



Effects of Host Blood on Fecundity and Longevity of Female *Anopheles* Mosquitoes

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Authors' contributions

This work was carried out in collaboration among all authors. Author JIC carried out the experiment drafted the manuscript. Authors AO and IOO designed the research and managed the literature search. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The effect of Host blood on the fecundity of female *Anopheles gambiae sensu lato* mosquitoes was studied under normal conditions of 64±2% Relative Humidity and 27±2°C Temperature.

Methods: Three-five day old (F1) female *Anopheles* mosquitoes were transferred into wooden cages (40x40x40 cm) and fed blood from the following sources: man, cattle, chicken, goat, pig and sheep through an artificial feeding membrane. Engorged females were observed and fecundity recorded. The entire experiment was replicated five (5) times.

Results: From the 1st to 4th gonotrophic cycle, mosquitoes fed human blood produced significantly greater ($p < 0.05$) number of eggs (Mean=121.90±1.18, 101.36±1.56, 64.12±1.54 and 29.66±1.69 respectively) than mosquitoes fed other blood meal sources. Across the six (6) blood meal trials (excluding that of sheep), there was a significant reduction ($p < 0.05$) in fecundity from the 1st to 4th gonotrophic cycles (1st>2nd>3rd>4th). There was no significant difference ($p > 0.05$) in fecundity between pigs, chicken and sheep. Total mean longevity and total mean fecundity was significantly greater ($p < 0.05$) in mosquitoes fed human and cattle blood than in mosquitoes fed the other blood sources.

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Conclusion: The results showed that blood meal source affects fecundity and longevity of female *Anopheles gambiae s. l* mosquitoes reared under laboratory conditions and that blood from humans as well as from other domestic animals is suitable for sustaining vectorial capacity in *Anopheles gambiae s. l* mosquitoes.

Keywords: *Anopheles* mosquitoes; haematophagous insects; vector diseases; host blood; *Anopheles gambiae s. l*.

1. INTRODUCTION

1.1 Background

Mosquitoes are haematophagous insects that vector diseases of both medical and veterinary importance. Malaria vectored by female *Anopheles* mosquitoes is responsible for millions of deaths worldwide. In 2015, 88% of worldwide malaria cases and 90% of deaths due to malaria occurred in Africa (World Malaria Report, [WHO] 2017). Elephantiasis a disfiguring and disabling disease, caused by a filarial worm is transmitted by *Anopheles* mosquitoes; in Africa, about 406 million people are at risk for elephantiasis parasite which causes kidney damage, pains, disfigurement and disablement of those infected (WHO, 2018). Mosquito vector is required for completion of the life cycle of malaria and elephantiasis, and mosquito vector control is aimed at transmission interruption. Mosquito vector control could be approached from different angles including the angle of reducing the habitat where they breed through environmental control, or by deterring their reproduction (fecundity), growth and longevity (life span). In nature female *Anopheles* mosquitoes may feed on humans and a range of animals including: cattle, horses, goat, sheep, pigs, dogs, cats, rabbits, chickens and ducks [1]. Olayemi et al. [2] reported that the source of a blood meal could affect fecundity, egg hatching rate and embryony period in *Anopheles gambiae sensu stricto*. Fecundity is an important factor in determining vectorial capacity of insects that vector diseases of medical importance (Van de Walle, 2010). It follows that the more abundant an insect vector is, the more likely the chances of host-vector contact and disease transmission. The fecundity of female *Anopheles* mosquitoes would therefore play an all important role in the epidemiology of malaria, filariasis and other diseases they vector.

Once ingested by a mosquito, malaria parasites and other parasites must undergo a developmental cycle also known as Extrinsic Incubation Period (EIP) within the mosquito before they are infectious to humans. Rajatikeka

et al. [3] reported higher susceptibility to insecticides in older mosquitoes in two *A. gambiae* populations. Aging leads to increased susceptibility to insecticides (Jones et al., 2012) even among blood fed mosquitoes (Glunt et al., 2011). A study in Western Kenya showed increased tolerance to pyrethroids in younger mosquitoes than in older mosquitoes, and significant reduction in mortality in blood fed mosquitoes than in unfed mosquitoes showing effect of age on *Anopheles* fitness and that blood meal confers fitness on *Anopheles* mosquitoes [4]. From the foregoing both longevity and fecundity are important parameters that could affect control of mosquito vector. This study was therefore aimed at determining the effects of blood meal on fecundity and life span (longevity) of *Anopheles* mosquitoes and the correlation between fecundity and longevity in laboratory reared mosquitoes feed different blood meal sources.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in a laboratory in University of Agriculture, Makurdi, Benue State, Nigeria under standard conditions of $80 \pm 2\%$ relative humidity and $27 \pm 2^\circ\text{C}$ temperature. Six (6) cubic wooden cages $40 \times 40 \times 40$ cm covered with white mosquito proof nets were constructed, and a stock of *Anopheles gambiae sensu lato* mosquitoes were used for the experiment. All mosquitoes were maintained on 15% sugar solution. After mating, 4-day old adults were transferred into cages, ten (10) females per cage for blood feeding. Only female mosquitoes were used for the blood feeding experiment.

2.2 Blood Collection, Feeding and Oviposition

Fresh blood from the following sources: cow, goat, chicken sheep, pig, were collected from an abattoir in sealed vacuum bowls and brought to the laboratory while self-blood was used as source of human blood (a trained specialist

performed the venipuncture to collect human blood). All blood used for the experiment were (heparinized) to prevent the blood from clotting. Stretched (powder free) latex gloves were used as artificial feeding membrane as described by Richards [5] and placed one per cage for the mosquitoes to blood feed. Non-engorged mosquitoes were aspirated out and only five engorged mosquitoes were randomly selected for oviposition. Four days after blood feeding [6], a plastic bowl containing filter paper at the bottom was placed in each cage and left overnight for the gravid mosquitoes to oviposit.

2.3 Egg Counting

In the morning, the petri dishes (oviposition sites) were removed, all filter papers containing eggs were photographed, and the images uploaded to a computer and counted using *Anopheles* egg counter software [7].

2.4 Subsequent Blood Feeding Trials

After the first gonotrophic cycle, the mosquitoes were again allowed to blood feed and oviposit and this was repeated all through their life span. The entire experiment was replicated five (5) times.

2.5 Fecundity

Fecundity of mosquitoes in each blood meal treatment was calculated as:

$$f = \frac{\text{Total no of eggs laid by mosquitoes that fed on a particular blood source}}{\text{Total no of mosquitoes that fed on a particular blood source}}$$

2.6 Longevity

Longevity of mosquitoes for each blood meal treatment was calculated as:

$$l = \frac{\text{Total no of days lived by mosquitoes that fed on a particular blood source}}{\text{Total no of mosquitoes that fed on a particular blood source}}$$

2.7 Statistical Analysis

Results were analyzed using One-way ANOVA to test for significant difference in fecundity from 1st to 4th gonotrophic cycles and difference in longevity between blood meal sources. A scattergram was plotted to show the correlation between fecundity and longevity in *Anopheles* mosquitoes.

3. RESULTS AND DISCUSSION

Prior to blood feeding, the female mosquitoes were maintained successfully on 15% sugar solution thus showing that sugar is capable of sustaining the life of *Anopheles gambiae s. l* mosquitoes and the reason for blood feeding by females in this species is to obtain a protein rich diet necessary for egg development. Gary et al. [8] opined that female *Anopheles* mosquitoes can survive in the wild on only plant nectar. Maia et al. [19] reported the effectiveness of attractive-toxic sugar baits in Tanzania for the control of mosquitoes.

Mosquitoes in each blood meal group readily fed on the blood meal they were offered within their cage compartment. Thus revealing that blood feeding behavior of *Anopheles* is determined by the availability of potential host as demonstrated by their ability to feed on a range of available vertebrate blood meals sources. This implies that not only humans should be protected from the mosquito vectors but animals as well as mosquitoes would take advantage of available blood source within their environment. The use of different hosts by *Anopheles* mosquitoes could have implications for disease transmission as could lead to a breakdown in the species barrier and transmission of diseases between species that share the same vectors. Higher fecundity in mosquitoes fed human and bovine blood could promote evolution of selective feeding in *Anopheles* mosquitoes as they may evolve a preference for host species that support maximum biological performance. This may in turn promote vector competence and a form of evolutionary divergence where mosquitoes that feed preferentially on humans would be likely vectors of diseases of medical importance while mosquitoes that feed on animals would be vectors of diseases of veterinary importance. Olayemi et al. [2] also reported higher fecundity in Anopheline mosquitoes fed human and bovine blood and opined that rearing of domestic animals close to homes could provide alternative blood meal source and therefore could effectively control malaria in communities. Basseri et al. [10] also described *Anopheles gambiae* as antropophilic having a selective preference for human hosts; and Swami and Srivastava [11] described *An. Annularis* as zoophagic based on presence of only bovine blood in the guts when dissected.

In contrast however, Takken and Verhulst, [12] opined that host-selection in mosquitoes is a deviation from the norm and that *Anopheles*

mosquitoes when starved will feed on available vertebrate hosts without showing host preference.

Chicken blood was the only non-mammalian blood meal source used in this study and it produced less fecund mosquitoes than any other blood meal source thus implying inferiority in quality of avian blood. In contrast *Culex quinquefasciatus* mosquitoes fed on live chicken blood had significantly higher fecundity than mosquitoes fed on bovine blood meal [13], Benhamed et al. [14] reported no significant difference in fecundity in *Anopheles maculipennis* fed cattle and chicken blood.

From our results, as *Anopheles gambiae* s. l. mosquitoes grew older, they became less fecund, thus implying a reduced fitness as mosquitoes age. Aging in mosquitoes has been reported to have effects on a mosquitoes fitness; older mosquitoes are more susceptibility to insecticides than their younger counterparts [3,15].

Average longevity was significantly higher ($p < 0.05$) in mosquitoes that fed on human and bovine blood meal. Such significant differences in longevity could have severe implications for disease epidemiology since it takes parasites like *Plasmodium* and arboviruses at least 10 days to complete their extrinsic developmental cycle within a mosquito and mosquitoes need to feed at least once to get infected and then to transmit

the parasite that has developed with it. By implication, therefore, mosquitoes that live longer would have higher vector competence than shorter lived mosquitoes; as they would survive long enough for the disease pathogens to develop within them and for disease transmission to take place. From our results, mosquitoes fed the different blood meals survived long enough to feed and oviposit at least three (3) times, showing vector competence across the different blood meal sources. Thus implying that the presence of humans as well as other animals poses potential problems for vector competence. A strong positive correlation was found between total mean fecundity and total mean longevity in *Anopheles* mosquitoes. Thus implying that short lived mosquitoes will produce less total number of eggs than their longer lived counterparts taking the same blood meal. This buttresses the importance of longevity as a biological parameter promoting vector competence and agrees with reports by McCann et al. (2009) who carried out two experiments in *Culex pipiens* fed on mouse blood. The experiments revealed overall higher mean fecundity in insects that lived longer and that had more gonotrophic cycles. Vezilier et al. (2012) on the other hand reported a negative correlation between fecundity and longevity in *Plasmodium* infected and non-*Plasmodium* infected *Culex pipiens* mosquitoes, and suggested that mosquitoes may reduce their egg laying as a tradeoff to ensure increased longevity.

Table 1. One-way ANOVA for different in fecundity from 1st - 4th gonotrophic cycle in mosquitoes fed different blood meal sources

Blood meal source	Gonotrophic cycle				P-value
	1 st	2 nd	3 rd	4 th	
Human	121.90±1.18	101.36±1.56	64.12±1.54	29.66±1.69	P<0.05
Cattle	92.36±0.78	62.92±1.87	41.16±0.95	0.00±0.00	P<0.05
Chicken	55.20±0.76	30.04±1.96	16.16±0.87	0.00±0.00	P<0.05
Goat	74.42±0.60	44.80±1.25	27.06±0.93	0.00±0.00	P<0.05
Pig	64.56±0.94	44.49±1.42	17.60±1.78	0.00±0.00	P<0.05
Sheep	0.00±0.00	36.38±2.29	21.21±1.15	0.00±0.00	P<0.05

Table 2. One-way ANOVA for differences in longevity of mosquitoes fed different blood meal sources

Blood meal source	Gonotrophic cycle				P-Value
	1 st	2 nd	3 rd	4 th	
Human	121.90±1.18	101.36±1.56	64.12±1.54	29.66±1.69	P<0.05
Cattle	92.36±0.78	62.92±1.87	41.16±0.95	0.00±0.00	P<0.05
Chicken	55.20±0.76	30.04±1.96	16.16±0.87	0.00±0.00	P<0.05
Goat	74.42±0.60	44.80±1.25	27.06±0.93	0.00±0.00	P<0.05
Pig	64.56±0.94	44.49±1.42	17.60±1.78	0.00±0.00	P<0.05
Sheep	0.00±0.00	36.38±2.29	21.21±1.15	0.00±0.00	P<0.05

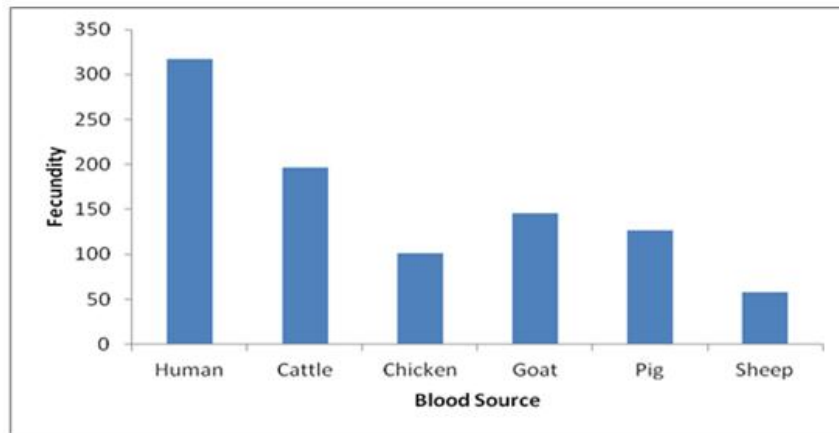


Fig. 1. Total average fecundity of mosquitoes for each blood meal source (n=25), (p<0.05)

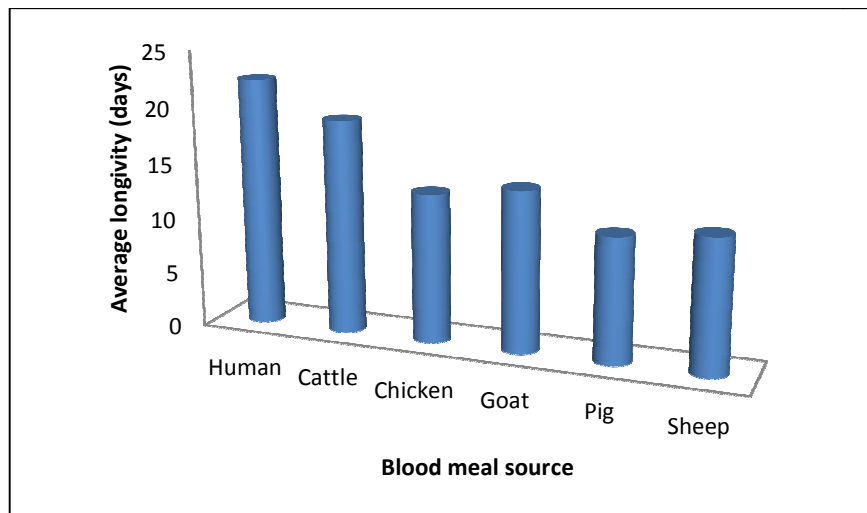


Fig. 2. Average longevity of mosquitoes per blood meal source (n=25), (p<0.05)

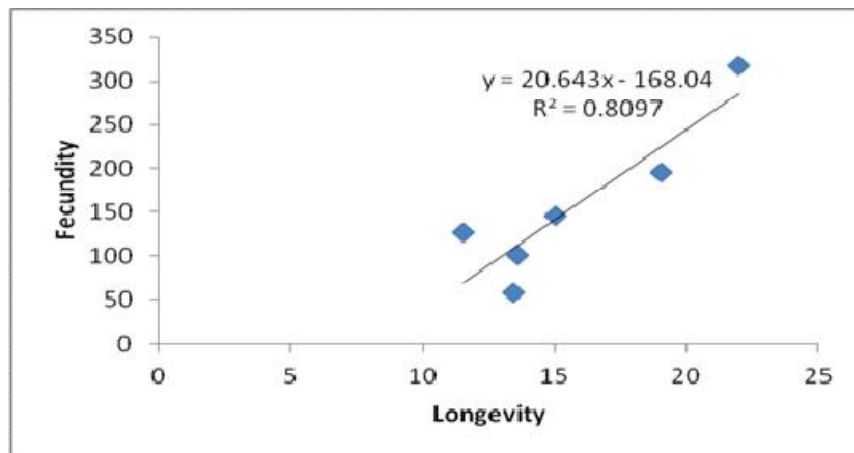


Fig. 3. Correlation between total mean fecundity and total mean longevity in *Anopheles* mosquitoes

Correlation is significant at (F = 0.014548)

With scarce resources for protection from mosquito vector, it could be suggested that priority be given to protecting humans and cattle from these noxious insects as their blood promotes higher longevity which translates to higher vector competence and overall higher fecundity. Resources should be channeled to protection of other animals as well as their blood is also capable of promoting fecundity and longevity in mosquitoes.

4. CONCLUSION

In conclusion, blood meal source affects fecundity and longevity in *Anopheles* mosquitoes reared under standard laboratory conditions and is expected to have similar effects in the wild. Both humans and animals dwellings should be mosquito proofed for effective vector and by extension, disease control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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