

Study on the reproductive efficiency of *Glossina pallidipes* and *Glossina fuscipes fuscipes* reared under laboratory conditions

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Abstract

Tsetse flies (*Diptera: Glossinidae*) are blood-feeder flies of the genus *Glossina* that belong to the family *Glossinidae*. SIT is based on the mass production, radiation-based sterilization and release of sterile male tsetse flies over a target area to suppress or locally eliminate a target tsetse population. The aim of this study was to assess the reproductive efficiency of *Glossina pallidipes* and *Glossina fuscipes fuscipes* reared under laboratory conditions in terms of productivity, and survival of flies fed on different animal blood. A total of 384 flies from each female *G. pallidipes* and *G. fuscipes fuscipes* were used to study the Reproductive Efficiency of *Glossina pallidipes* and *Glossina fuscipes* in terms of productivity and survival of flies obtained from stock colony of Kality Tsetse Research Center and fed on animals' blood of bovine, camel, ovine and caprine for a period of 3 months. Duncan multiple range test was performed to analyze pupae per initial female, pupae production, fecundity, survival test fed on different blood diets using Stata computer software (version 12.0). The result revealed that Pupae per initial female recorded in *G. pallidipes* fed on the blood of caprine was significantly lower compared to those flies fed on the blood of the other animals. On the other hand, female flies of *G. fuscipes fuscipes* which fed on the blood of camel had significantly higher mean pupae per initial female than those flies fed on the blood of ovine, bovine, and caprine. Moreover, pupae quality as measured by weight class showed that flies fed caprine blood had also more small weight pupae (class A and B) compared to flies fed camel blood. However, in *G. pallidipes*, a statistically significant difference was not recorded in their survival rate in females flies fed camel, bovine and ovine blood. But in general camel blood was found to be the most suitable blood followed by ovine, bovine, and caprine in terms of productivity, pupae production, fecundity, and pupae weight of flies. Camel blood meal should be used for *in vitro* feeding purpose of both *G. pallidipes* and *G. fuscipes fuscipes* species of tsetse flies under laboratory condition to ensure better production.

Introduction

Background of the study

Tsetse flies (*Diptera: Glossinidae*) are medically and economically important insects of the genus *Glossina* that belong to the family *Glossinidae* [1]. They are confined almost exclusively to the sub-Saharan African continent, between 5°N to 20°S, latitudes, but the two species are found in southwest Saudi Arabia [2]. Over 31 species of tsetse flies are present in the continent of Africa. However, only five species namely, *Glossina pallidipes*, *G. m. submorsitans*, *G. f. fuscipes*, *G. tachinoides*, and *G. longipennis* are known in different parts of Ethiopia (Amhara, Benishangul Gumuz, Oromia, Southern and Gambella) region of Ethiopia are infested with more than one species of tsetse fly [3,4]. In Africa, the presence of tsetse flies and parasites is considered a major cause of poverty in humans (sleeping sickness) and animals (Nagana) [5,6].

Vaccinating against the African animal Trypanosomiasis is unsuccessful due to the sophisticated and evasive nature of the parasite. The parasites are shrouded in a thick glycoprotein coat and they can intermittently change resulting in the immune system being in a constant state of catch-up to identify the ever-changing parasite [7], and existing trypanocidal for chemotherapy is not always effective due to a reduction in efficacy as a result of increasing drug resistance in the parasites [8]. Currently, the vector control methods are sequential aerosol spraying technique (SAT), ground spraying, aerial spraying, insecticide-treated targets or live baits, the use of impregnated traps, and the sterile insect technique (SIT) [9].

SIT is based on the mass production of tsetse flies; radiation-based sterilization and the release of sterile insects over a target area to reduce or eradicate the target tsetse fly population [10]. As compared to other tsetse control methods, the SIT has a non-residual effect on the environment and does not affect the non-target organisms, is species-specific, and can easily be integrated with biological control and chemical methods [11]. The technique has been effectively used for the eradication of the new world screwworm (NWS) (*Cochliomyia hominivorax*) from North and then Central America to Panama [12] and tsetse (*G. austeni*) from Unguja Island in Zanzibar [13].

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 general objective was to create a tsetse-free zone in a 25, 000 square kilometer area suitable for agricultural development. To achieve this objective, the Kality Tsetse Fly Mass Rearing and Irradiation Center was inaugurated on 3 February 2007 with the mission of creating a zone free of tsetse fly in the Southern Rift Valley of Ethiopia [14].

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Key words: granules, ibuprofen, oral administration, pharmacokinetics

Received: December 18, 2025; **Accepted:** January 15, 2026; **Published:** January 22, 2026

Mass rearing of tsetse flies (*Glossina* spp.) in the laboratory is dependent on the high availability of blood and free of microbiological contaminant blood diet [15-18]. The use of host animals for live feeding for tsetse flies in mass-rearing insectariums is a risk to animals. For this reason, in the laboratory, it is necessary to develop effective and standardized daily tsetse fly feeding methods without using live animals for daily blood feeding [19]. Therefore, the use of membrane (*in vitro*) feeding technique to successfully produce mass tsetse flies and economically less risky.

Materials and methods

Description of the study area

The present experimental study was carried out at Kality Tsetse Fly Research Center of Insectarium located in Addis Ababa.

Blood collection and processing

Before the collection day, the containers, stirring material, and any other materials that were in contact with the blood were washed and sterilized in the oven for 24 hours. Blood of bovine (cattle), ovine (sheep), camel, and caprine (goat) was collected during the first week of January of 2023 from the cut of blood vessel of each host type in hygienically and separately collected from Addis Ababa Municipal Abattoir, in 10-liter stirring containers that allow defibrinating using the blood using a magnetic stirrer to prevent blood from clotting. The collected blood of each animal was sieved and poured separately into four-liter bottles and immediately taken to Kality Tsetse Fly Research Center. The blood of each animal in each bottle was labeled and stored in a cold room (-20°C) [20].

Blood processing procedures and irradiation

The collected blood was checked for microbial contamination, and bioassay for its nutritive value, and proportioned into bottles with a manageable size to be irradiated and fed to tsetse flies [21]. After four days of storage, all the 4 liter bottles that were collected on the same day were thawed. Blood was mixed into a 100 liter container, previously sterilized, to obtain one overall sample of the blood collected each day. This sample was later used for the bioassay (also called 25 day feeding test) and was composed of 25 vials labeled carefully. One single bioassay was carried out for each collection day. After sampling for the bioassay, the blood in the 100 liter container was proportioned into a volume of 2 liters bottle, and was labeled. The labeled bottles were frozen until the result of the bioassay was available. During the proportioning, a sample of the blood of each bottle must be taken and labeled this samples were kept together with its original bottle and was later be used for microbial screening [20].

Blood preparations were performed under UV laminar fans to reduce microbial contamination. Each frozen blood group with 2 liter bottles to were irradiated at 1- 1.5 kilogray (kGy) in the gamma cell 220 excel. After irradiation the blood was tested for bacteria by inoculating 3ml of a blood sample with syringe onto the petri Dishes and mixed with agar and the samples were incubated at 37°C for 48 -72 hours and read at 48 hrs and 72 hrs. The results were checked for bacterial colonies after 48 and 72 hrs. Blood with accepted colony number were passed for tsetse feeding (<10 colony number) and the rest discarded [21]. The bioassay samples were irradiated, after which, the 25 day feeding test was conducted by feeding the blood diet for tsetse flies at Kality Tsetse Fly Research Center. The bioassay test was done for 25 days checking feeding response, survival and number of pupae production. The parameter was used for to calculate the quality factor the QF

greater than one. Bioassay used for blood diet suitable for in colony maintenance for tsetse flies. The irradiated blood was transferred in to the working laboratory room and thawed in water until it changed in to liquid blood at $+4^{\circ}\text{C}$ refrigerators for feeding purpose.

Experimental flies

The tsetse fly species used for the study were *G. pallidipes* and *G. f. fuscipes* of teneral flies obtained from the stock colonies of Kality Tsetse Fly Research Center.

Research design

An experimental study was conducted at Kality Tsetse Fly Research Center. The experiment composed of two groups:

A. Fecundity, Pupae per Initial Female (PPIF), survival rate and pupae production and weight class

The sample sizes of the test flies used for the specific types of experiments carried out were based on [21]. Accordingly, for

- a. *Feeding and productivity test (fecundity, PPIF and pupae production) experiment:* 384 female and 96 male flies of *G. pallidipes* and 384 female and 96 male flies of *G. fuscipes fuscipes* (i.e., 32 females and 8 male flies per cage)
- b. *Survival (mortality) rate experiment:* 384 female and 96 male flies of *G. pallidipes* and 384 female and 96 male flies of *G. fuscipes fuscipes*.

All the activities performed in the study were conducted following the center's standard operating procedure.

Experimental procedures

Pupae per initial female (PPIF) and fecundity test of female G. pallidipes and G. fuscipes fuscipes

Thirty-two teneral female flies and 8 male flies (4:1 ratio) of *G. pallidipes* and *G. fuscipes fuscipes* were randomly selected from the stock colonies and were separately placed in three cages (diameter of 20 cm and width of 5 cm). The flies in each cage were assigned to feed on different blood sources (treatments): bovine, camel, ovine and caprine, for 90 days. The feeding schedule was five days per week (Monday, Tuesday, Thursday, Friday and Saturday) for ten minutes and the temperature of the blood was adjusted to $35-37^{\circ}\text{C}$ each time. Each treatment was replicated three times.

Female productivity was measured as pupae per initial female (PPIF), the total number of pupae produced in a given time divided by the number of initial females. PPIF is commonly used to assess the health of the *Glossina* colonies [21].

Evaluation of pupae production and pupae weight

The pupae produced by the flies were collected daily from larviposition cups throughout the experimental period starting from day 16-20 after emergence expected and were sorted into normal and abort larvae three by visual observation recorded [22]. Normal pupae were collected in to a separate Petri Dish, labeled according to the cage number and kept for 24 hours before weighing. Then the normal pupae were categorized into five weight classes using pupae balance. The pupae were sorted in to the standard system A (smallest, below 23 mg) to E (largest, above 37 mg) for *G. pallidipes* and A (smallest below 22 mg) to the (largest, above 36 mg) for *G. f. fuscipes* length of the collection area was adjusted to correspond the five weight classes previously defined to pupae of *G. pallidipes* and *G. f. fuscipes* (Table 1). The pupae collected from the 3 cages of one experimental group were pooled together [22].

Table 1. Definition of pupae weight classes for different *Glossina* species

Species	Pupae weight class (mg)				
	A	B	C	D	E
<i>Glossina pallidipes</i>	< 23	23 –28	29 –32	33 –36	> 36
<i>Glossina fuscipes</i>	< 22	22 –27	28 –31	32 –35	> 34
<i>Glossina morsitans</i>	< 18	18 –21	22 –25	26 –29	> 29
<i>Glossina tachinoides</i>	< 14	14 –16	17 –18	19 –20	> 20

Note: < = less than, > = greater than

Table 2. Mean (± SD) pupae per initial female of *G. pallidipes* and *G. fuscipes fuscipes* fed on the blood of different animals

Treatment	Tsetse species	
	<i>G. pallidipes</i>	<i>G. f. fuscipes</i>
Bovine	2.332 ± 0.489 ^{ab}	2.02 ± 0.193 ^a
Camel	2.98 ± 0.325 ^{bc}	3.35 ± 0.52 ^c
Ovine	2.5 ± 0.605 ^{abc}	2.15 ± 0.55 ^{ab}
Caprine	0.79 ± 0.435 ^d	1.60 ± 0.64 ^{ad}

Means in a column followed by the same letter are not significantly different from each other at 5 % level of significance (DMRT)

Table 3. Means in a column followed by the same letter are not significantly different from each other at 5 % level of significance (DMRT)

Treatment	Tsetse species	
	<i>G. pallidipes</i>	<i>G. fuscipes fuscipes</i>
Bovine	76.6 ± 16.60 ^{ab}	67.3 ± 6.50 ^{ab}
Camel	96.6 ± 7.62 ^{ac}	113.3 ± 16.8 ^c
Ovine	83.0 ± 22.53 ^a	69.3 ± 14.8 ^{ab}
Caprine	24.66 ± 12.89 ^d	52.66 ± 21.19 ^b

Means in a column followed by the same letter are not significantly different from each other at 5 % level of significance (DMRT)

Evaluation of survival rate of female *G. Pallidipes* and *G. fuscipes fuscipes*

Mortality was recorded daily for each test group throughout the experimental period. Mortality rate in each cage was checked every day starting from day 2 after emergence up to the end of experimental period. Dead flies were recorded into blood-fed and starved fly mortalities. The mortality rate was calculated according to Standard Operating Procedures for Mass-Rearing of Tsetse flies. Survival was calculated by subtracting the number of dead flies from the previous day recorded of the total number of survival flies in each cage [21].

The two *Glossina* species were maintained under different optimum environmental conditions of insectariums at a temperature of 23 - 25°C and RH of 75%-80 % for *G. pallidipes* and 23-25°C and RH of 80 - 85 % for *G. f. fuscipes* [17].

Data analysis

Duncan's Multiple Range Test (DMRT) was performed to analyze PPIF, pupae production, fecundity, and survival of flies fed on different blood diets using Stata computer software (version 12.0) at 5 % significance level. Results were presented using tables Pupae weight class percentages were analyzed compared with KTFRC's standard operating procedure (SOP) [21].

Results

Pupae per initial females (PPIF)

Pupae per initial females of *G. pallidipes* and *G. f. fuscipes* are shown in Table 2. Significantly lower (P < 0.05) PPIF was recorded in *G. pallidipes* fed on the blood of caprine compared to flies fed on blood

of the other animals. However, female of *G. fuscipes fuscipes* fed on the blood of camel had significantly higher mean (P < 0.05) PPIF than flies fed on the blood of ovine, bovine and caprine. Where there was no significant (P > 0.05) difference among these flies (Table 2).

Average pupae production by *G. pallidipes* and *G. fuscipes fuscipes*

Female flies of *G. pallidipes* fed on caprine blood significantly showed lower (P < 0.05) pupae production than flies fed on the blood of other animals (Table 3). On the other hand, *G. f. fuscipes* female flies fed on the blood of camel showed significantly higher (P < 0.05) mean pupae production compared to those flies fed on the blood of bovine, ovine and caprine (Table 3).

Pupae weight Class (%) *G. pallidipes* and *G. fuscipes fuscipes*

In *G. pallidipes* the highest pupae weight (class E, > 37) was recorded on camel (51 %), followed by bovine blood diets (class E, > 37) (29 %) (Table 4). In *G. f. fuscipes* the highest pupae weight class (class C, (45 %) was recorded on camel blood diets than other blood diets (Table 5).

Fecundity

Fecundity of females of *G. pallidipes* fed on the blood of caprine was significantly lower (P < 0.05) than flies fed on the blood of camel and ovine (Table 6). In addition there was no significant difference (P > 0.05) between the caprine and bovine blood. Unlike *G. pallidipes*, female flies of *G. f. fuscipes* fed on the blood of camel had significantly higher fecundity (P < 0.05) compared to those flies fed on other blood of animals (Table 6).

Table 4. Mean % of pupae produced by female of *G. pallidipes* fed on different blood of animals sorted in to different pupae weight class

Treatment	Pupae weight class (%)				
	A	B	C	D	E
Bovine	4	7	26	34	29
Camel	2	3	14	30	51
Caprine	16	32	30	14	8
Ovine	4	9	28	34	25

Pupae classes represented by letters A to E indicate weight in ascending order where 'A' refers to small pupae while 'E' refers to large pupae

Table 5. Mean % of pupae produced by female of *G. fuscipes fuscipes* fed on different blood of animals sorted in to different pupae weight class

Treatment	Pupae weight class (%)				
	A	B	C	D	E
Bovine	29	55	13	3	0
Camel	8	30	45	14	2
Caprine	17	31	30	14	8
Ovine	19	56	22	3	0

Pupae classes represented by letters A to E indicate weight in ascending order where 'A' refers to small pupae while 'E' refers to large pupae

Table 6. Mean fecundity of female *G. pallidipes* and *G. fuscipes fuscipes* fed on different blood diets during the survival day

Treatment	Tsetse species	
	<i>G. pallidipes</i>	<i>G. f. fuscipes</i>
Bovine	0.676 ± 0.037 ^{ab}	0.526 ± 0.015 ^a
Camel	0.786 ± 0.138 ^{bc}	0.93 ± 0.149 ^c
Ovine	0.771 ± 0.118 ^{bc}	0.61 ± 0.175 ^{ab}
Caprine	0.54 ± 0.056 ^a	0.55 ± 0.130 ^a

Means in a column followed by the same letter are not significantly different from each other at 5 % level of significance (DMRT)

Table 7. Mean (\pm SD) of survival rate of female of *G. pallidipes* and *G. fuscipes fuscipes* fed on blood of different animals

Treatment	Tsetse species	
	<i>G. pallidipes</i>	<i>G. fuscipes fuscipes</i>
Bovine	0.218 \pm 0.13 ^{ab}	0.354 \pm 0.117 ^b
Camel	0.239 \pm 0.06 ^{ab}	0.17 \pm 0.20 ^{ab}
Ovine	0.104 \pm 0.017 ^{ab}	0.291 \pm 0.23 ^{ab}
Caprine	0.062 \pm 0.082 ^a	0.062 \pm 0.107 ^a

Means in a column followed by the same letter are not significantly different from each other at 5 % level of significance (DMRT)

Female survival rate of *G. Pallidipes* and *G. fuscipes fuscipes*

There was no significant difference ($P > 0.05$) in weekly survival rate of female *G. pallidipes* fed on different blood of animals (Table 7). In contrast, significantly ($P < 0.05$) higher survival rate of *G. f. fuscipes* female was recorded on those fed on bovine blood diet, while lower survival rate was observed on caprine (Table 7).

Discussion

For a colony to sustain- the minimum PPIF should be above 2.1 for 13 weeks and fecundity (as pupae per female per ovarian cycle) should be above 0.6 [23]. However, pupae production below 3 per initial female results in no effective colony growth, or even decline [24]. Based on this fact, the results of this study indicated that camel blood diets in *G. f. fuscipes* flies resulted in the highest mean PPIF (3.35) and fecundity(0.93) in which they were found to be above the standard PPIF (PPIF=3)and fecundity(F=0.6) value required to have a steady colony growth (effective colony growth). Similarly, the mean PPIF and fecundity, of *G. pallidipes* in camel, ovine and bovine blood diets, and *G. f. fuscipes* flies under ovine blood diets, were found to be above the standard (PPIF =2.1) and fecundity (F=0.6) value- required to sustain or survive the colony. However, caprine blood diets in *G. pallidipes* and both bovine and caprine blood diets in *G. f. fuscipes* resulted in extremely lower PPIF and fecundity values below the standard minimum values required to sustain or survive the colony compared with the rest diet.

In the present study, the highest PPIF and fecundity of flies indicative of the overall performance and the nutritional quality of the blood diets [21]. Several studies have revealed that insufficient nutrition of the female fly would lead to abortions. Increased abortion rates and reduced productivity resulting from an inadequate diet would hamper colony growth [21,25,26]. However, the reproductive performance of *G. pallidipes* flies *in vitro*-fed on goat blood was significantly lower compared with flies fed on others sources of blood.

In both species the overall results obtained from the pupae quality test were consistent with the findings of the reproductive test. *G. pallidipes* flies maintained feeding on caprine blood diets not only produced a low quantity of pupae but also a poor quality of small and light pupae which fell mostly into the smaller size categories A–B weight class. Similarly, *G. f. fuscipes* flies maintained feeding on camel blood diet produced a higher quantity with a good quality of pupae which fell mostly into the heavier weight categories C–E weight class. These results were in line with the findings of [27] who found that the weight of pupae depends on the amount and quality of blood fed by a female during pregnancy, with a highly significant correlation between puparia weight and quality of blood ingested. It is a fact that pupae class is a good overall quality indicator of the effectiveness of colony maintenance where each weight class can be defined using a weight-sorting machine. The mean pupae weights should approximate the values developed by [22], and no more than 10% of the puparium should be in weight class 'A'(21). Consequently, the results of this study indicated that *G. pallidipes* flies

maintained on camel, bovine and ovine blood diets,-the percentage of class 'A' pupae were found to be in the acceptable range which was less than 10 %. In contrast, *G. f. fuscipes* flies maintained in all diets except camel blood, the percentage of class 'A' pupae were found to be in the unacceptable range which was greater than 10 %. Both species of flies maintained on camel blood diets resulted in good quality of pupae while flies reared on caprine blood produced a poor quality pupae. This finding is in agreement with the previous report by [28] on female *G. pallidipes*, where the highest percentage of (A class) pupae were found on flies that were maintained under caprine blood diets compared with ovine and bovine blood diets. The reason for this finding could be due to the nutrient shortfall needed for the required performance in flies fed caprine blood diets and due to presence of better nutritive value in camel blood diets that supports the development of large pupae weight class as stated by previous findings, which stated pupae quality is an indication of the nutritional status of the fly and is reflected by pupae weight and size. High fecundity and low mortality is correlated with pupae size and weight. Very small and light pupae resulted in low emergency rate, a low number of strong and viable flies. The size and weight of pupae are reflection of the maintenance and feeding of female flies, and her ability to transfer the nutrients to her offspring [14].

According to the present study, *G. f. fuscipes* showed a higher mean survival percentage under *in-vitro* feeding of bovine than caprine blood diets. The result obtained in *G. f. fuscipes* is in agreement with the report of [28] that the females of *G. pallidipes* fed on caprine blood diets showed lower average survival rate than flies fed on bovine, ovine and pig blood diets. However, we did not find significant differences in the survival rates of females of *G. pallidipes* flies fed on camel, bovine, ovine and caprine blood diet. This contradicts with the finding of [28] that *G. pallidipes* fed on caprine blood diets showed lower average survival rate than flies fed on bovine, ovine and pig blood diets. The low survival rate in case of caprine blood may be due to the differences in host specificity of *G. f. fuscipes* and a range of physiological adaptations to the specific host blood feeding. *G. f. fuscipes* female flies do not prefer to feed on caprine blood diet both at the field level and under laboratory in ideal ecology.

Although tsetse flies, including *G. pallidipes*, are selective in their choice of hosts (except for the palpalis group, like *G. f. fuscipes*) [29], the present study shows that *G. pallidipes* can be maintained on less preferred hosts like camel blood. This is also in agreement with [30-32]. It is now well known that *G. morsitans morsitans*, *G. palpalis*, *G. tachinoides*, and *G. pallidipes* can be reared easily on less preferred hosts. Based on this fact, the results of this study indicated that *G. pallidipes* flies can be maintained under feeding of camel blood diets. This finding disagreed with the report of [21] that rearing of tsetse flies using *in vitro* membrane feeding of bovine, porcine, or a mixture of both blood have been a method of routine for colony maintaining. Tests using only bovine blood (at FAO /IAEA laboratories, Sibersdorf, Austria) also demonstrated that most tsetse species could be maintained by feeding bovine blood alone. The result also clearly revealed that the overall performance of *G. pallidipes* colonies maintained on *in vitro* feeding of camel blood diets were nearly as good as maintained on bovine and ovine blood diets showing that camel blood diets can be absolutely useful for maintaining and rearing of both *G. f. fuscipes* and *G. pallidipes* colonies under laboratory condition at Kaliti Tsetse Fly Research Center laboratory. It would appear, therefore, that tsetse flies have a physiological capability to digest and utilize as efficiently the blood of several mammals, including some on which they do not normally feed, such as wildebeest [33,34].

According to the result, the PPIF and fecundity of female *G. pallidipes* fed on bovine and ovine blood was almost comparable to that

of camel blood fed flies, there were no statistical difference among them. This result agreed with the report of [28] who reported *G. pallidipes* colonies flies of the same strain (at Kality Tsetse Fly Research Center laboratory) maintained on bovine and ovine blood diets were produced significantly more pupae than those fed on caprine, porcine and mixed blood diets. According to [35] after prolonged maintenance, he found that a population of *G. swynnertoni* fed on sheep gave far better results than one fed on guinea pigs in Geigy racks; he also found that from the beginning of maintenance *G. pallidipes* did far better on sheep [36]. It is known that the host preference of *G. pallidipes* in nature may include, in addition to bovines, smaller mammals such as bush pigs [37]. Porcine- and bovine blood, and various combinations thereof, were therefore evaluated as rearing diets for these species. However the reproductive performance of *G. pallidipes* flies *in vitro*-fed on goat blood was significantly lower compared with flies fed on others sources of blood. According to the result, the PPIF (0.79) and fecundity (0.54) value of *G. pallidipes* flies fed on goat blood diet was extremely lower than the minimum standard value of PPIF (2.1) and fecundity (0.6). This result also agreed with the report of [28] who reported both the PPIF and fecundity value of the same strain of *G. pallidipes* colonies flies fed on caprine blood diet was lower than the standard value (2.1) and 0.6 respectively.

According to [28], *G. pallidipes* colonies flies maintained on bovine and ovine blood were also produced significantly more pupae than those fed on caprine, porcine and mixed blood diets. This also agreed with the PPIF reported on generation F3 *G. austeni* and *G. pallidipes* fed on reconstituted freeze-dried caprine blood [23]. This showed that the caprine blood diet highly reduces *G. f. fuscipes* female fly production. This was due to a lower nutritional value of caprine blood that supports the development of pupae in *G. f. fuscipes* resulting in lower pupae production and in the pregnancy cycle larval development is adversely affected by a nutritional shortfall. The quantity and weight of pupae depends on the amount of blood fed by a female during pregnancy, with a highly significant correlation between pupae weight and quality of blood diet fed [28,38].

The overall performance of the *G. f. fuscipes* flies fed camel blood diets was superior as compared to bovine, ovine and caprine blood diets which are most commonly used blood diets for rearing purposes in various laboratories showing that camel blood diets also can be absolutely useful for maintaining and rearing of *G. f. fuscipes* colonies under laboratory condition at Kality Tsetse Fly Research Center laboratory. The tsetse fly *Glossina palpalis* (Diptera: Glossinidae, including *G. f. fuscipes*) probably has no match among haematophagous insects in its vertebrate host range. According to [39] this fly can feed on any vertebrate it contacts. It is also not responsive to host derived odors presently being evaluated as olfactory baits and incorporated in trapping technology strategies for tsetse flies [40]. However, *G. f. fuscipes* colonies fly *in vitro*-fed on bovine, ovine and caprine blood diets were invariably survived and reproduced. This result was disagree with the report of [28] who reported the PPIF female *G. pallidipes* fed on bovine blood was almost comparable to that of ovine blood fed flies (PPIF=2.77) but agreed with the report of [28] who reported the PPIF value of *G. pallidipes* colonies flies fed on caprine was lower than the standard value (2.1). The difference was due to the fact that the two tsetse species belong to different groups of *Glossina* species (riverine and savannah) possibly exhibiting difference in host preferences [41,42].

Conclusion

In conclusion, overall performance of the *G. f. fuscipes* flies fed on camel blood diets was superior as compared to bovine, ovine and caprine blood diets. In addition overall performance of *G. pallidipes*

colonies maintained on *in vitro* feeding of camel blood diets were nearly as good as maintained on bovine and ovine blood diets, showing that camel blood diets can be useful for maintaining and rearing of both *G. f. fuscipes* and *G. pallidipes* colonies under laboratory condition. The result encourages the use of blood diets for tsetse mass rearing in Ethiopia, considering the presence of large number of camel in the country, which are slaughtered in large numbers at Addis Ababa (Akaki Kality) Municipal Abattoirs near to Addis Ababa where the rearing facility is located. On the other hand, the low performance of the *G. pallidipes* flies fed caprine blood, particularly low PPIF, low fecundity and high proportion of lighter pupae, low longevity and low survival rate, indicated that caprine blood diet is nutritionally poor to be used for colony development. Similarly, *G. f. fuscipes* flies fed on goat, bovine and ovine blood diets showed low performance compared to camel blood indicating that these diets have low nutritional quality and therefore not qualified as a preferred diet for tsetse flies feeding.

Recommendation

Therefore, based on the above results, the following recommendations have been forwarded:

1. Camel blood diet was superior as compared to bovine, ovine and caprine blood showing that camel blood diets can be absolutely useful for maintaining and rearing of *G. f. fuscipes* and *G. pallidipes* tsetse fly colonies to ensure better production under laboratory conditions.
2. Bovine blood diet, which has been used *in vitro* feeding techniques at Kality Tsetse Fly Research Center, produced not only a low quantity of pupae but also a poor quality of pupae. The highest overall percentage of inferior pupae quality (class A) (29 %) recorded in *G. f. fuscipes* species fed on bovine blood diet were found to be in the unacceptable range which was greater than 10 % showing that bovine blood diet is unsuitable for maintaining and rearing of *G. f. fuscipes* colonies. Hence camel blood could be considered as an alternative colony feeding in order to assure better tsetse production at Kality Tsetse Fly Research Center.
3. Further investigations should be done to assess the nutritional contents of camel diets for tsetse fly rearing.
4. The mass-rearing of tsetse remains challenging, especially when more than one species is involved. The optimal rearing diet may differ between colonies and tsetse species and might need to be customized for each production unit. Decisions on the most suitable rearing diet will not only depend on the biological requirements of the flies involved but will also be influenced by the availability of a suitable blood source on a continuous and economic basis. Quality control and research on factors to optimize the diet needs to be done continuously.

Acknowledgments

The authors thank the Adama Science and Technology University and staffs of KTFRC insectaries for their technical support and their cooperation.

Conflicts of interest

The authors declare no conflict of interest.

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