Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(2): 335-339

Received: 25-01-2016
Accepted: 28-02-2016

Djamila Benhamed
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Fatiha Bendali-Saoudi
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Noureddine Soltani
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Correspondence
Fatiha Bendali-Saoudi
Laboratory of Applied Animal Biology Department of Biology, Faculty of Sciences, Badji Mokhtar University of Annaba 23000-Annaba, Algeria

Effect of two blood meal types on reproduction and developmentin the mosquito *Anopheles maculipennis*

Djamila Benhamed, Fatiha Bendali-Saoudi, Noureddine Soltani

Abstract
Mosquitoes play an important role in global disease transmission. The diseases that they transmit impact directly on the human and animal health. Our study aimed the evaluation of two blood meals (Cattle blood and Chicken blood), on the duration of the development, the biochemical composition of ovaries and the reproductive parameters in *Anopheles maculipennis* Meigen, 1818. Data showed that the fecundity was significantly higher (p<0.001) with the Cattle blood (192.9±77.37 eggs laid/female) as compared to the Chicken blood (95.8±33.35 eggs laid/female). However, there was no significant difference (p=0.155) between the two tested bloods on the fertility (Cattle blood 69.25±17.84 % and Chicken blood 62.65±16.89%). In addition, the Chicken blood significantly reduced the duration of embryonic development, fourth larval instar and pupal stage, and increased the amounts of main biochemical components as compared with the Cattle blood.

Keywords: Mosquitoes, *Anopheles maculipennis*, Hematophagy, Reproduction, Development

1. Introduction
The mosquito species belonging to *Aedes*, *Culex* and *Anopheles* play an important role in global disease transmission. The diseases that they transmit impact directly on the human and animal health. Malaria transmitted by female mosquitoes of *Anopheles*, affects more than half of the world population [1, 2]. with 300 million infections per year and 1.5 to 2.7 million of deaths per year [3, 4]. In Algeria, mosquitoes were the subject of a great number of studies dealing with systematic, biochemistry, morphometry, chemical and biological control [5-12]. The physiology of blood sucking insect's remains poorly studied [13]. Therefore, fundamental studies are needed in order to better control vectors [14].
The influence of nutritional status on the alimentary research behavior among hematophagous insects has been extensively studied in female mosquitoes, and the studies have focused in particular on the interaction between the nutritional status and reproductive status [15, 16]. Furthermore, the evolution of the essential biochemical parameters of the yolk was studied by a quantitative determination of main biochemical components according to the blood type. The present study prompted us to perform a mass breeding of the *Anopheles maculipennis* Meigen 1818 (Diptera, Culicidae) under laboratory conditions, taking into account the breeding conditions and blood types (Mammals and Birds), examining their impact on the duration of larval and pupal stages. The better control of human disease resulting from the transmission of parasites by some hematophagous insects requires a good knowledge of the biology of these vectors. One of important aspect in the control of these insects is the seeking behavior of the specific host.

2. Materials and methods
2.1Presentation of the study area
The studied species was sampled from the lake of Birds, which is a wetland of universal importance, located outside the boundaries of the National Park of ElKala (PNEK), during the period ranging from May 2012 to June 2012. It is a freshwater lake with 120 hectares in winter and 70 hectares in the dry season. This lake is by its location in the North of State Road 44, a privileged natural center for environmental education. Its water is naturally done by run of surface water from the water shed and groundwater [17].The water of the lake is characterized by its alkalinity with pH varying between 7.68 and 8.13, its temperature ranged between 27 and 29.5 °C, and electric conductivity from 300 and 800 µS [18].

2.2 Sampling and rearing of mosquitoes

Our study was conducted on *An. maculipennis*. Mosquito adults were collected in barns and stables which are located near the Lake. Sampling was carried out using a glass tube that gently deposited on the top of the female engorged of blood and at rest, the latter flies to the bottom of the tube, which will be opened inside the cage. The breeding of *Anopheles* was carried out in the laboratory in incubators (15x15x15cm) with a wooden frame covered with tulle. A grape cluster (each one is cut in half) was introduced in each cage to provide sugars for mosquito adults. Pyrex storage jars containing 200 ml tap water were also placed in each cage to collect the eggs [5].

In the adult case: Females of *Anopheles* species collected from stables were identified according to the informatic software [19], eggs criteria [20] as well the dichotomic key of Culicidae of Morocco [21]. The Females were selected according to the type of blood (Cattle and Chicken). The blood was taken via intravenous way and preserved in heparin tubes. A tube is put in each cage during 48 hours, which is the necessary time for the engorgement of females. Thirty females for each blood type were isolated individually in thirty cages and nourished by grapes. Water containers are placed in the cages serving as egg-laying environment.

In larval case: The eggs are harvested and placed into breeding containers containing dechlorinated water. After hatching, the larvae will eat algae: 0.04 g every 2 to 3 days. Three days after hatching, the water should be changed weekly. The physicochemical parameters of water were as follow: light (12 to 14 hours), temperature (29 to 30 °C), food quality (algae) and larval density per unit area or volume of water (0.5 larvae / cm^2) [22]. The obtained pupae were placed in cages where the mosquito adults emerge. During the experiment, the egg hatchability (percentage of neonates that emerged from eggs), the number of larvae reaching the pupal and adult stages were determined.

2.3 Characteristics of blood meal types

We chose the Cattle blood for Mammals and Chicken blood for Birds. The main organic (such as total proteins, glucose, total cholesterol) and mineral (Mg, Ca, Na, K) constituents of plasma and some biophysical parameters (pH, viscosity) of these two types of blood has been previously determined [23-25].

2.4 Reproductive parameters

The insects were examined daily until adult emergence and the mortality at various developmental stages scored. The fecundity (number of eggs laid per female) and the egg hatchability (percentage of neonates that emerged from eggs) were determined.

2.5 Extraction and quantification of ovarian biochemical components

Paired ovaries were collected from engorged females and analysed. The extraction of principal biochemical components of the ovaries from *An.maculipennis* adult females carried [26] and homogenized in 1ml of trichloracetic acid (20%). Afterwards the first centrifugation (5000 x g for 10 min), the supernatant obtained is used for carbohydrate determination [27], while the precipitate1is added to the mixture ether and chloroform (1V/1V). The second centrifugation gives another supernatant that is used for lipid determination [28], while the precipitate 2 that was suspended in 1 ml of NaOH (0.1N) and used for total protein determination. Ovarian proteins were quantified using the Coomassie Brilliant Blue G 250 (BBC) [29] as a reagent and bovine serum albumin (BSA) as standard (Sigma). The reading of absorbance was performed at a wavelength of 595 nm. The determination of carbohydrates was performed on an aliquot (100 µl) [30]. This method uses anthrone as a reagent and glucose (Sigma) as standard. The absorbance was read at a 620 nm. Lipid amounts were estimated based on the vanillin method [31]. Data on ovarian biochemical components were expressed in µg/mg ovaries.

2.6 Statistical analysis

Results are presented as mean ± standard deviation (SD). All analyzes were performed using MINITAB Software (version 16, PA, Penn State College, USA). The number of individuals tested and repeats are given with results. Statistical significance was set at $p<0.05$ level.

3. Results

3.1 Effect of blood meal types on fecundity and fertility:

The biochemical and biophysical characteristics of the two blood tested were previously determined [23- 25] and presented in table 1. The Chicken blood is relatively rich in sugars and poor in total proteins and lipids (total cholesterol) in comparison with Cattle blood.

Table 1: Plasma constituents and biophysical parameters of the two types of blood (Cattle's blood and Chickens' blood).

Plasma constituants	Cattle blood	Chicken blood
Glucose (mmol/l)	2.5 - 7.2	8.4 - 10
Urea (mmol/l)	0.5 - 2.2	0.3
Creatinin (µmol/l)	18 - 230	80 -160
Total Bilirubin (µmol/l)	1.7-12	0 -3.4
Uric acid (µmol/l)	≤ 160	147- 481
Total cholesterol (mmol/l)	2 - 44	1.3 - 3.8
Total proteins (g/l)	60 - 85	52 - 69
Albumins (g/l)	25 - 42	21 -35
Alpha-1 globulins (g/l)	6 - 12	4.5 - 10
Alpha-2 globulins (g/l)	3.5 - 9.5	3.5 - 12
Béta-globulins (g/l)	4 - 11	5 -18
Gamma-globulins (g/l)	7 - 26	4.6 - 10
Sodium (mmol/l)	132 - 152	148 - 163
Potassium (mmol/l)	4 - 7	4.6 - 6.5
Chlorine plasma (mmol/l)	97 - 110	116 - 140
Bicarbonates (mmol/l)	18 - 36	17.6 - 30
Phosphates (mmol/l)	1.5 - 3	2 - 2.5
Calcium (mmol/l)	2.3 - 3	2.2 - 6
Magnesium (mmol/l)	0.5 - 1.5	0.5 - 1.5
Alkaline phosphatase (Ul/l)	94 - 170	25 - 45
Acid phosphatase (Ul/l)	1 - 18	23 - 42
LDH (Ul/l)	8 - 300	88 - 310
Ph	7.6 - 7.9	7.34 - 7.37
Viscosity	1.3	2.14

The fecundity of females engorged with Cattle blood are significantly ($p>0.001$) higher than those of the females Engorged with Chicken blood (Table 2).

Table 2: Effect of two blood meal types (Cattle, Chicken) on fecundity (number of eggs laid/female) and fertility (percentage of egg hatchability) in *An.maculipennis* (Mean ± SD; (min-max); N = 30).

Reproduction	Cattle blood	Chicken blood	P
Fecundity	192.9 ± 77.37 (84 - 384)	95.8 ± 33.35 (37-160)	< 0.001
Hatchability (%)	69.25 ± 17.84 (35.18 - 100)	62.65 ± 16.89 (30-90)	0.155

3.2 Effect of blood types on the duration of larval and pupal development:

The table 3 summarizes the duration of larval and pupal stages. The duration of the larval and pupal stages within the larvae of the females engorged with Chicken blood was found to reduce significantly the duration of the fourth larval stage $p>0.004$ as compared with Cattle blood stages.

Table 3: Effect of two blood meal types (Cattle, Chicken) on the duration (days) of larval and pupal stages of *An.maculipennis* (Mean ± SD; N= 30).

Blood type / Duration (days)	Cattle blood	Chicken blood	P
Larval stage 1	2.83 ± 1.48	2.32 ± 0.92	0.586
Larval stage 2	2.60 ± 1.05	2.36 ± 0.97	0.712
Larval stage 3	3.30 ± 1.34	2.92 ± 0.79	0.202
Larval stage 4	7.16 ± 1.68	5.96 ± 1.45	0.004
Pupal stage	2.03 ± 0.18	1.96 ± 0.18	0.161

3.3 Effect of blood types on the biochemical composition of ovaries:

The biochemical measurements are presented in table 4. Results showed that the blood type had no significant effect on the ovarian amounts of proteins (p=0.349), lipids (p=0.299) and carbohydrates (p= 0.289).

Table 4: Effect of two blood meal types (Cattle, Chicken) on the ovarian amounts (µg/mg tissue) of proteins, lipids, and carbohydrates in *An. maculipennis* (Mean ± SD; N= 5). For each component, values followed by the same letter are not significantly different ($p>0.05$).

Blood types	Cattle	Chicken	P
Proteins	3.848 ± 1.42 a	5.794 ± 1.93 a	0.349
Lipids	1.676 ± 0.67 a	2.752 ± 0.90 a	0.299
Carbohydrates	3.096 ± 1.14 a	4.954 ± 1.58 a	0.289

4. Discussion

Reproductive physiology of female mosquitoes was analyzed at different levels because of their role as disease vectors of diseases. Considerable progress has been made in understanding the behavioral studies on host-seeking [16], their endocrinology [32-34], blood meal digestion and utilization [35, 36], and biology and ecology of mosquitoes [37, 38]. The breeding of *Anopheles* mosquitoes in the laboratory has a global issue. This kind of mosquito is very delicate and very sensitive to environmental conditions (Temperature, humidity, photoperiod...) for adults and the water quality (dechlorinated water, moist soil, organic matter...) for the larvae [39]. *An. maculipennis* maintained in the laboratory allowed us to determine the duration of larval and pupal stages, the main biochemical components of ovaries, and the reproductive events as function the blood meal types.

It is suggested that the duration of the fourth larval stage in the larvae engorged by Cattle blood is longer compared to those engorged by chicken blood, this is due to the composition of the cholesterol of the former. In addition, fecundity is more important in the females engorged by Cattle blood that may be due to the composition of the latter which is richer in proteins compared to the chicken blood. Therefore, this is probably related to the difference in biochemical and physical composition of the tested blood types [23- 25].

The host-seeking behavior is inhibited until, depending on species, egg maturation or oviposition [40]. Other studies indicate that in certain species of mosquitoes (*An.gambiae*) [40], several blood meals take place during the same gonotrophic cycle. The acquisition of nutrient resources necessary for survival is indeed, in the majority of species, the trigger event in the reproduction process; it requires adaptation to the life styles of host species. However, no detailed physiological study seems to exist and actual measurements of larval parameters are rare [41]. The reproduction process need an accumulation of various energetic materials like lipids (triglycerides, lipoproteins), carbohydrates (glycogen), proteins and salts mineral [42]. The vitellus constitutes the energy reserves used by the embryos during the embryonic development. It is produced by the mother's body and accumulates in the oocyte during oogenesis. Proteins play a fundamental role in the organization of all known living biological species [43]. Carbohydrates are also the energy component of the body plays an essential role in the physiology of insects. They form part, with proteins and lipids, essential constituents of living beings and their nutrition since; they are one of the main biological intermediate storage and energy consumption. Ovarian lipids are an important part of reserves; weakly synthesized by oocytes and follicles, they originate from fatty substances via the hemolymph form lipoprotein [42]. Our study on *An.maculipennis* showed that the type of blood meal had no effect on the ovarian content of proteins, carbohydrates and lipids.

5. Conclusion

The overall results show that the blood type can affect the development and the reproductive events in *An. maculipennis.* The Chicken blood significantly reduced the duration of both embryonic development, fourth larval and pupal stages, the fecundity and the fertility, as compared with the Cattle blood. However, the biochemical measurements showed that the blood type had no significant effect on the ovarian amounts of proteins, lipids and carbohydrates.

6. Acknowledgements

This work was supported by the National Fund for Scientific Research to Pr. N. Soltani and the Ministry of High Education and Scientific Research of Algeria (CNEPRU and PNR projects).

7. References

1. Kaufman PE, Mann RS, Butler JF. Insecticidal potency of novel compounds on multiple insect species of medical and veterinary importance. Pest. Manag. Sci, 2011; 67:26-35.
2. Who. Guildeness for the treatment of malaria. World Health Organization, Geneva, 2006.
3. Who. Global Program to eliminate lymphatic filariasis: annual report on lymphaticfilariasis. Weekly Epidemiological Record, 2007; 82:361-380.
4. Nuttall I. Web pages, Division of Control of Tropical Diseases. World Health Organization, Geneva, Switzerland, 1997.

5. Bendali-Saoudi F. Study of *Culex pipiens*. Anatogene, systematic, biology, fight (*Bacillus thuringiensis israellensis* serotype H14, *Bacillus sphaericus*, 1953) and 2 species of hydracariens. Magister thesis. Badji Mokhtar University of Annaba, 1989, 114.
6. Bendali-Saoudi F, Djebbar F, Soltani N. Compared effectiveness of some fish species with regard to various stages of *Culex pipiens*. Under laboratory conditions. Parasitica, 2001; 57(4):255-265.
7. Bendali-Saoudi F. Bioecologic, Systematic and Biochemical study of Culicidae (Diptera- Nematocera) of the area of Annaba. Biological fight anticulicidiènne. Doctorate thesis of State in natural science, University of Annaba, 2006, 224.
8. Boudjellida H, Bouaziz A, Soin T, Smmaghe G, Soltani N. Effects of ecdysone halofenozide against *Culex pipiens*. Pest. Biochem and physiol, 2005; 83:115-123.
9. Tine-Djebbar F, Soltani N. Biological activity of an agonist non-steroïdien of the moult hormone on *Culiseta longiareolata*: morphometric analyses, biochemical and energy. Synthesis, 2008; 18:23-34.
10. Tine Djebbar. Bioecology of the mosquitos of the Tébessa area and evaluation of two growth regulator (halofenzide and méthoxyfenzide) with regard to two species of mosquitos *Culex pipiens pipiens* and *Culiseta longiarolata*: toxicological, morphometric, biochemistry and reproduction. PhD Thesis. University of Annaba, 2009, 170.
11. Messai N, Berchi S, Boulknafed F, Louadi K. Systematic inventory and biological diversity of Culicidae (Diptera: Nematocera) in the area of Mila (Algeria). *Faunistic Entomology*, 2011; 63(3):203-206.
12. Tine-Djebbar F, Bouabida H, Soltani N. Morphometric and biochemical characterization of certain mosquitos species inventoried in the area of Tébessa. *Bull. Soc. zool. Fr*, 2011; 136(1-4):177-185.
13. Adams TS. Hematophagy and hormone release. Annals of Entomological Society of America. 1999; 92(1):1-3.
14. Bodin A. Modulation of the behavior of research of the host in the insect's hematophagic: Importance of the endogenous factors. Memory for obtaining doctorate. University of Tours François – Rabelais, 2008, 145.
15. Kelly DW. Why Some People are bitten more than others. Trends in Parasitology, 2001; 17(12):578-581.
16. Klowden MJ. Endocrine aspects of mosquito breeding. Archives of Insect Biochemistry and Physiology, 1997; 35:491-512.
17. Forestry Branch. Atlas of the 26 Algerian humid zones, 3rd edition, Algiers, Algeria, 2002, 86.
18. Necib A, Rezig H, Boughdiri L. The bio-indication of watery pollution by the microalgues (case of the l'Oued «Bounamoussa» and the Lake of the Birds). Rev. Sci. Technol., Synthesis, 2013; 27:6-14.
19. Schaffner F, Angel G, Geoffroy B, Hevry JP, Rhaiem A, Brunhes J. Moustique d'Europe. Institut de recherche pour le développement IRD. Logiciel d'identification, 2001.
20. Gutsevich AV, Monchadski AS, Shtakelberg AA. Fauna of the U.S.S.R., Diptera Family Culicidae, III, 1974; 4:408.
21. Himmi O, Dakki M, Trari B, Elagbani MA. Les Culicidae du Maroc. Clés d'identification avec donnés biologiques et écologiques. Trav. Inst. Sci. Zool, Rabat, 1995 ; 44:50.
22. Hervy JP, Coosemans M. The breeding of *Aedes* and *Anopheles* realization and practice. Coordination and cooperation organization. For the fight against major diseases, 1979.
23. Fontaine M. Vade mecum du vétérinaire, 15ème édition, Paris, Vigot, 1987, 838-842.
24. Sanchez A, Plouzeau M, Rault P, Picard M. Muscular and cardio respiratory function growth in broilers. INRA Prod. Anim, 2000; 13(1):37-45.
25. Coriat P, Amour J. Heart and Anesthesia: Constraints circulatory and cardiac complications. Arnette Blackwell, 2005, 178.
26. Shibko S, Koivistoinen P, Tratyneck C, New Hall A, Friedman L. A quantitative method for the sequential separation and glycogen from rat liver homogenate, a single gold from a subcellular fraction, Analyt. Biochem, 1966; 19:415-428.
27. Timmermann SE, Briegel H. Molting and metamorphosis in mosquito larvae: a morphometric analysis. Mitteilyngender Schweizewchen Entomologische Gesellschaft, 1998; 71:373-387.
28. Gäde G, Hoffman KH, Spring JH. Hormonal regulation in insects: Facts, gaps, and future directions. Physiol. Rev, 1997; 77:963-1032.
29. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem, 1976; 72:248-254.
30. Duchateau A, Florkin P. The trehalose insect molting and meaning. Arch. Insect. Physiol. Biochem, 1959; 67(2):306-314.
31. Goldsworthy GJ, Mordue W, Guthkelch J. Studies on insect adipokinetic hormones. *Genr. Compar. Endocrinol,* 1972; 18:545-551.
32. Dhadialla TS, Raikhel AS. Endocrinology of mosquito vitellogenesis. In: Davey, KG, Peter, RE, Tobe, SS (Eds), Perspectives in Comparative Endocrinology. Nat. Res. Council of Canada, Ottawa, 1994, 275-281.
33. Raikhel AS, Lea AO. Control of follicular epithelium development and vitelline envelope in the mosquito: role of juvenile hormone and 20-hydroxyecdysone. Tissue and Cell, 1991; 23:577-591.
34. Koller CN, Raikhel AS. Initiation of vitellogenin uptake and protein synthesis in the mosquito (*Aedes aegypti*) ovary in response to a blood meal. Journal of Insect Physiology. 1991; 37:703-711.
35. Kelly DW. Why Some People are bitten more than others. Trends in Parasitology, 2001; 17(12):578-581.
36. Lounibos LP, Rey JR, Frank JH. Ecology of mosquitoes. Proceeding of a workshop. Florida Medical Entomology Laboratory, Vero Beach, Florida, USA, 1985.
37. Cassier P, Lafont R, Descamps M, Porchet M, Soyez D. Reproduction of the invertebrates: strategies, methods and regulation. Interest fundamental and applied Masson, 1997, 354.
38. Lea AO, Briegel H, Lea HM. Arrest, resorption, or maturation of oocytes in *Aedes aegypti*: dependence on the quantity of blood and the interval entre blood meals. Physiological Entomology, 1978; 3:309-316.
39. Assako Assako RJ. Contribution of social sciences and entomology in the analysis of the endemicity of malaria in HEVECAM, an agricultural processing industry of South-Cameroon, 2005.
40. Klowden MJ, Briegel H. Mosquito gonotrophic cycle and multiple feeding potential: contrasts between *Anopheles* and *Aedes* (Diptera: Culicidae). Journal of Medical Entomology. 1994; 31:618-622.

41. Clements AN. The biology of mosquitoes. Development, nutrition and reproduction. Chapman and Hall, London, 1992.
42. Cassier P, Lafont R, Descamps M, Porchet M, Soyez D. Reproduction of the invertebrates: strategies, methods and regulation. Interest fundamental and applied Masson, 1997, 354.
43. Jacob F, Monod J. Genetic regulatory mechanism in the synthesis of proteins. J Biol. Med. 1961; 3:318-321.