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Effect of Different Blood Meal on Feeding Rate and Fecundity of *Aedes* and *AnophelesSpp* (Diptera: *Culicidae*)

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Abstract
This study investigates the extent of reproduction and effect of different blood meals on feeding rates and fecundity on Aedes and Anopheles Spp. Mosquitoes were reared with the use of standard techniques, such as natural climatic conditions, temperature and humidity control which are probably the most important factors in successful rearing and also the use of cloths over cage provide humidity and maintained cycle of 12hr of light and 10hrs of dankness. Aedes, and Anopheles fed on three different blood sources including Human blood (H/B), Sheep blood (S/B) and Chicken blood (C/B) respectively. Female mosquitoes utilizing human blood (H/B) has the highest feeding rates of 67.3% related to other blood source fed on Aedes Spp. Human blood (H/B) with 53.0% feeding rate as the highest in Anopheles spp. Female Anophelesfed directly on chicken blood (C/B) 34.0% and human blood 81.0% (H/B) were higher than sheep blood. Aedesand Anophelesfed on human blood (H/B) with fecundity range from 52.5% to 47.5% and 60.0% to 40.0%. Fecundity attributes differed significantly (P<0.05) among blood meals types. However reproductive performance was significantly lower with Aedesfed on chicken blood (C/B) respectively. This study proves that blood meal is unavoidable for successful reproductions of mosquitoes with human blood induce highest level of productivity. These findings indicated that Anopheles and Aedes Spp to some extent reproduce successfully by utilizing blood meals other than human host.

Keyword Index: Blood source, Feeding rate, Fecundity, *Anopheles, Aedes*

INTRODUCTION
When rearing mosquito vectors in the laboratory, researchers are often challenged by the method of feeding and by the source of blood that will effectively and efficiently facilitate predictability and success in terms of both colony maintenance and experimentation. Indeed, females of most species of mosquito must take a vertebrate blood meal to acquire protein for egg production. In the development of a system for mass-rearing insects, efficiency and economics are of the utmost importance (Bailey *et a1.*, 1980). *Anopheles* and *Aedes* are colonized in the Insectary at the Department of Biochemistry Bayero University Kano. These mosquitoes are reared to support research on malaria control, as well as to support repellent testing and pesticide resistance evaluations. Two techniques are commonly used for feeding female

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mosquitoes in the laboratory. One such technique, direct feeding, has several disadvantages, including the added expense and inconvenience associated with maintaining laboratory animals (Thomas *et al*., 1985). A more effective technique is feeding via artificial membranes. Such a method is low in cost, more standardized, and limits the involvement of animals (Cosgrove and Wood 1996, Benzon and Apperson 1987, O'Meara *et al* 1993). Our insectary at BUK uses membrane feeding techniques to rear mosquito colonies. There are several published studies on different aspects of mosquito membrane feeding (Samish *et a*l, 1995, Robert 1998). However, there are no published reports comparing the relative impact of blood sources (i.e., human, sheep, and chicken via direct feeding) on feeding, fecundity, and egg hatch rates in laboratory colonies. The insectary historically utilizes sheep blood to rear *Aedes spp*. and human blood for rearing *Anopheles* species because both are economical, locally acquired, and have provided adequate results in our past efforts to rear mosquitoes. In this study, we test the hypothesis that blood source influences feeding success, female fecundity, Feeding rates in two colonized mosquito species. Blood sources were selected because they are also available from our current blood.

MATERIALS AND METHOD

Origin of Blood Sources

The study utilized three blood sources (sheep, chicken, and human). Commercial defibrinated sheep blood and were purchased from the Kano Animal slaughtering centre, abattoir Nigeria. Commercial human blood was purchased from Blood Bank via EDTA, Malam Aminu Kano Teaching Hospital (AKTH).

Mosquito Rearing

Aedes and *Anopheles* colonies were maintained and all experiments were carried out at a constant temperature of 25 ± 2^{o}C and 80 ± 10% relative humidity. Eggs were submerged in 1.5 litres of distilled water in plastic trays (size 30x35x5 cm). After eggs hatched, ground fish food was added (0.1g,- 0.3 g for 3rdinstar larvae and 0.5 g for 4thinstar larvae at 08:00 and 16:00 each day) to each tray for the successive two weeks until pupation of all larvae. Pupae were transferred to plastic containers with fine mesh netting at the top where healthy adults emerged and assumed resting positions on the side of the container. Healthy emerged adults were provided with soaked cotton balls containing a 5% multivitamin solution (commercially available vitamin syrup, containing 50% sucrose). The vitamin-saturated cotton balls were removed from the holding containers for 12h prior to blood feeding and replaced with a water-soaked cotton ball.

Membrane-Feeding Technique

Sausage casing membranes were stretched across the bottom of the feeders and secured with a rubber band (glove). Petri dish containing mosquitoes were placed under the feeders, all the while ensuring that the bottom of each feeder was in contact with the mesh netting fitted to the top of the cup. Blood was added (10 ml) to the feeder well. After 30 mins feeding, the number of mosquitoes that successfully fed was recorded and mosquitoes that did not feed or only partially fed were discarded.

Determination of Engorged Females and Survival Rates Post-Feeding`

On day 1, five to seven-day-old females were placed into 8.5x8cm wooden cage. Each container had one open end and a piece of nylon mesh or net was fitted over this open end to facilitate feeding. Mosquitoes were allowed to feed for 30 mins either through the sausage casing membrane or via direct feeding as described previously. All fully-engorged

mosquitoes were counted and recorded, while those that failed to engorge were removed and discarded. Feeding rates of engorged females were recorded on days 1, 3, and 7. The sequence was repeated per blood source including direct feeding on human blood.

Fecundity and Egg hatching rate

Two days after a 30-min period of blood feeding, engorged females were selected at random from each species (Yang *et al*., 1963) *Aedes* and *Anopheles Spp*, were allowed to mate naturally. Each blood-fed and mated female was held in an individual glass vial containing a piece of moist filter paper as an oviposition substrate. Numbers of eggs laid were recorded for each female up to 48 h post-feeding. All eggs of engorged females per species were immersed in plastic rearing trays with water for hatching observation. Two days later, the number of newly hatched larvae was recorded.

Developmental Period and Survival Rate of Offspring from Blood-Fed Adults

Larval and pupal periods were determined and time spent from first instar to pupation was recorded for all hatched larvae. Immediately after adult emergence, the number of adults were counted and compared with the number of eggs to compute the survival rate of offspring(Akoh *et al*.,1992)

Laboratory Investigation and Reproductive Performance

Laboratory investigation in successfully rearing required attention 24hr/7 days a week (Spitzen *et al.,* 2005). Reproductive performance such as Blood feeding and fecundity were determined based on the following such as; Blood feeding rate (BF) was determined by the proportion of female mosquitoes presented with number of mosquitoes tested, Fecundity rate (F) is total number of egg laid per number of engorged female x 100%. It was determined by the technique of Edillo *et al.,* (2004) as well as described by Olayemi and Ande (2009).

Statistical Analysis

All result analysis was computed using SPSS (version 21) ($p< 0.05$)Chi-Tests were used to determine if there were significant differences between treatment in rate of feeding on human blood, chicken blood and sheep blood. Analysis of variance was used to determine significant differences in fecundity between treatment and feeding trials.

RESULTS

Engorgement and adult post-feeding survival rates

Aedes

The mean percentages of engorgement from feeding on human blood (91%) were significantly higher relative to chicken and sheep blood (51. 9% and 13.5%, respectively) ($P<0.05$) (Table 2). By day 7 post-feeding, engorged females utilizing human blood had the highest survival rate (100%) relative to other blood sources, although not significantly higher than chicken blood. In contrast, the survival rate of engorged females post-feeding on sheep blood (26.1%) was significantly less than other blood sources ($P<0.05$) (Table 3).

Anopheles

The mean percentage of engorged females from direct feeding on human (95.3%) was higher than the other blood sources; sheep blood (17.1%), chicken blood (67.0%), (Table 2). At 7 days post-feeding, the survival rate with human blood was 100%. Human blood were showing survival rates at 98.3, and 96.2 respectively, although there were no statistically significant differences among the blood sources ($P>0.05$) (Table 3). Presumably, survival rate is not affected by the blood source for this particular species.

Table 1: Mean Percentage Engorged/Feeding Rate of Female Mosquitoes Fed Various Blood Sources

	Mean percentage Engorge Female	
Blood Source	*Aedes*	*Anopheles*
Human Blood	91.0±41.0[b]	95.3±87.6[b]
Chicken Blood	51.9±6.4[b]	67.0±2.71[b]
Sheep Blood	19.5± 7.0[b]	17.1±21[b]

Percentages within the same are not significantly different (P>0.05, by One-way **ANOVA** and X^2 test).

Fecundity and egg hatching rate

For *Anopheles* the mean number of eggs laid by direct feeding on a human (218 eggs +75.0) was not significantly different from on Chicken blood (118 eggs +4.8) (Table. 1). However, the number was higher than what was observed with chicken blood (151.3 eggs +3.2) and sheep blood (95.0 eggs +2.3) (Table 2). The hatching rate was significantly lower with sheep blood (19.5%) relative to the other blood sources (Table 2). For *Aedes*, the mean number of eggs laid and hatching rate associated with a sheep blood diet (12 eggs +16.5, 64.0%) was significantly less than what was observed with human and chicken blood.

Table.2 Mean Comparison of the Number of Eggs Laid for Each Blood Source

Blood source	*Aedes* **No. of Eggs ±SD**	*Anopheles* **No. of Eggs ±SD**
Human Blood	16.5±6.3 (12)	7.5±1.96 (218)
Chicken Blood	74.0±72.6(110)	4.8±5.6 (118)
Sheep Blood	N/A	2.3±10.7(95)

Percentage /numbers; Significant different (p<0.05, by one-way ANOVA; N/A=NOT available

Developmental period and survival rate of Offspring

The mean developmental time from egg to adult of offspring from *Anopheles* parents fed on one of three blood sources was 11.3 days (human blood), 12.3 days (chicken), and 13.7 days (sheep blood) (Table 1). Mean developmental time to adult after parents were fed on human blood was significantly longer relative to the developmental times of other blood sources (P<0.05). The survival rate of offspring originating from adults fed with sheep blood (19.2%) was significantly less than that observed with other blood sources: chicken blood (84.8%), and human blood (80.1%). The mean developmental time for offspring from *Aedes* fed human blood observed for (13.7 days). This result proved to be significantly longer than the 11.3 days observed for offspring from adults fed chicken, blood. No significant differences were found among the survival rates of offspring as a result of adult feeding on human (93.9%), chicken blood (87.3%).

DISCUSSION

The results of this study showed the blood meal source has impacts on feeding rates, adult survival, fecundity, hatching rates, and developmental times of *Aedes* and *Anopheles* mosquitoes. Sheep blood had a significant, negative impact on the survival rates of *Aede s*and *Anopheles*. The mean number of eggs laid was reduced relative to the other blood sources when *Aedes* adults were fed sheep blood. Sheep blood did not induce the production of eggs for *Aedes*. Previous studies have in fact shown that mosquito fecundity is affected by the following factors: mosquito species (Briegel, 1990), body size (James, B. and Done, W.,(2016).), host (Taylor and Hurd, 2001), size of the bloodmeal (Roitberg and Gordon, 2005) and amino acids from erythrocytes (Hurd, 2003).

Furthermore, the offspring of *Anopheles* fed with sheep blood survived for a shorter period of time relative to what was shown with the other blood sources. The results shows that blood meal sources impact feeding rates, survivorship, and fecundity, and are consistent with those of other studies. (Lutomia *et al*., 2017) studied the effects of nutrition from a blood meal on survival and calorie intake in mosquitoes when females were fed a meal of chicken blood.

Efficiency of blood ingested for survival was highest in *Aedes.* Xue (2008) reported that the survival of female *Anopheles* that were fed on blood of two different hosts (double meal; 60.2%) was higher than the females fed on one host (single meal; 55.5%) and produces significantly more eggs. Kweka *et al*. (2010) investigated the feeding and mortality rates of *Cx.quinquefasciatus*, *Ae. Aegypti* and three different hosts (rabbits, guinea pigs, and mice) also studied previously.

Other studies shows mosquito mortality was 54.9% for those introduced to mice as a host, 34.3% for the guinea pig, and 10.8% for those introduced to the rabbit. Survivorship in mosquitoes is related to many factors including temperature and available nutrition (Clement, 1992). Also, human blood was the most ideal for oviposition rate (79.2%), while chicken were the least favourable (46.8 and 48.1%, respectively). In addition, Richards *et al.* (2012) reported the impact of blood meal source on feeding and reproduction in *Aedes* when mosquitoes were fed on different blood meal sources (chicken blood and human blood).

Fecundity and fertility of *Anopheles* were greater in mosquitoes fed with chicken blood than the other blood source. Offspring of parents reared under low food availability produced more eggs than the offspring from parents reared under high food conditions. Harre *et al*., (2001) found no differences in feeding success, mortality rates, egg numbers, and egg viability when sand flies (*Phlebotomus papatasi*) were fed on blood from different mammals (human, horse, cow, pig, dog, rabbit, guinea pig, and hamster). Our study demonstrates that sheep blood has a negative impact on adult survival, egg-laying, and hatching rates of Anopheles mosquitoes. Future studies will elucidate some of the mechanisms for these findings in terms of the chemical and physical composition of sheep blood or digestion processes.

CONCLUSION

Feeding rate (BFR) was significantly highest in group of mosquitoes fed with human blood. Also human blood was the most ideal for egg production rate (Oviposition), while sheep blood and Chicken bloods are least favourable respectively. Fecundity rate (FR) has distribution pattern has hatching was generally high on mosquitoes fed with human blood and chicken blood. Blood feeding rate (BFR) defines the proportion of female mosquitoes that successfully blood feed. Oviposition rate (OR) define the proportion of blood- fed female mosquitoes that oviposited or Determine as the proportion of engorged female mosquito that eventually laid eggs. Fecundity rate (FR) determine as the total number of eggs laid per

number of female mosquitoes. This study proves that blood meals are unavoidable for successful reproduction of mosquitoes. Blood meals significant affect feeding rates and fecundity, with human blood induce highest level of productivity. These findings indicate that *Anopheles* and *Aedes* produce successfully by utilizing blood meals other than human host.

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