in the two feeding regimes (F4 and F5) was less than the acceptable daily mortality rate (1.20%) suggested by the IAEA (2007). In contrast to this, the daily mortality for F3 was above the acceptable level of daily mortality, highlighting that applying this feeding regime affects the steady growth of the tsetse colony. These results are in agreement with the previously obtained data on other *Glossina* species, *G. pallidipes* and *G. morsitans morsitans*, which proved the lowest survival and productivity of three times per week feeding as compared to four and six times per week feeding regimes indicating a positive correlation between feeding frequencies and adult fly survival as well as fecundity (Langley and Stafford, 1990). According to these authors, the minimum number of blood meals per cycle (an inter-larval period of 9 days) which did not cause a decline in reproductive performance was 4 times for *G. m. morsitans* and five for *G. pallidipes*. Gaston *et al.* (1993) also stated that the flies fed every third day, and always engorged fully at every opportunity, whereas flies offered food every second day refused to feed at all or did not engorge fully at every opportunity. Furthermore, previous studies indicated that in the laboratory, female flies which take a full blood meal every third day perform less (survival and productivity) than those which take on average rather smaller blood meals every second day (Langley and Pimley, 1979). The best outcomes for F4 in nearly all parameters evaluated here could be because the meal, which is critical for normal larval development, was more likely to be attained with the 4 meals per week regime and hence the flies have more opportunity to have a meal at the critical time. On the other hand, even though a feeding frequency of five times per week optimized the number of blood-fed flies, it is important to note that the tsetse fly needs on average 2 days to completely digest a blood meal (McCue *et al.*, 2016).

Conclusions

In conclusion, the best results for almost all parameters evaluated in this study were obtained in treatment F4 (4 blood-feedings per week), and the lowest results were obtained in treatment F3 (3 blood feedings per week) indicating that feeding flies four times per week instead of five or more times per week will have no adverse effect on colony production performance. In contrast to this, treatment F3 (3 blood-feedings per week) led to significant negative effects on female survival and production parameters although maintenance remained acceptable in some regards. It is, therefore, recommended to feed tsetse flies, particularly *G. f. fuscipes* species, four times per week in the tsetse flies' factory. Furthermore, the effects of the feeding regime on tsetse fly emergence rate and progeny quality need to be assessed.

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