Evaluation of the effect of horse blood supplemented with human blood and vitamin on the performance of *Glossina morsitans morsitans colony*

Berisha Kapitano

National Institute for the control and Eradication of Tsetse and Trypanosomosis(NICETT), Tel 0911093600, email:-berishakapitano@gmail.com, P.O.Box 474, Hawassa, Ethiopia

Abstract

The study was conducted to evaluate the effect of horse blood supplemented with human blood and vitamin on the performance of *Glossina morsitans morsitans* colony. Three feeding groups were established and a total of 144 female *G. m. morsitans* flies were assigned to each group. The first group was entirely maintained on defibrinated horse blood, while the second and third groups fed horse blood with one feed per week on human blood and vitamin supplement respectively. The result of the study showed no difference in mortality between the flies fed human blood and vitamin supplement ($p = 0.807$) but vitamin supplement ($p = 0.002$) and human blood supplement ($p = 0.006$) produced significantly greater mortalities than the horse blood. The flies fed horse blood performed better than human blood supplement and vitamin supplement in terms of female survival ($p=0.002$), pupal weight ($p< 0.001$) and emergence ($p = 0.029$). Abortion was low throughout and not significantly different ($p = 0.548$) between the three feeding groups. Similarly no difference was found in pupae production between the flies fed horse blood and human blood supplement but both of these produced significantly more pupae than vitamin supplement ($p = 0.002$). The flies fed human blood supplement produced large numbers of pupae but of lighter weight and with low emergence. The emergence of the flies from the pupae produced by the flies fed horse blood, vitamin supplement and human blood were 97.2%, 91.1% and 90.1% respectively. According to the study, horse blood was the best diet of the three and recommended for colony feeding while human blood supplement is nutritionally poor. On the other hand, vitamin supplement did not improve the nutritional quality of horse blood, but on balance deteriorated the nutritional quality of the meal.

Key words: Colony performance, Feeding regime, *Glossina morsitans morsitan,* Horse blood, Human blood supplement, Vitamin supplement.

http://dx.doi.org/10.4314/evj.v19i2.4

Introduction

Tsetse flies are large biting insects that belong to the genus *Glossina*. Both sexes of tsetse flies feed on the blood of vertebrate animals. The flies become infected by ingesting blood meal from an infected host and remains infected throughout their life time. The trypanosomes undergo a cycle of development in the tsetse fly and transmitted to healthy animals during the fly's subsequent feeding on different animals, causing sleeping sickness in human and *nagana* in cattle (Jordan, 1986; Vreysen, 2001). Trypanosomosis control was undertaken using various trypanocidal drugs but confidence on drugs was lost due to drug resistance and vector control with various techniques remained the most desirable option to control trypanosomosis (Allsopp, 2001; Leak, 1999). However, in spite of extensive international collaboration and considerable expenditure on mechanisms to control the vector and the disease, the impact of trypanosomosis shows little sign of reduction (Allsopp, 2001). Suppressed tsetse fly populations recover from residual pockets and/ or re-invasion occurs from infested neighbouring areas creating challenge to the sustainable creation of tsetse free areas(Feldmann *et al.*, 2005).

Thus Sterile Insect Technique (SIT) which involves sustained and systematic release of sterile males over a period of about 3-4 years, was considered as another option to complement conventional techniques and mop-up the remaining population to achieve eradication (Feldmann and Hendrichs, 2001). The technique requires establishment of tsetse mass rearing facilities to produce tsetse flies in a larger number under artificial environment (Williamson *et al.*, 1983). A more successful mass rearing of *G.austeni* , maintained on blood collected from animals in slaughter house, was achieved in Tanga, Tanzania, with eradication of the species from an Island of Unguja, Zanzibar, using SIT (Vreysen *et al.*, 2000). Efforts of tsetse mass rearing and SIT are being organised by different African countries including Ethiopia, based on Zanzibar's experience (Wood, 2005) with very limited or no success.

Therefore, the aim of this study is to evaluate the effect of horse blood supplemented with human blood and vitamin on the performance of *Glossina morsitans morsitans* colony by comparing the mortality, abortion, survival, pupae production, pupal weight and emergence to contribute to the success of these efforts.

Materials and methods

Experimental flies

The tsetse fly species used for the study was *Glossina morsitans morsitans*, a typical savanna species, obtained from the colony established at the Liverpool School of Tropical Medicine (LSTM) for research and academic purposes.

Study design and experimental setup

The study was conducted using nine PVC cages of 15 cm diameter and 5 cm width. The cages were labelled B1, B2, B3, B4, B5, B6, B7, B8 and B9, together with date and number of male and female flies in the cage. Separate plastic trays of 19 cm diameter and 6 cm depth were used for holding individual cages. The bottom of the small trays were thinly covered with fine sand and two small bamboo sticks (15 cm each) were placed in the bottom of the trays between the cage netting and bottom of the tray to create space for larvae to freely crawl into the underlying tray and pupate. The individual trays were placed in a big black plastic tray (97cm x 37cm) which has the capacity of holding 10 individual trays and horizontally placed on a shelf in the main fly holding room.

A total of sixty newly emerged flies (12 males and 48 females, i.e., 1:4 ratios) (Gooding et al,. 1997) were placed in each cage and left together. Three feeding regimes were used for the experiment and three cages were allocated for each feeding regime. The flies in group one (Cages B1, B2 and B3) were allocated to feed on sterile defibrinated horse blood obtained from TCS Biosciences in 25ml aliquots and the flies in group two (cages B4, B5 and B6) were allocated to feed on horse blood as in group one but also offered human blood obtained from Liverpool regional blood bank and prepared in to 25ml aliquots instead of horse blood once a week, while those in group three (cages B7, B8 and B9) were allocated to feed on horse blood as in group one but also offered vitamin supplement instead of horse blood alone once a week. MEM vitamin solution was prepared into 30 µl aliquots in a 1.5 ml Appendorf tubes and added into 10 ml of defibrinated horse blood under sterile condition as indicated in the Life Technologies catalogue no.11120-052. Horse blood was used for *G.m.morsitans* colony established in LSTM and human blood, for which authorization was obtained by the school, was used for other research purposes related to human diseases. Therefore human blood was considered to evaluate its effect on the performance of the flies when supplemented to horse blood and the same was done with vitamin supplement.

The flies in all groups were allowed to feed every other day for ten minutes. The temperature of the feeding mat was adjusted to $37^0C \pm 1^0C$ throughout the feeding period to keep the blood at body temperature. The temperature and relative humidity of the room (insectary) were maintained on average at $26^0C \pm 1^0C$ and 70% ± 5% respectively (Gooding et al., 1997). The light of the room was kept constant under fluorescent lighting from 6 a.m. to 6 p.m. (during the day) and automatically switched off to maintain darkness from 6 p.m. to 6 a.m. (during the night). The same room was used for both feeding and breeding purposes.

Data collection

Fly mortality and abortion

Mortality in each cage was monitored every day starting from day 2 after emergence throughout the study period. The dead flies in each cage were daily removed by holding the cages upright with the small access hole facing downward to let the dead flies fall down into a tray. The flies were identified by sex and recorded according to the cage number. Survival was calculated by subtracting the number of dead flies (by sex) from the last record (previous day) of the total number of life flies in each cage. Abortion from individual trays was checked every day during the mortality check, starting from 7^{th} day post emergence (first ovulation expected) (Zelger and Russ, 1976) throughout the study period.

Pupae production and weight

Each cage was monitored every day starting from day 14 post emergence (first larviposition expected) (Zelger and Russ, 1976)for the presence of pupae. The pupae were checked whether soft or normal and recorded. Normal pupae were collected in to a separate Petri dish, labelled according to the cage number and kept for 24 hours before weighing. The pupae were individually weighed and classified into "A" (≤ 18mg), "B" (18-22mg), "C" (22-26mg), "D" (26-30mg) and "E" (>30mg) (Zelger and Russ, 1976) according to their weight. The pupae obtained from 3 cages of one feeding regime were pooled together and put according to the weight class in a row of weighing boats temporarily prepared on a shelf close to the big tray.

Pupal emergence

When the pupae were about 22 days old after larviposition, they were transferred into 60 ml disposable universal tubes and temporarily closed with netting using plastic bands instead of cap. The tubes were labelled according to the feeding regime and pupal weight class, and kept in the same room with the colony to emerge. The new emergents were chilled for 10 minutes in the cold room (at 4^0C) and sorted into male and female flies (Parker, 2005). Both sexes were counted and recorded according to the feeding regime and pupal weight class. The pupae were allowed to stay in the universal tube until no more fly emerges.

Data analysis

The three feeding regimes were computed: a) Chi-square tests for categorical and binary data (e.g mortality, pupae classes, etc) and b) one way analysis of variance for weights, with least significant difference (LSD) tests to examine difference between pairs of feeding regimes. Statistical significance was set at the conventional 5% level for all analysis. The SPSS and Stata computer programs were used to carry out the computation.

Results

Fly mortality

The study showed high overall mortality in the flies supplemented with human blood (36.1%) and vitamin (38.2%) than those fed only horse blood (20.8%). When the mortality of the flies fed horse blood is compared to those supplemented with vitamin ($p = 0.002$) and human blood supplement ($p = 0.006$) a highly statistically significant difference was observed. However, the difference in the mortality between the flies fed human blood and vitamin supplements was not statistically significant ($p = 0.807$). No difference was observed in the mortality between the flies fed human blood and vitamin supplement but both of these produced significantly greater mortality than the horse blood. The trend of mortality in different age groups is shown in Fig. 1.

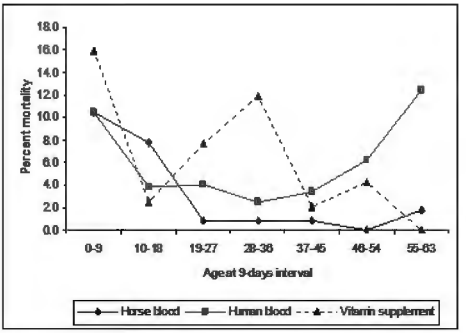

Figure 1. Mortality in different age groups for the three feeding regimes.

Abortion

The proportion of abortion recorded in the flies fed horse blood, human blood supplement and vitamin supplement were 4.2%, 5.6% and 2.8% respectively (Fig.2). This is equivalent to the daily abortion of 0.07%, 0.08% and 0.04% in that order. However, the difference is not statistically significant ($p = 0.548$).

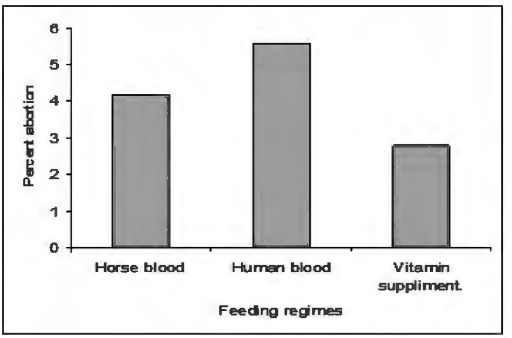

Figure 2. Abortion in the three feeding regimes.

Fly survival

When the proportion of the flies survived until the end of this experiment (63 days) was plotted against Age Group Period (AGP), the flies fed horse blood showed better survival (79.2%) than the flies supplemented with human

blood (63.9%) and vitamin (61.8%) (Fig.3). Unlike the difference between human blood and vitamin supplements (p=0.807), the overall difference in the proportion of survival (p = 0.002), the difference between horse blood and human blood supplement (P= 0.006), and horse blood and vitamin supplement (P = 0.002) are statistically significant. The proportion of fly survival between the three feeding regimes at different age group periods showed no significant difference at the age of 0-9 days (p = 0.2950, 10-18 days (p = 0.608) and 19-27 days (p = 0.295), but significant at the age of 28-36 days (p = 0.006), 37-45 days (p = 0.008), 46-54 days (p = 0.003) and 55-63 days (p = 0.002).

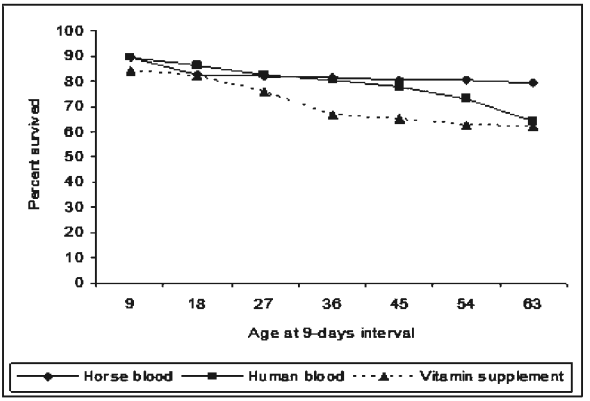

Figure 3. Fly survival at different age groups for the three feeding regimes.

Pupae production

The flies have started producing pupae regardless of the feeding regimes at the age of 17 days, although many of them started at the age of 18 days. The flies fed human blood produced high number of pupae (n = 453) compared to the flies fed horse blood (n = 415) and vitamin supplement (n = 352) respectively. The pupae produced per initial female were 2.88, 3.14 and 2.44 for horse blood, human blood supplement and vitamin supplement in that order. The trend of pupae production by the flies maintained on the three feeding regimes corresponding to the age of the flies is shown in Fig. 4. The flies fed horse blood and human blood had no difference but both of these produced significantly more pupae than vitamin supplement (p = 0.002).

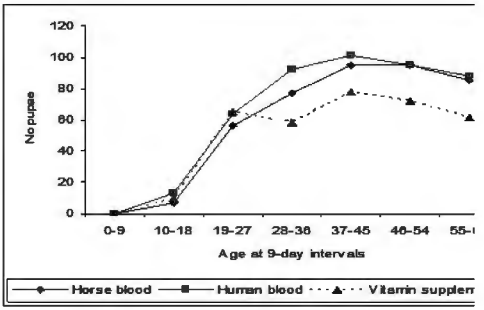

Figure 4. Pupae production at 9-day intervals for the three feeding regimes.

Pupal weight

The pupae collected on a daily basis from the three feeding regimes were sorted into five different weight classes (Fig. 5). For the flies fed horse blood and vitamin supplement, 60% of pupae were at classes D and E compared to 40% in human blood supplement which is statistically highly significant ($p < 0.001$). On the other hand, the overall difference in pupal weight between the three feeding regimes was highly different ($p< 0.001$). When each pair of feeding regimes were compared (using the LSD post hoc test), horse blood and vitamin supplement were statistically different from human blood supplement ($p< 0.001$) but no significant difference was found between horse blood and vitamin supplement ($P = 0.240$). Adjusting for differences between cages did not alter these findings. The p-value for the horse blood vs vitamin supplement was even less significant ($p = 0.296$).

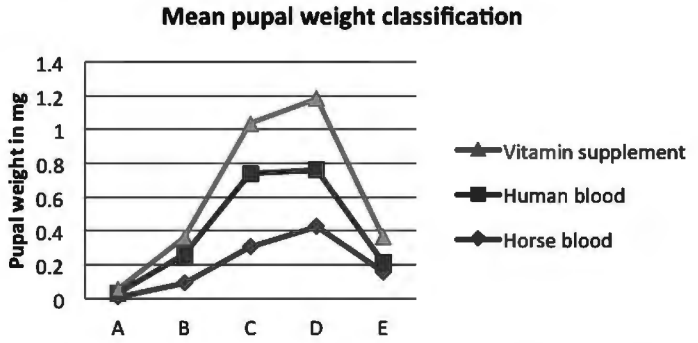

Figure 5. Mean pupal weight for the three feeding regimes

Pupal emergence

Pupal emergence was started on the same day at 26 days post larviposition regardless of the feeding regimes. Only females emerged during the first 2 days and males predominantly emerged during the last 3 days, while both sexes were mixed at the middle of the emergence period. The emergence of pupae produced by the flies fed horse blood, vitamin supplement and human blood supplement were 97.2%, 91.1% and 90.1% (Fig 6) and sex ratio at emergence was 1:1, 1:1.10 and 1:1.16 (male: female) respectively. Out of the pupae produced by the three feeding regimes, 9.9% of human blood supplement, 2.8% horse blood and 8.9% vitamin supplement were not emerged. When unemerged pupae were dissected, about 67% of them were empty (lysed) and the remaining 33% had a formed adult stages but none of them were found alive or showing a sign of movement. The flies emerged from weight class "A" were smaller than other weight classes, although flies with slightly similar sizes were emerged from weight class "B". The flies emerged from weight class "E" were generally bigger. The overall pupal emergence showed no statistically significant difference (p= 0.065). However, both human and vitamin supplements resulted statistically significant difference with horse blood (p = 0.029), and no difference was found between human blood and vitamin supplements (p= 0.835)

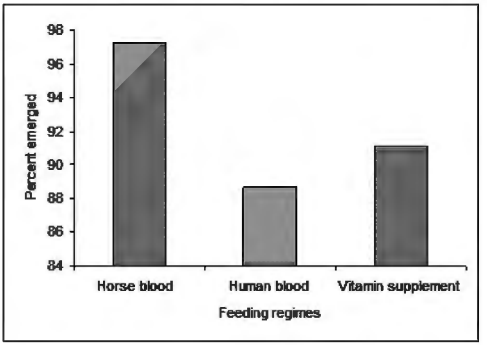

Figure 6. The proportion of pupae emerged from the three feeding regimes.

Discussion

The present study showed that the daily mortality rate recorded in the three feeding regimes were far below the acceptable daily mortality rate (1.2%) suggested by IAEA (2002) showing that the colony is doing very well. The mortality recorded in all feeding regimes during the 1st AGP was in agreement

with Wetzel and Luger (1978) who indicated that this period is critical for the survival of the flies. The high mortality recorded in the 3rd and 4th AGPs of the flies fed vitamin supplement had coincided with the period where the flies are at a high productive age. The proportion of abortions recorded in all feeding regimes, were lower than the abortion (8.72%) reported by Moloo *et al.* (1988) in *G. m.morsitans* maintained on the blood of different species of wild mammals. So, it could be concluded that the flies maintained on all feeding regimes performed better in relation to abortion, although the abortions of the flies fed human blood supplement looks slightly higher than vitamin supplement and horse blood probably due to its poor nutritional quality.

Despite the speculation that additives could profoundly increase the performance of the flies, feeding vitamin supplement with horse blood or feeding human blood once a week had no effect in improving the survival of the flies. This is in agreement with similar findings by Mews *et al.* (1976) where the addition of high concentration of ATP in a haemolysed blood did not improve the survival of the flies. The relatively better overall survival observed in the flies fed horse blood compared to vitamin and human blood supplements was directly related to the high number of deaths occurred during the 1st and 4th AGPs in the flies fed vitamin supplement and 6th AGP in the flies fed human blood supplement. The low performance of the flies fed vitamin supplement was due to poor survival of the flies which had an obvious implication on the reduction in pupae production compared to the flies maintained on horse blood and human blood supplement. Unlike the standard proportion (>95%) (Wetzel and Luger, 1978) to be attained at 5th AGP, the proportion of flies reached this age were 83.3%, 86.1% and 82.6% for horse blood, human blood supplement and vitamin supplement respectively.

The age at which the flies started producing pupae is in agreement with the findings of other similar studies (Wetzel and Luger, 1978; Gooding *et al.*, 1997) which indicated that the flies rarely start producing pupae before the end of the 2nd AGP and increases until the flies reach maximum productive age at 5th AGP before declining. The number of pupae produced per female regardless of feed source was within the range (2-3 pupae per female) which agrees with the number suggested by Wetzel and Luger (1978) who also indicated that the number is sufficient to maintain the same number of females in the colony.

Pupal quality is an indication of the nutritional status of the fly and is assessed in terms of pupal weight and size (Jordan and Langley, 1991; IAEA, 2002). The

pupae produced by the flies fed human blood supplement were lighter than those produced by the flies fed horse blood and vitamin supplement. However, it is still above the minimum acceptable weight (24 mg) (Wetzel and Luger, 1978). The weight of pupae produced at the end of the 2nd AGP and during 3rd AGP was lighter compared to the 4th and above AGPs regardless of the feeding regimes. This is in agreement with the findings of Wetzel and Luger (1978) who described that the weight of large number of pupae produced until the 3rd AGP fall in weight class B and C and then shift to weight class C and D from 4th to the end of this experiment (7th AGP). Younger females of *G. m. morsitans* fed horse blood and vitamin supplement produced lighter pupae than older females, the weight being increasing with age, while that of human blood supplement remained constant. This is in the contrary to the findings reported by Wetzel and Luger (1978) who stated that females younger than 50 days produce heavier puparia irrespective of blood source than females aged 50 and above days.

Although the flies fed human blood supplement produced high number of pupae compared to horse blood and vitamin supplement, there was significantly low number of flies emerged from the pupae produced by the flies supplemented with human blood and vitamin compared to the horse blood. The emergence was slightly higher for the flies fed horse blood than the emergence (95%) suggested by (Zelger and Russ, 1976) while others were below this performance limit. The large number of pupae produced by the flies fed human blood supplement could not compensate lower performance of pupae in terms of weight and emergence. Another observation was that the large proportion of unemerged pupae recorded in the flies fed human blood supplement had coincided with large proportion of lighter pupae (<22 mg) produced by the flies fed human blood supplement (18.54%) compared to 10.36% and 12.12.5% in horse blood and vitamin supplement respectively. So, the conclusion that could be drawn from this is that the nutritional quality of human blood supplement was lower than horse blood which could have directly contributed to the reduction of pupal weight and emergence. Similarly, the low pupal emergence recorded in the flies fed vitamin supplement showed that the addition of vitamin supplement did not improve or on balance deteriorated the nutritional quality of horse blood with low pupal weight and emergence compared to horse blood.

Unlike the standard (1:1) sex retio (IAEA, 2002) the study showed a slight difference in sex ratio which could be assumed to be due to the difference in pupal emergence but not actual variation in sex ratio among the flies maintained on

the three feeding regimes. In addition, dissection of unemerged pupae revealed high proportion (67%) of lysed pupae with only 33% that had formed adult stage. This is in agreement with the findings of Moloo *et al.* (1988) and Gooding *et al.* (1997). Since all the handling and environmental conditions were similar for all feeding regimes, the difference in the proportion of unemerged pupae were likely to be the effect of nutritional quality of the meal.

Conclusion

In conclusion, the overall performance of the flies fed horse blood was superior as compared to human blood and vitamin supplements showing that horse blood can be absolutely useful for the fly rearing. The result encourages the use of equine blood for tsetse mass rearing in Ethiopia, considering the presence of large number of equines in the country, which are thrown away when aged. On the other hand, the low performance of the flies fed human blood, particularly high proportion of lighter pupae and low pupal emergence, indicated that human blood is nutritionally poor to be used for colony development. Similarly, the flies fed vitamin supplement showed low performance compared to horse blood indicating that vitamin supplement did not improve the nutritional quality of horse blood, but probably deteriorated, and therefore not qualified as a preferred diet for tsetse flies feeding. Moreover, feeding every other day showed good overall performance with horse blood with an additional advantage of reduced work load and could be considered as a standard colony feeding procedure.

Acknowledgement

I am grateful to Professor M. J. Lehane, Dr. Paul Bates and all teachers and staff of Liverpool School of Tropical Medicine who directly or indirectly assisted me during the study. My special thanks go to Dr E. B. Faragher who assisted me in data analysis. I also thank the International Atomic Energy Agency for the sponsorship, and Ethiopian Science and Technology Commission and the Southern Tsetse Eradication Project for granting me the opportunity.

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