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Study on the Reproductive Efficiency of *Glossina Pallidipes* and *Glossina Fuscipes Fuscipes* Reared under Laboratory Conditions

Masresha Yehualashet* and Mintesnot Tsegaye

Animal Health Institute, Kality Tsetse Fly Research Center, Ethiopia

***Corresponding author:** Masresha Yehualashet, Animal Health Institute, Kality Tsetse Fly Research Center P.O.Box 19917 Sebeta, Ethiopia

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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 rang test was performed to analyze pupae per initial female, pupae production, fecundity, survival test fed on different blood diets on the survival days using Stata computer software (version 12.0) pupae per initial female was recorded in *G. pallidipes* fed on the blood of caprine 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. The research center should use the camel blood meal 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]. The tsetse flies' distribution is commonly found in the southern southwestern and northwest regions of Ethiopia, between longitudes of 33°and 38°E and latitudes of 5° and 12°N [3]. 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, Benishangu lGumuz, Oromia, Southern and Gambella) region of Ethiopia are infested with more than one species of tsetse fly [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]. So far, many chemical and biological methods of tsetse fly control have been devel-

oped each of which has its advantages and limitations. Currently, the vector control methods are sequential aerosol spraying techniques (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 tests; 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 control of Trypanosomiasis within an area-wide integrated tsetse management approach (AW-ITM) using the sterile insect technique. 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]. Mass rearing of tsetse flies (*Glossinaspp*.) 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. Kality Tsetse Fly Research Center was inaugurated on 3 February 2007 with the mission of creating a tsetse flies free zone in the Southern Rift Valley of Ethiopia when the center has a quality of equipment and operational successfully also the facility is to have a colony capacity of approximately seven million female flies and was able to produce 700,000 released sterile male flies per week to treat approximately 7000-kilometer square at a time [14].

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 days 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-litter container was proportioned to 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. These 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 were irradiated at 1- 1.5kilogray (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 was passed for tests 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 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 into the working laboratory room and thawed in water until it changed into liquid blood at +4 °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 is 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 °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]. Fecundity was expressed as the number of pupae produced per female per 10 days, by considering day 18 after immediately they emerged from pupae stage as the first larva position day [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 into 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. fuscipes fuscipes* length of the collection area was adjusted to correspond the five weight classes previously defined to pupae of *G. pallidipes* and *G. fuscipes 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*.

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

Note: < = less than; > = greater than

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 survival fed on different blood diets on the survival days 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).

Results

Pupae per Initial Females (PPIF)

Pupae per initial females of *G. pallidipes* and *G. fuscipes 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).

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

	Tsetse species	
Treatment	*G.pallidipes*	*G.fuscipesfuscipes*
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}

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. fuscipes 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).

Table 3: Mean (± SD) pupae production of *G. pallidipes* and *G. fuscipes fuscipes* fed on blood of different animals.

	Tsetse species	
Treatment	*G.pallidipes*	*G.fuscipes fuscipes*
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}

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. fuscipes fuscipes* the highest pupae weight class (class C, (45 %) was recorded on camel blood diets than other blood diets (Table 5). 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. 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 4: Mean % of pupae produced by female of *G. pallidipes* fed on different blood of animals sorted into 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

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

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

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

Treatment	Tsetse species	
	G.pallidipes	*G.fuscipesfuscipes*
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}

Female Survival Rateof *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 feds on bovine blood diet, while lower survival rate was observed on caprine (Table 7).

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

Treatment	Tsetse species	
	G.pallidipes	*G.fuscipesfuscipes*
Bovine	0.218±0.13^{ab}	0.354±0.117^{b}
Camel	0.239±0.06^{ab}	0.17±0.20^{ab}
Ovine	0.104±0.017^{ab}	0.291±0.23^{ab}
Caprine	0.062±0.082^{a}	0.062±0.107^{a}

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, both (*G. pallidipes* and *G. f. fuscipes* colonies reared and maintained by *in vitro* feeding of bovine, ovine, caprine and camel blood, regardless of holding conditions or experimental variations, camel, bovine and ovine blood were found to be suitable for rearing and maintaining of *G. pallidipes flies* compared with caprine blood diets while camel blood diet was found to be suitable for-*G. f. fuscipes* colonies compared with ovine, bovine and caprine blood diets. 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 pupae while flies reared on caprine blood produced a poor-quality pupa. 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.fuscipesis* 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 *Glossina morsitansmorsitans*, *Glossina palpalis, Glossina tachinoides*, and *G. pallidipes* can be reared easily on less preferred hosts. Based on this fact, the results of this study indicated that *G. pallidpes* 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 bloods have been a method of routine for colony maintaining. Tests using only bovine blood (at FAO /IAEA laboratories, Sibersd of, 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 Kality Tsetse Fly Research Center laboratory. It would appear, therefore, that tsetse flies have a physiological capability to digest and utilize 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 that 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 haematophagus 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 and Recommendations

In conclusion, the 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 the overall performance of *G.pallidipes* colonies maintained on *in vitro* feeding of camel blood diets was 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 conditions. The result encourages the use of blood diets for tsetse mass rearing in Ethiopia, considering the presence of large number of camels 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. 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* colonies at Kality Tsetse Fly Research Center in order to assure better production tsetse fly.

2. Bovine blood diet, which has been used *invitro* 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 tests 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 availably 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.

References

1. Radostits OM, Gay CC, Hinchcliff KW, Constable PD (2007) Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th. London, UK.
2. Warnes M, Van den Bossche P, Chihiya J, Mudenge D, Robinson T, et al. (1999) Evaluation of insecticide treated cattle as a barrier to reinvasion of tsetse to cleared areas in northeastern Zimbabwe. Medical and Veterinary Entomology 13(2): 177-184.
3. Kotye FD (2006) A comparative study on the ecology of tsetse flies (*diptera*: *glossinidae*) in the wabe and walga river systems. Addis Ababa University.
4. Langridge WP (1976) A tsetse and trypanosomiasis survey of Ethiopia. Ministry of Overseas Development of British and Ministry of Agriculture of Ethiopia. Addis Ababa, Ethiopia, p. 97.
5. Dyck VA, Hendrichs J, Robinson AS (2021) Sterile insect technique: principles and practice in area-wide integrated pest management: Taylor & Francis.
6. Feldmann U, Dyck V, Mattioli R, Jannin J, Vreysen M (2021) Impact of tsetse fly eradication programmes using the sterile insect technique Sterile Insect Technique (pp. 1051-1080): CRC Press. For cosmetics. Journal of Cosmetic Science 69(3): 187-202.
7. Jeremy S, Samuel B, Stefan M (2010) African Trypanosomiasis: New insights for disease control. Parasitology 137: 1975-1975.
8. Stany G, Peter H, Oumar D, Mark E (2001) African bovine Trypanomosis: the problem of drug Resistance. Trends Parasitol 17(1): 25-28.
9. (2011) WHO. Vector control, Human African Trypanosomiasis.
10. Dyck VA, Hendrickx G, Robinson AS (2005) Sterile insect technique. Springer, International Atomic Energy Agency, Vienna, Austria.
11. Leak SG (1999) Tsetse biology and ecology: their role in the epidemiology and control of Trypanosomiasis.
12. Richard W, David S (1997) Myiasis. In: Veterinary Ectoparasites: Biology, Pathology, and Controlm (2nd Edn.)., London.
13. Vreysen M (2001) Principles of area-wide integrated tsetse fly control using the sterile insect technique. Médecine Tropicale 61(4-5): 397-410.
14. (2007) IAEA. Technical cooperation report for 2006. Report by the director general, international atomic agency.
15. Nash TAM, Jordan AM, Boyle JA (1968) The large-scale rearing of G. austeni (Newst) In the laboratory. The final technique, Annals of Tropical Medicine and Parasitology 62: 336-341.
16. Van der Vloedt A (1982) Recent advances in tsetse mass rearing with particular reference to Glossina palpalispalpalis (Rob.-Desv.) fed *in vivo* on guinea pigs. Paper presented at the sterile insect technique and radiation in insect control: proceedings, International Symposium on the Sterile Insect Technique and the Use of Radiation in Genetic Insect Control, held in Neuherberg.
17. Williamson D, Dame D, Gates D, Cobb P, Bakuli B, et al. (1983) Integration of insect sterility and insecticides for control of Glossina morsitans-morsitans Westwood (*Diptera*: *Glossinidae*) in Tanzania. V. The impact of sequential releases of sterilized tsetse flies. Bulletin of Entomological Research 73(3): 391-404.
18. Olandunmade M, Takken W, Tenabe S, Onah J, Dengwat L, et al. (1990) Eradication of Glossina palpalispalpalis (Robineau-Desvoidy) (*Diptera*: *Glossinidae*) from agro pastoral land in central Nigeria by means of the sterile insect technique Sterile insect technique for tsetse control and eradication of Radiation in Genetic Insect Control, held in Neuherberg.
19. Wetzel H, Luger D (1978) *In vitro* feeding in the rearing of tsetse flies (*Glossina m. morsitans* and *G.ppalpalis*, *Diptera*: *Glossinidae*). Tropenmedizin und Parasitologie 29(2): 239-251.
20. Feldmann U (1994c) Guidelines for the rearing of tsetse flies using the membrane feeding technique. In: Techniques of insect rearing for the development of integrated pest and Vector management strategies: In: Ochieng-Odero JPR (Edt.)., ICIPE science press, pp. 449-471.
21. (2006) FAO/IAEA. Food and Agriculture organization of the United Nations and International Atomic Energy Agency. In: Standard Operating Procedures (SOP) for Mass-Rearing Tsetse flies.
22. Zelger R, Russ K (1976) Untersuchungenuber die mechanischeTrennung von Mannchen und Weibchen der KirschfruchtfliegeimPuppenstadium. Z ang Zool 63: 257-266.
23. Opiyo MA, Ngowo HS, Mapua SA, Mpingwa M, Nchimbi N, et al. (2021). Sub-lethal aquatic doses of pyriproxyfen may increase pyrethroid resistance in malaria mosquitoes. PLoS One 16(3): e0248538.
24. (2003) IAEA. Automation for tsetse mass rearing for use in sterile insect technique programmes. Final report of a coordinated research project 1995–2001, May/2003.Iaea-Tecdoc1353.Iaea, Vienna, Austria, p. 1-45.
25. Mellanby H (1937) Experimental work on reproduction in the tsetse fly, Glossina palpalis. Parasitology 29(1): 131-141.
26. Ward RA (1970) Tsetse fly colonization (*Diptera*: *Muscidae*, *Glossina spp*.). Bulletin of the ESA 16(2): 111-115.
27. Kettle DS (1995) Medical and veterinary entomology (2nd Edn.)., CABI Publishing, Wallingford, United Kingdom
28. Assefa K (2012) Study on the survival and reproductive performance of female *G. pallidipes* (*Diptera*: *Glossinidae*) at Kality Tsetse Fly Rearing and Ir-

radiation Center, Addis Ababa. MSc Thesis, Addis Ababa University School of Graduate Studies, Department of Biology, p. 26.

29. Jaenson TGT, Takken W (1980) Rearing of Glossina pallidipes using membrane-feeding technology. Entomologia Experimentaliset Applicata 27(1): 102-104.
30. Buxton PA (1955) The Natural History of Tsetse flies. An Account of the Biology of the Genus Glossina (Dipterans). The Natural History of Tsetse Plies. An Account of the Biology of the Genus Glossina (Dipterans) (10).
31. Langley PA (1968) The effect of host pregnancy on the reproductive capability of the tsetse fly, Glossina morsitans, in captivity.Journal of Insect Physiology 14(1): 121-133.
32. Moloo S, Grootenhuis J, Kar S, Karstad L (1988) Survival and reproductive performance of female Glossina morsitansmorsitans when maintained on the blood of different species of wild mammals. Medical and Veterinary Entomology 2(4): 347-350.
33. Weitz B (1963) The feeding habits of Glossina. Bulletin of the world health organization 28(5-6): 711.
34. Moloo SK (1971) An artificial feeding technique for Glossina. Parasitology 63(3): 507-512.
35. Willett KC (1953) The laboratory maintenance of Glossina. I. Parasitology 43(1-2): 110-130.
36. Geigy R (1948) Rearing of Glossina palpalis. Acta Tropica 5(3): 201-218.
37. De Beer CJ, Venter GJ, Vreysen MJ (2016) Improving the diet for the rearing of Glossina brevipalpisNewstead and Glossina austeniNewstead: Blood source and collection–processing–feeding procedures. Plos One 11(12): e0168799.
38. Langley PA, Pimley RW (1975) Quantitative aspects of reproduction and larval nutrition in Glossina morsitansmorsitansWestw. (*Diptera*, *Glossinidae*) fed *in vitro*. Bulletin of Entomological Research 65(1): 129-142.
39. Jordan AM, Lee-Jones F, Weitz B (1962) The natural hosts of tsetse flies in Northern Nigeria. Annals of Tropical Medicine & Parasitology 56(4): 430-442.
40. Willemse LPM, Takken W (1994) Odor-induced host location in tsetse flies (*Diptera*: *Glossinidae*). Journal of Medical Entomology 31(6): 775-794.
41. Clausen, Adeyemi, Bauer, Breloeer, Salchow, Staak (1998) Host preferences of tsetse (*Diptera*: *Glossinidae*) based on blood meal identifications. Medical and veterinary entomology 12(2): 169-180.
42. Auty H, Morrison LJ, Torr SJ, Lord J (2016) Transmission dynamics of Rhodesian sleeping sickness at the interface of wildlife and livestock areas. Trends in Parasitology 32(8): 608-621.

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