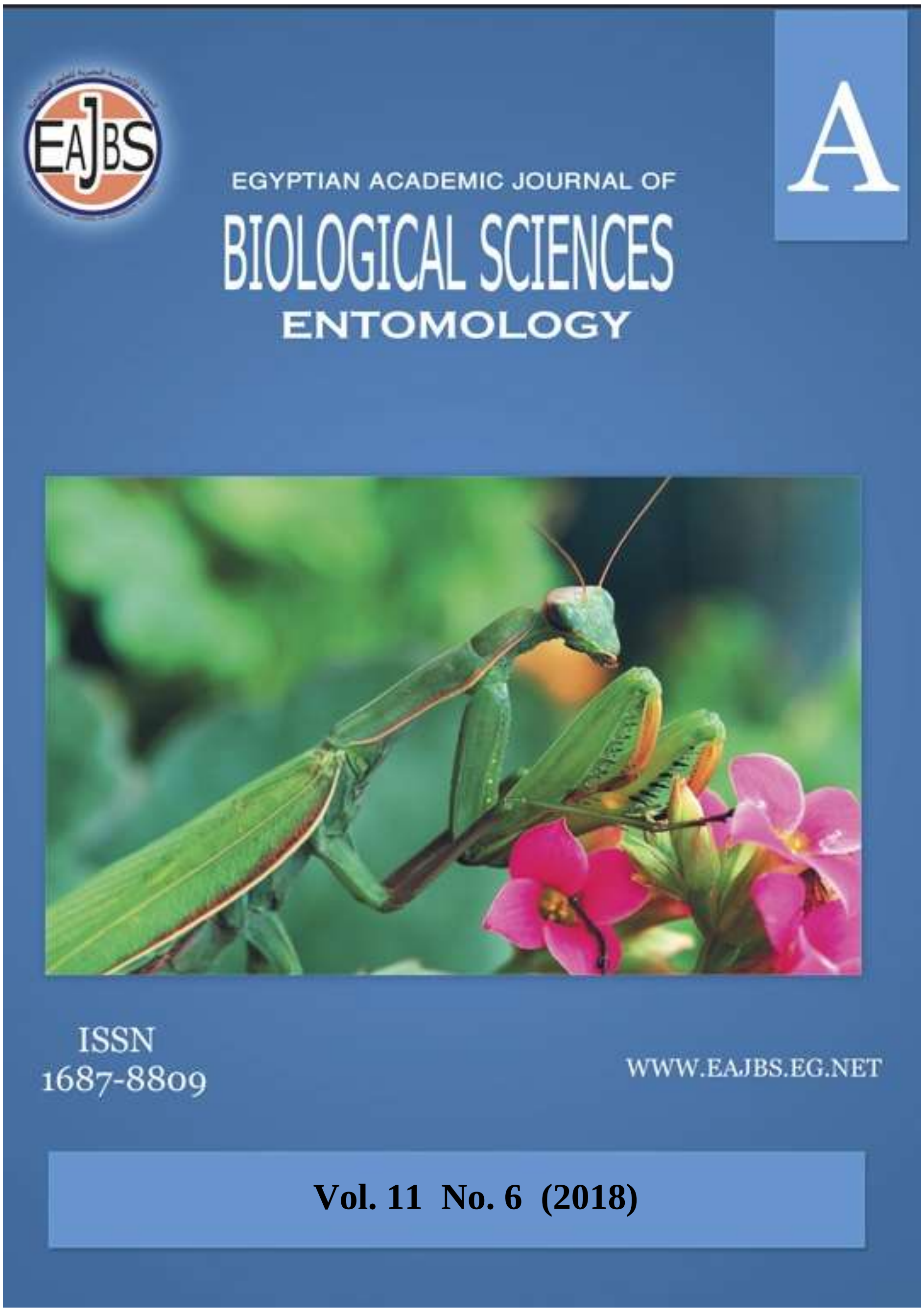

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Feeding Rate and Reproductive Performance of Three Mosquito Species as Influenced by Different Blood Meal Sources

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ABSTRACT

The present study evaluated the effect of different blood meal sources (human volunteer, pigeon, guinea pig and Egyptian mastigure) on feeding rate and reproductive performance of laboratory colonized *Culex pipiens* L., *Anopheles sergentii* Theobald and *Culex antennatus* Becker mosquitoes during two sequential gonotrophic cycles. The obtained results showed that, mosquitoes tested exhibited a special feeding rate to human blood, as the mosquitoes fed on human blood exhibited higher feeding, fecundity and fertility rates than those fed on either type of blood sources. Concerning with feeding rates, the highest percentages of engorged females during a first blood meal (82.3, 88.1 and 86.3) were recorded when *Cx. pipiens*, *An. sergentii* and *Cx. antennatus* females fed on the pigeon and human hosts. Meanwhile, the highest percentages of engorged females during second blood meal (88.4, 93.5 and 92.7) occurred when *Cx. pipiens*, *An. sergentii* and *Cx. antennatus* females fed on the human host, respectively. Also, fecundity and fertility rates of mosquitos fed on different hosts were varied between gonotrophic cycles, as at the first gonotrophic cycle fecundity and fertility of tested mosquitoes were greater than those at the second gonotrophic cycle. In addition, feeding on human blood recorded higher fecundity and fertility rates in tested mosquito species as compared with feeding on other hosts.

INTRODUCTION

Mosquitoes are arthropods of public health importance because many species are vectors of diseases (Gillij *et al.*, 2008). In Egypt, mosquitoes have a wide distribution and play an important role in disease transmission, as *Culex pipiens* is the vector of Rift Valley fever virus (Meagan *et al.*, 1980; Darwish and Hoogastrall, 1981), lymphatic filariasis caused by *Wuchereria bancrofti* (Khalil *et al.*, 1930; Gad *et al.*, 1996) and Western Nile virus (El-Bahnasawy *et al.*, 2013). *Culex antennatus* is the main vector of Rift Valley Fever virus during an outbreak in the Nile Delta of Egypt (Hanafi *et al.*, 2011). Also, the role of *Anopheles sergentii* in malaria transmission in Egypt is well documented based on epidemiological evidence and also on the finding of naturally sporozoite-infected females (Kenawy, 1988). Egg development is initiated by blood feeding in anautogenous mosquitoes (those that require a blood meal before producing eggs) (Hurd *et al.*, 1995). Ecology, climate and behavior are factors affecting the vectorial capacity of mosquito (Olayemi *et al.*, 2011), as well as the blood source which is pivotal to female mosquitoes in order to acquire amino acids from erythrocytes and plasma protein digestion to synthesize yolk proteins for egg production (Hurd, 2003; Roitberg and Gordon, 2005). Although little is known about how different blood sources affect the life table characteristics of mosquitoes (Richards *et al.*, 2012), Barlow, (1955), Colless and Chellapah, (1960) reported that, the fecundity (actual number of eggs laid) of *Aedes hexodontus* and *Ae. aegypti* was dependent on the weight of the blood consumed. Subsequently, Bennett, (1970) illustrated that *Aedes aegypti* mosquito that fed on human blood had greater fecundity (number of eggs produced) than those fed on mammalian blood differing with Suleman and Shirin, (1981) who reported that, *Cx. quinquefasciatus* that fed on mammalian blood had greater fecundity than those fed on avian blood.

Also, studying the effect of different blood sources on feeding rates and reproductive performance of mosquito helps to understand the relationship between mosquito and pathogenic agents, as well as, its ability to transmit disease. Thus, the present study aimed at evaluating the influence of human, pigeon, guinea pig and Egyptian mastigure blood on the feeding rates and reproductive performance of *Cx. pipiens*, *An. sergentii* and *Cx. antennatus* mosquitoes during two sequential gonotrophic cycles.

MATERIALS AND METHODS

Mosquitoes Rearing:

Larvae of *Cx. pipiens*, *An. sergentii* and *Cx. antennatus* after collected from its natural habitats identified according to Harbach, (1988) and Glick, (1992) and maintained continuously for several generations in Medical Entomology Insectary, Animal House, Department of Zoology, Faculty of Science, Al-Azhar University, under controlled conditions of temperature (27±2^{o}C), relative humidity (70±10%) and (12-12) light-dark regime. Eggs were submerged in 1.0 liter of distilled water in plastic trays (size 30×30×6cm) (Phasomkusolsil *et al.*, 2013). After eggs hatched, a piece of bread was added to larvae emerged until pupation. Pupae were transferred to plastic containers with fine mesh netting at the top where adults emerged and assumed resting positions on the side of the container. Emerged adults were transferred to standard cages provided with cotton pads soaked in 10% sucrose solution. Cotton pads were removed from the cages for 12h prior to blood feeding.

Mosquitoes Feeding and Hosts:

Female *Cx. pipiens*, *An. sergentii* and *Cx. antennatus* (3-5 days old) were starved of sugar for 12h before feeding experiments to induce hunger. Blood sources conducted were the human volunteer, pigeon, guinea pig and Egyptian mastigure. Before human volunteer feeding, the volunteer asked to wash his arm skin with unscented soap. One hundred *Cx. pipiens*, *An. sergentii* and *Cx. antennatus* females were allowed to feed on different hosts (first blood meal) for 4h. After eggs deposition completed the females were allowed to take a second blood meal from the selected hosts. Unfed (percentage of mosquitoes that did not feed), partially fed (percentage of mosquitoes that imbibed a partial blood meal) and fully- engorged mosquitoes (percentage of mosquitoes that fully engorged) were recorded, while unfed females were removed and discarded. Three replicates used and mean values were taken.

Mosquito Oviposition:

After two days of first blood feeding, ten engorged females were selected by random from each feeding group using an electric aspirator recommended by WHO and held in an individual glass vial containing cotton pads soaked in 10% sucrose solution and plastic tray as an oviposition substrate (Phasomkusolsil *et al.*, 2013). Two days after the second blood feeding, ten engorged females were also selected for testing the oviposition process as previously described. The numbers of eggs laid were counted for each female up to 72h post-feeding using a binocular stereomicroscope and then mean value was taken. The non-hatched eggs were classified into embryonated and non-embryonated eggs by the apparent confirmation of the presence of an embryo under a dissecting microscope (Hassan *et al.*, 1996). Hatched and non-hatched embryonated eggs were considered as fertilized, while non-hatched and non-embryonated eggs were regarded as unfertilized ones (Rak and Ishii, 1989).

Statistical Analysis:

Data was subjected to a three-way analysis of variance (ANOVA) using Statistical Package for Social Science (IBM SPSS) software version 23 (SPSS 2007), pairwise comparisons based on Tukey's HSD (Honestly Significant Difference) test were carried out.

RESULTS

For both first and second blood meals, the number of partial and fully engorged female mosquitoes was varied according to the blood source, as well as tested mosquito species (Table 1). In general, mosquitoes feeding rates in first blood meal was higher than those in the second blood meal. *Culex pipiens* females recorded the highest percent of fully- engorged females in the first blood meal of pigeon blood (82.3%) followed by Egyptian mastigure (79.8%), guinea pig (79.3%) and human blood (73.2%) (Fig. 1), *Anopheles sergentii* recorded 88.1, 76.9, 77.1 and 70.5% fully- engorged females for human, pigeon, guinea pig and Egyptian mastigure blood, respectively (Fig. 2). Also, the highest and lowest *Culex antennatus* fully-engorged females in the first blood meal recorded by human (86.3%) and Egyptian mastigure blood (83.5%), respectively, as compared with other groups (Fig. 3). On the other hand, at the second blood meal, *Cx. pipiens* recorded 88.4, 87.0, 86.2 and 85.7 fully- engorged females percentages by human, pigeon, guinea pig and Egyptian mastigure blood, respectively (Fig. 1A). Meanwhile, *An. sergentii* fed on human more than other tested hosts, as the number of fed females (partial and fully engorged) was higher than those recorded by other blood sources (Fig. 1B). Also, the percent of *Cx. antennatus* fully- engorged females in second blood meal recorded 92.7, 86.0, 85.5 and 91.0% for human, pigeon, guinea pig and Egyptian mastigure blood, respectively (Fig. 1C).

Both fecundity (mean number of eggs laid/female) and fertility (mean number of larvae hatched/eggs) were affected by gonotrophic cycle and blood meal source. The number of eggs laid per one female during the first gonotrophic cycle was greater than those during the second gonotrophic cycle for all tested groups (Table 1). Also, the fertility of eggs deposited during the first gonotrophic cycle was greater than the fertility of eggs deposited during the second gonotrophic cycle for all tested mosquito species and blood sources. At first gonotrophic cycle, human blood showed the highest fecundity (267.6±13.8 eggs/female) and fertility (94.7±1.6% hatchability) in *Cx. pipiens* mosquito followed by pigeon (242.0±6.3 eggs/female and 93.4±2.5% hatchability), guinea pig (228.3±15.8 eggs/female and 91.6±2.1% hatchability) and Egyptian mastigure blood (198.2±14.0 eggs/female and 86.3±1.4% hatchability), respectively. Also, human blood showed the highest fecundity and fertility in *An. sergentii* as compared with other blood sources. Meanwhile, Pigeon blood recorded the highest fecundity and fertility in *Cx. antennatus* as compared with other blood sources (Table 1). On the other hand, human and Egyptian mastigure blood showed the highest and lowest fecundity and fertility during the second gonotrophic cycle, respectively in all tested mosquito species as compared with other blood sources (Table 1).

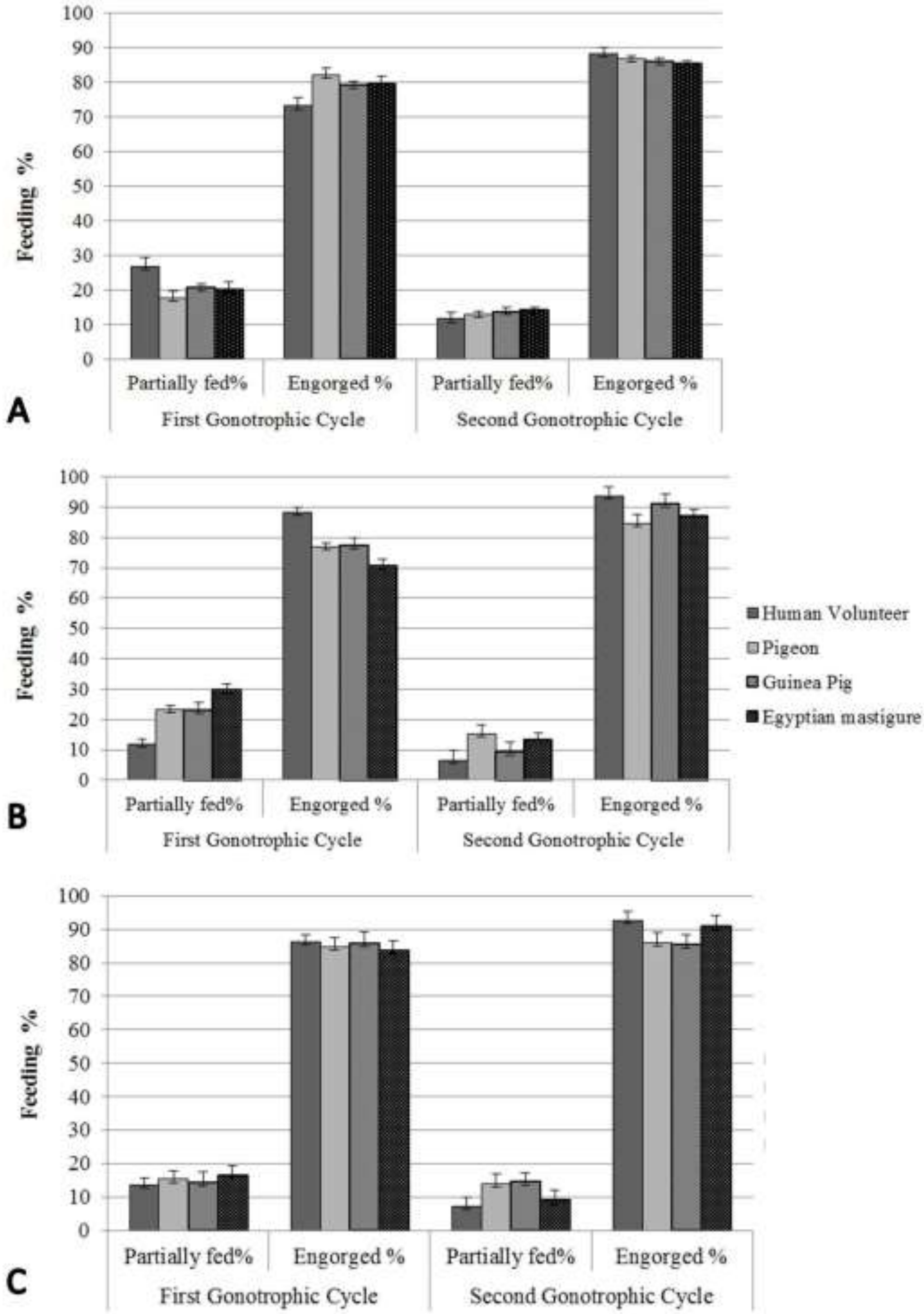

Fig. 1: Feeding rates of tested mosquito species that fed on different types of blood- meal sources over first and second blood meals. A) *Culex pipiens*, B) *Anopheles sergentii* and C) *Culex antennatus*.

Table 1: Fecundity and fertility of tested mosquito species for each blood- meal source during two gonotrophic cycles.

Sp.	Blood Meal Source	No. of eggs/female (Mean±SD)	Hatched eggs (%±SD)	Non- Hatched eggs	
				Embryonated (%)	Non-Embryonated (%)
First Blood Meal					
Cx. pipiens	Human Volunteer	267.6±13.8^{a}	94.7±1.6^{a}	2.9±0.5	97.1±0.5
	Pigeon	242.0±6.3^{b}	93.4±2.5^{a}	5.4±0.9	94.6±0.9
	Guinea Pig	228.3±15.8^{c}	91.6±2.1^{ab}	5.6±0.9	94.4±0.9
	Egyptian mastigure	198.2±14.0^{de}	86.3±1.4^{de}	10.0±0.4	90.0±0.4
An. sergentii	Human Volunteer	99.4±9.3^{k}	91.6±1.0^{ab}	1.7±0.1	98.3±0.1
	Pigeon	91.2±4.8^{kl}	89.9±1.5^{bc}	4.5±0.3	95.5±0.3
	Guinea Pig	83.0±4.9^{l}	85.3±1.8^{e}	4.9±0.5	95.1±0.5
	Egyptian mastigure	81.2±5.8^{l}	80.0±1.7^{f}	10.6±1.0	89.4±1.0
Cx. antennatus	Human Volunteer	207.8±7.6^{d}	86.9±1.7^{cde}	11.5±0.8	88.5±0.8
	Pigeon	209.6±12.4^{d}	89.1±1.3^{bcd}	11.7±1.3	88.3±1.3
	Guinea Pig	189.8±7.1^{ef}	77.3±0.8^{fgh}	13.2±1.7	86.8±1.7
	Egyptian mastigure	176.9±7.4^{fg}	76.6±0.6^{gh}	15.0±1.1	85.0±1.1
Second Blood Meal					
Cx. pipiens	Human Volunteer	193.1±7.8^{e}	78.2±1.5^{fg}	5.4±0.7	94.6±0.7
	Pigeon	175.9±5.7^{g}	70.3±1.3^{i}	4.8±0.4	95.2±0.4
	Guinea Pig	176.3±9.0^{g}	64.2±1.9^{j}	7.6±0.7	92.4±0.7
	Egyptian mastigure	158.4±5.6^{h}	60.5±1.9^{k}	10.9±1.2	89.1±1.2
An. sergentii	Human Volunteer	56.9±3.4^{m}	74.7±1.2^{h}	10.1±0.9	89.9±0.9
	Pigeon	56.5±3.9^{m}	69.9±2.3^{i}	12.8±0.9	87.2±0.9
	Guinea Pig	51.8±3.6^{mn}	51.8±2.7^{l}	15.0±1.1	85.0±1.1
	Egyptian mastigure	40.6±6.7^{n}	45.5±3.6^{m}	17.6±0.7	82.4±0.7
Cx. antennatus	Human Volunteer	138.0±4.7^{i}	44.8±2.1^{m}	6.9±0.8	93.1±0.8
	Pigeon	123.2±4.1^{j}	44.8±1.5^{m}	5.1±0.7	94.9±0.7
	Guinea Pig	115.7±3.9^{j}	37.3±1.8^{n}	9.6±0.8	90.4±0.8
	Egyptian mastigure	102.4±6.2^{k}	35.0±2.9^{n}	14.7±1.3	85.3±1.3

Means that do not share a letter are significantly different (P<0.05).

DISCUSSION

The present results showed that different blood meal sources induced variations in feeding, fecundity and fertility rates of tested mosquito species. In general, mosquitoes fed on human blood exhibited higher feeding, fecundity and fertility rates than those fed on either type of blood sources tested and this may reflect the superiority of human blood in mosquito oogenesis (Olayemi *et al.*, 2011). The variation in mosquito fecundity attributed to genetic differences in mosquito populations (Richards *et al.*, 2012), mosquito species (Briegel, 1990), host (Taylor and Hurd, 2001), size of the blood meal (Roitberg and Gordon, 2005) and amino acids from erythrocytes (Hurd, 2003). The obtained results of fecundity and fertility consistent with the previously reported by Downe and Archer, (1975) who found that, *Culex tarsalis* Coquillet shows higher fecundity when fed on chickens compared to guinea pigs or snakes, possibly due to different rates of digestion and/or nutrition for different blood sources, however, the source of the blood meal did not influence the hatchability of the eggs. Also, Clement, (1992), Islam and Ferdousi, (1999) recorded a strong positive correlation between blood meal source and reproductive output of mosquito, especially, fecundity and hatchability rates, Richards *et al.*, (2012) reported the impact of different blood meal sources (chicken blood and bovine blood) on feeding and reproduction of *Cx. quinquefasciatus* mosquitoes, where, the fecundity and fertility of *Cx. quinquefasciatus* were greater in mosquitoes fed chicken blood than the other blood source and Phasomkusolsil *et al.*, (2013) found that feeding rates differ among blood sources within mosquito species, as fecundity and hatchability rates were lower in all *Anopheles* species and *Aedes* *aegypti* after membrane feeding on sheep blood, as well as, *An. minimus* and *An. sawadwongporni* laid no eggs by seven days post-feeding with sheep blood, while *An. dirus* and *An. cracens* produced significantly fewer numbers of eggs and demonstrated significantly lower hatching rates relative to what was observed with the other blood sources.

Also, the obtained results revealed that fecundity and fertility rates during the first gonotrophic cycle were greater than those laid during the second gonotrophic cycle and this may be due to increased chronological and/ or gonotrophic age of the mosquito. Such these results are in agreement with previously recorded by McCann et al., (2009) who found that, increasing in *Cx. quinquefasciatus* chronological age at the time of the first blood meal on a chicken reduced fecundity, as mosquitoes that were 5 d old at the time of the first blood meal produced (laid and retained) 115- 230 eggs, while mosquitoes that were 13 d old at the first blood meal produced only 25-200 eggs. Also, Walter and Hacker, (1974), Suleman, (1979) and Akoh *et al.*, (1992) recorded a reduction in *Cx. quinquefasciatus* fecundity with increasing age when fed repeatedly on a mouse or chicken, Awahmukalah and Brooks, (1985) found that, *Cx. pipiens* fecundity declined with increasing age when fed repeatedly on a chicken and Richards *et al.*, (2012) recorded a significant decrease in *Cx. quinquefasciatus* fecundity and fertility rates from the first to the second blood feeding.

Conclusion:

The results of the present study confirm that blood meal source influences feeding success, female fecundity, egg hatching and survival rate of tested mosquito species. Each blood- meal source (the type of blood meal) contains certain amino acids that play an important role in oogenesis and egg- hatchability. Thus, blood-meal types consequently affect the success of mosquito vectors in disease transmission.

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ARABIC SUMMERY

مُعدل التغذية والأداء التناسلى لثلاثة أنواع من البعوض المُتأثِر بمصادر وجبة دم مختلفة

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قيّمت الدراسة الحالية تأثير إختلاف مصادر وجبة الدم (متطوع البشري، حمام، خنزير غينيا و الضب المصري) على معدل التغذية والأداء التناسلى لبعوض *كيولكس بيبنز*، *انوفيليس سيرچنتى وكيولكس انتيننانس* المُربى معملياً خلال دورتى تغذية متتابعتين. النتائج المُتحصل عليها أوضحت أن البعوض المُختبر أظهر معدل تغذية خاص لدم الإنسان، حيث كانت معدلات التغذية، إنتاجية وخصوبة البيض أعلى من التي سجُلت للبعوض المُتغذى على مصادر الدم الأخرى. بالإشارة إلى مُعدلات التغذية، أعلى نسبة مئوية للإناث ممتلئة البطن خلال دورة التغذية الأولى (٨٢,٣، ٨٨,١ و٨٦,٣%) سُجلت لإناث *كيولكس بيبنز*، *انوفيليس سيرچنتى وكيولكس انتيننانس* المُتغذية على الإنسان والحمام. بينما أعلى نسبة مئوية للإناث ممتلئة البطن خلال دورة التغذية الثانية (٨٨,٤، ٩٣,٥ و٩٢,٧%) سُجلت لإناث *كيولكس بيبنز*، *انوفيليس سيرچنتى وكيولكس انتيننانس* المُتغذية على الإنسان. أيضاً، معدلات إنتاجية البيض والخصوبة تفاوتت بإختلاف دورات التغذية، حيث كانت معدلات إنتاجية البيض والخصوبة خلال دورة التغذية الأولى أعلى من مثيلاتها خلال دورة التغذية الثانية. بالإضافة إلى ذلك، سجلت إناث أنواع البعوض المُتغذى على الإنسان أعلى معدل إنتاجية وفقس للبيض مُقارنةً بالمُتغذية على العوائل الأخرى.