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Study on feeding frequency and its associated effects on production and survival rate of *glossina fuscipes*

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Abstract
The sterile insect technique (SIT) is an efficient genetic-based tsetse fly control method that depends on the high production of male flies within laboratories, sterilization, and release of these flies in the target area. Production of flies in insectary/laboratory requires a sustainable supply of quantitative and qualitative blood for *in vitro* feeding. The need for a regular supplement of large amounts and high blood quality is among the main challenges in tsetse fly mass-rearing insectaries that increase the cost of production. For this reason, optimized feeding strategies that can optimize production and minimize the cost of production are mandatory in any given insectary facility. However, an optimized feeding strategy for this species is lacking. Therefore, this study aimed to identify the best feeding frequency that could optimize production and minimize the cost of production in a mass-rearing insectary facility. Three laboratory experimental groups were established based on the frequency of fly feeding regimes. The first group was fed three times per week, the second group was fed four times per week, and the third group was fed five times per week. The results showed that feeding frequency had an important effect on fly production and survival. The lowest outcomes for all parameters were obtained in flies which fed three times per week and relatively the best results were obtained in the four-times-per-week feeding regime.

Keywords: Feeding regime, G.f. fuscipes, Mass-Rearing Productivity, Survival

Introduction
"Tsetse flies are blood-sucking flies of the genus *Glossina* that belong to the family Glossinidae (Radostitis *et al*., 2007) [1]. They are found exclusively on the African continent, between 5°N to 20°S latitudes" (Warnes *et al*, 1999) [2].

These flies may be ranked as some of the maximum detrimental pests and are the vectors of the causative agents for napping illness in human beings and African Animal Trypanosomosis (AAT) or Nagana in livestock (Vreysen, 2001) [3]. Both diseases cause many direct and indirect losses, which represent a major obstacle to sustainable development in endemic countries. The direct effect is that the infected livestock may have a high mortality rate if not treated. The indirect effect is that nagana is a wasting disease and the affected animals are chronically unproductive in terms of milk, meat, manure, and traction (FAO, 2000) [11].

Disease control with control of the tsetse flies has been found the most effective strategy due to the lack of effective vaccines and the high costs of disease treatment. So far, many chemical and biological methods of tsetse fly control have been developed, each of which has its own advantages and limitations. Currently, Insecticides are used in vector control interventions through the sequential aerosol spraying technique (SAT), ground spraying, insecticide-treated animals used as live baits, traps, and the sterile insect technique (SIT) (WHO, 2011) [11].

SIT is an efficient genetic-based tsetse control method developed so far. It involves the mass production of male flies in a laboratory, sterilization, and release of these flies onto the target area. As compared to others, the SIT is on-intrusive to the terrain, has no adverse goods on non-target organisms, is species-specific, and can fluently be integrated with natural control styles similar to parasitoids, bloodsuckers a, and pathogens (Leak, 1999) [6]. There's no trouble of resistance development to the goods of sterile males, handed that acceptable quality assurance is assured during the production process and that the sterile insects cannot get established in released areas as is the case with other natural control programs (Vreysen, 2001) [3].

The success of SIT project depends on the quality and the number of produced male tsetse flies in the laboratory/insectary. The quality of the insects may be challenged by many factors in the rearing facility including overcrowding, procedures of rearing, food, number of insects within the colony, deterioration of the strain, an adaptation of laboratory, genetic change, and other techniques (Simmons *et al*., 2010) [7].

Feed and feeding strategy are among the vital factors that determine the success of mass production in tsetse fly's insectary facilities. A sustainable supply of blood is required for the maintenance of fly colonies (IAEA, 2000) [8] *in vitro*. But, the need for a regular supplement of larvae mounts and of high-quality blood is among the main challenges in tsetse fly mass-rearing insectaries increasing the cost of production. For this reason, optimized feeding strategies that can optimize production and minimize the cost of production are mandatory in any given insectary facility. However, a trial-based verified feeding regime for *G. fuscipes* was lacking here in Ethiopia. Therefore, the main objective of this study was to evaluate the effect of three, or four five-times-per-week feeding frequencies on survival and productivity in order to point out the best feeding regime, both in cost-effectiveness and optimizing the production of *fuscipes* in tsetse mass-rearing facilities.

2. Materials and Methods

2.1. Study Area

"This experimental study was conducted at Kality Tsetse Fly Mass Rearing and Research Center within the capital, Addis Ababa. Addis Ababa is the capital city of Ethiopia with a geographical location of 8° 53' 44.9916" N and 38° 47' 20.9832" E latitude and longitude, respectively" (Seifu and Stellmacher, 2021) [9].

The Southern Tsetse Eradication Project (STEP), which the Ethiopian government launched with the assistance of the International Atomic Energy Agency (IAEA), was established in the Southern Rift Valley in 1997 with the goal of eradicating the tsetse fly from the area. When fully operational and equipped, the facility was expected to have a colony capacity of about 7 million female flies and be able to eradicate the tsetse fly from the Southern Rift Valley of Ethiopia (IAEA, 1957-2007) [10].

2.2. Study population and study period

The study was conducted from March up to May 2022 to estimate the effects of feeding frequency on G.f. recipes. The experimental canvases used for the purpose of this study were attained from an analogous product batch of nymphs that began from the colonies of the insectary (Kality Tsetse Fly Mass Rearing and Irradiation Center) and a total of 450 (360 females and 90 males) flies. Canvases were rendered to immobility by using a bite (4 °C) incontinently after they had surfaced from incubated nymphs; coitus-sorted and placed independently in colony coops with 50(40 ladies per 10 males) flies per pen called colonies, following the predefined coitus rate (41) and kept in a replicated coops with 20 cm periphery and 5 cm range of netting on top and nethermost for feeding and collection of the incubated naiads (FAO/ IAEA, 2006) [11].

2.3. 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 Kality Tsetse Fly Mass Rearing and Irradiation Center. 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 three mating cages with 50 (40 female and 10 male) flies per cage. All groups were kept in the same optimum environmental conditions and controlled insectarium similar to the general colony of the center, at a temperature of 23-25 ℃ and an RH of 75-80% (FAO/ IAEA, 2006) [11]. 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, 1957-2007) [10]. The feeding system was performed grounded on the environmental conditions of 25 ± 1 ℃ and 50 ± 5 moisture for all superintendence. The canvases were maintained in the feeding room for lower than 30 twinkles grounded on environmental conditions (FAO/ IAEA, 2006) [11]. The holding room temperature and moisture were acclimated and covered daily from the data jack set in the parenting room. Nymphs were collected daily and a mortality check was performed daily. The weekly datasets of the colonies, in different feeding regimes, were measured by testing of 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 larva larvae position day" (FAO/ IAEA, 2006) [11].

Pupae sorting and grading test: The collected Pupae larva larvae were positioned in 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 labelled, A (smallest) to E (largest); the length of the collection area has been adjusted to correspond to the five weight classes that previohave has been defined to tsetse pupae of *G. fuscipes"* (Zelger and Russ, 1976) [12].

Mortality Test: Mortality was recorded daily for each test group throughout the experimental period. Dead oils were sorted into blood-fed and starved-cover mortalities. The

mortality was rate calculated according to Standard Operating procedures (fix) for Mass-Rearing Tsetse oils (FAO/ IAEA, 2006) [11].

2.4. 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 position of "lower than 5" for analyses.

3. Results

3.1. Pupae production and pupal size class distribution

The first larvae position day "the day on which the first larva was deposited "was the same for all blood-feeding treatment groups (3 times per week (F3), 4 times per week (F4), and 5 times per week (F5),) and was at the age of 18 days. 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).

The outcome indicated 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 67.1% and 68.2% 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 (10.3,6%) and the least was produced by F5 (9.7%) and the reverse was true for the largest pupal class (class E) covering 6.9%, 1.8% and 1.2% of the total pupal production of their respective group, F5, F 4, and F3 respectively. The pupal size classes' distribution for each test group is shown in figure 1.

Table 1: Pupae production, Pupal size distribution and PPIF of test groups; F3 (three blood meals per week), F4 (four blood meals per week) andF5 (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(27.8%)	105(38.7%)	36(13.3%)	3(1.12%)	2.26
F4	386	40(10.36%)	87(27.54%)	137(35.5%)	115(29.8%)	7(1.8%)	3.22
F5	390	38(9.7%)	86(22%)	112(28.7%)	127(32.6%)	27(6.9%)	3.25

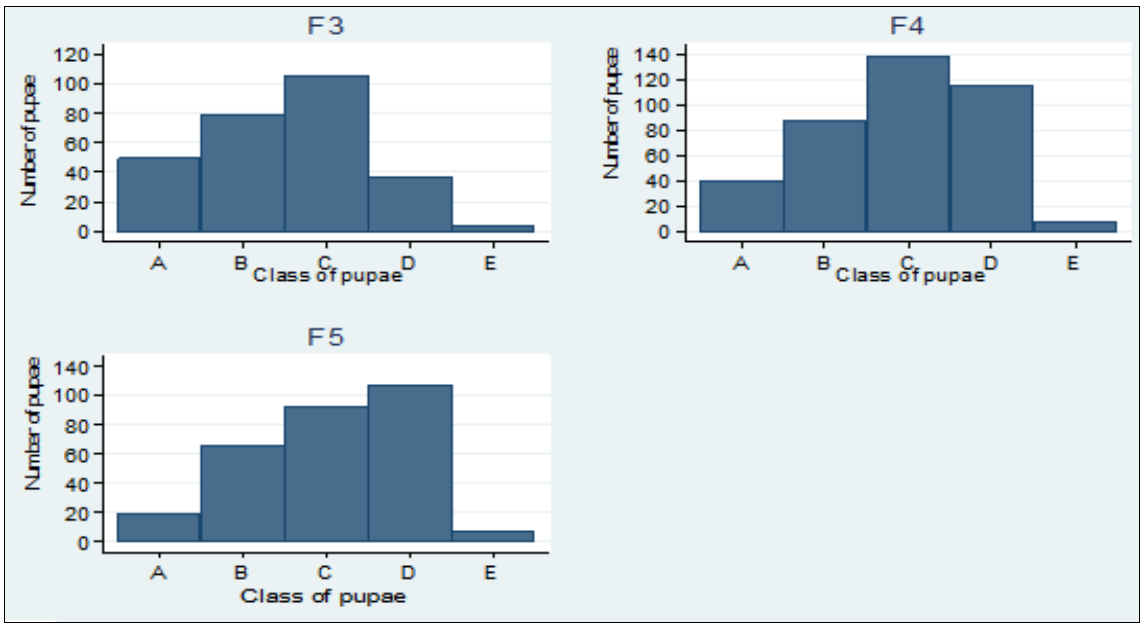

Fig 1: Pupal size class distributions of test groups, F3 (three blood meals per week), F4(four blood meals per week), F5 (five blood meals per week).

3.2. 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 (3.22PPIF) and least was by group F3 (2.26). Similarly, the fecundity, which is defined as pupae per female per 10 days (P/F/10 days) was assessed for each group and the lowest fecundity was obtained for the feeding treatment group F3 with a mean fecundity of 0.559 ± 0.15 pupae/females/10days compared to both F4 and F5 with a mean fecundity of 0.721± 0.15 and 0.697± 0.01 pupae/females/10days in that order. A statistically significant link was investigated 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. The trend of PPIF for the three treatment groups over the study period is shown in figure 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%_Conf	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

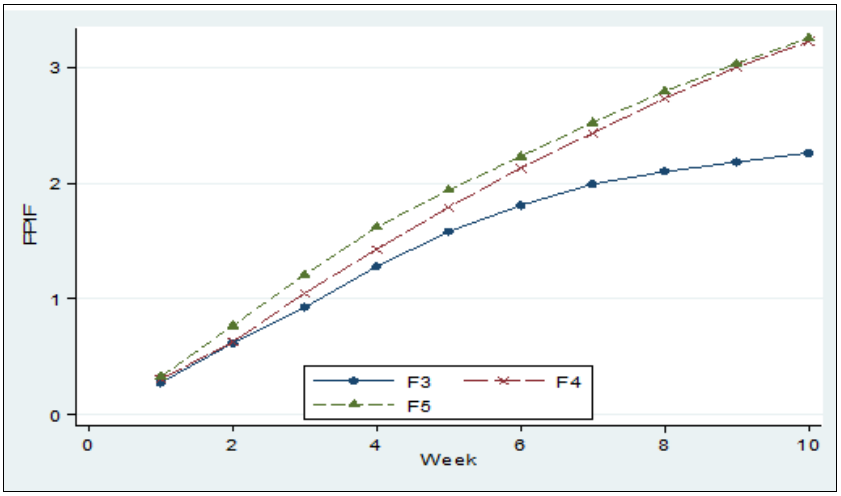

Fig 2: The trend of PPIF for the three treatment groups, F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week

3.3 Fly Mortality and Survival

The overall Female mortality was highest for females fed 3 times per week (41.6%) followed by females fed 5 times per week (29.8%) and the lowest was for flies fed 4 times (28.6%). The dissection result of the dead female flies indicated that only 1.8% 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 relationship between mortality and the causes of mortality (P>0.05). Nonetheless, the frequency of blood-feeding had a significant result on womanish survival and/ or mortality (ANOVA, p>0.044 df = 2). Females mortality was highest for females fed 3 times per week (41.6%) followed by females fed 5 times per week (29.8%) and the lowest was for flies fed 4 times (28.6%) 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 both F4 and F5. However, there was no statistically significant difference in females' mortality rate of treatment groups F4 and F5 (Table 3). The trend of survival rate for the three feeding frequency groups over the study period is shown in figure3.

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%_Conf	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

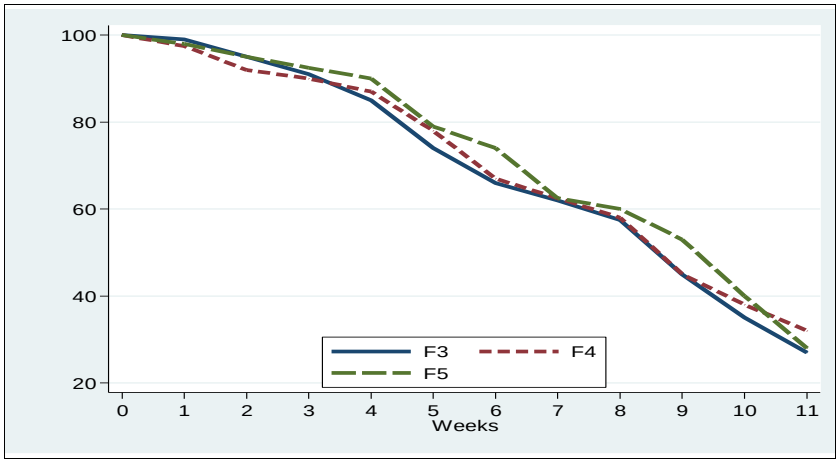

Fig 3: The trend of survival rate for flies fed at different frequencies per week, F3 (three blood meals per week), F4 (four blood meals per week), and F5 (five blood meals per week)

4. Discussion

In this study, the effect of different feeding frequencies on the survival and production of *G. f. fuscipes* was assessed with the aim of identifying 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 commonly 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 study revealed that feeding frequency had a crucial effect on the productivity and survival rate of G. f. *fuscipes* subjected in this 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 agrees with other Glossina species, G. *pallidipes*, by Tsegaye *et al.* (2020) [13] who reported low pupal production for flies fed three times per week compared to five times per week.

Concerning the size class distribution of the produced pupae in each test group, this study indicated 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 size pupae in F3 feeding treatment group, covering about 84.58% of the total pupae produced by the group while class C-E size pupae constituted the highest percentage in F4 and F5 feeding treatment groups taking about 67.1% and 68.2% of their overall production respectively. Additionally, the highest number of the smallest size class puparial (class A) was produced by F3 (18.0%) followed by F4 (10.36%) and the least was produced by F5 (9.7%) and the reverse was true for the largest puparial class (class E) covering 6.9%, 1.8% and 1.2% of the total pupal production of their respective group, F5, F4 and F3, respectively.

"It is an established 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 Zegler and Russ, and no more than 10% of the puparium should be in weight class A" (FAO/IAEA, 2006) [11]. Based on this fact, the results of this study clearly 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) are highest in F5 followed by F4 although, the difference between the two was not significant. These results were in line with Kettle, (1995) [15] who reported that the mass of a nymph depends on the quantum of blood taken by a womanish tsetse canvases during gestation, which has a significant relationship. 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 who stated, Pupal quality is an indication of the nutritional status of the fly and is reflected by pupal weight and size (IAEA, 2002) [14].

Regarding PPIF, the overall commutative Pupae per Initial Female (PPIF) for the group of flies subjected to five times per week feeding regime produced 3.25PPIF 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 3.22PPIF. On the other hand, flies subjected to the three times per week feeding frequency regime produced an overall PPIF of 2.26 PPIF which was significantly lower than both. Similarly, the lowest fecundity was obtained for the feeding treatment group F3 with a mean fecundity of 0.559 ± 0.15 pupae/females/10days 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 wide ranges of PPIF depending different factors. Results ranging from 4-7 using different food sources were obtained by (Langley and Pimley, 1979) [16] 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, 7 pupae per initial female was reported when flies were fed on cow blood, and 6.8–7.8 when fed on rabbits (Mews *et al*, 1976). "According to Jordan, the values can vary greatly, from 1.6 to 14 and the PPIF required for the establishment of a colony should be ≥3" (Jordan, 1980) [18]. 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 was lower than the limit required to establish a fly colony in a given insectary facility.

On the other hand, studies on tsetse fly rearing facilities stated that colony's daily mortality was kept below 1% and the fecundity (pupae/female/10 days) was above 0.6 (Jordan, 1980) [18]. Thus, the fecundity of F4 and F5 groups under this study were 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 (41.6%) followed by females fed 5 times per week (29.8%) and the lowest was for flies fed 4 times (28.6%) 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 (1.2%) suggested by the IAEA (2002) [14]. 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. morsitansmorsitans*, 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 relationship between feeding frequencies and adult fly survival as well as fecundity (Langley and Stafford, 1990) [19]. According to these authors, the minimal quantum of blood refection\ per cycle which didn't beget a decline in reproductive performance was four times for G.m. morsitans and five for G. pallidipes. Gaston *et al* (1993) [20], also stated that the canvases fed every third day, always engorged completely at every occasion, whereas canvases offered food every alternate day refused to feed at all or didn't engorge completely at every occasion. Furthermore, previous studies indicated that in the laboratory, female flies which take a high amount of blood feed every third day have less survival and productivity than those which take smaller blood meals every second day (Langley and Pimley, 1979) [16]. The best outcomes for F4 in nearly all parameters

evaluated here could be due to the fact that the feed is important for normal larval growth and was more likely to be attained with the 4 meals per week regime hence the flies have more chance to have a meal at the required time. On the other hand, despite the fact that a feeding frequency of five times per week optimized the number of blood-fed canvases, the tsetse cover needs 2 days to fully digest a bloody mess (McCue, *et al*. 2016) [21].

5. Conclusion and Recommendations

In conclusion, the stylish issues for nearly all parameters estimated in this study were attained 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) lead to significant negative changes on female survival and production factors although maintenance remained acceptable in some regards. Therefore, feeding tsetse flies particularly *G. f. fuscipes* species, four times per week in tsetse flies factory is recommended. Furthermore, the effect of the feeding regime on tsetse fly emergence rate and progeny quality needs to be assessed.

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