Revista Colombiana de Entomología 32(1): 57-60 (2006) https://doi.org/10.25100/socolen.v32i1.9359

Caloric content of the sand fly *Lutzomyia ovallesi* (Diptera: Psychodidae) vector of *Leishmania*

Contenido calórico del flebotomíneo *Lutzomyia ovallesi* (Diptera: Psychodidae) vector de *Leishmania.*

PEDRO NOGUERA, MARITZA RONDON, ELSA NIEVES*

Abstract. Females of the sand fly *Lutzomyia ovallesi* (Ortiz) (Diptera: Psychodidae) were fed with blood from various species of vertebrates and analyzed to determine energy reserves under laboratory conditions. *L. ovallesi* specimens were allowed to artificially feed to satiation through chicken membranes on blood from horse, dog, cow, chicken, goat, pig or human. Caloric reserves were calculated spectrophotometrically after females were homogenized in a solution of sodium dichromate and sulfuric acid. The caloric content of *L. ovallesi* varied according to the type of vertebrate blood on which it had fed. The highest content (cal/insect) was found in females fed on human blood (0.33), followed in decreasing order by dog, pig, cow, chicken, goat and horse (0.26). Statistical analysis showed significant differences ($P < 0.05$) among sources. The results showed that human and dog blood meals were more nutritionally efficient. The most inefficient diet for *L. ovallesi* was horse blood manifested by its poor nutritional quality.

Key words: Sand flies, caloric reserves, biological potential, bloodmeal, insect vectors.

Resumen. Hembras del flebotomíneo *Lutzomyia ovallesi* (Ortiz) (Diptera: Psychodidae) fueron alimentadas con sangre proveniente de varias especies de vertebrados y analizadas para determinar las reservas energéticas en condiciones de laboratorio. Ejemplares de *L. ovallesi* se alimentaron artificialmente a repleción a través de membrana de pollo con sangre de caballo, perro, vaca, gallina, chivo, cerdo o humano. Las reservas calóricas se estimaron espectrofotométricamente, después de homogenizar las hembras en una solución de dicromato de sodio en ácido sulfúrico. El contenido calórico de *L. ovallesi* varió de acuerdo con el tipo de sangre con que se alimentaron. El mayor contenido calórico (cal/insect) fue encontrado en hembras alimentadas con sangre de humano (0,33), seguido en orden decreciente: perro, cerdo, vaca, pollo, chivo y caballo (0,26). El análisis estadístico mostró diferencias significativas ($P<0.05$) entre las fuentes. Los resultados mostraron que la sangre de humano y perro fueron más eficientes nutricionalmente. La dieta más ineficiente para *L. ovallesi* fue la sangre de caballo manifestada por su pobre calidad nutricional.

Palabras clave*:* Flebotomínos, contenido calórico, potencial biológico, fuentes sanguíneas, insectos vectores.

Introduction

The ability of any hematophagous insect to survive and transmit pathogens depends principally on its caloric reserves (Van Handel 1972; Magnarelli and Modi 1988; Briegel *et al.* 2001). The energy requirements of female phlebotomine sand flies (Diptera: Psychodidae) are supplied by three sources: caloric reserves built up during the larval stage and sugar and vertebrate blood ingested as an adult (Van Handel 1972, 1984; Magnarelli and Burger 1984; Magnarelli and Modi 1988; Mostowy and Foster 2004).

Fecundity variations in sand flies according to bloodmeal source may be attributed to significant differences in the caloric content of carbohydrates, lipids, and proteins from the ingestion and metabolization of blood. Large caloric reserves could provide greater potential energy for egg production, oviposition survival, and flight capacity (Magnarelli and Modi 1988; Harre *et al*. 2001), increasing the biological potential of a specific sand fly population, resulting in increased transmission of *Leishmania* (Kinetoplastida) (Schlein *et al*. 1983; Daba *et al*. 1997; Schlein and Jacobson 1998; Hurd 2003). When available energy reserves in both sexes of the sand flies *Lutzomyia longipalpis* (Lutz & Neiva) and *Phlebotomus papatasi* (Scopoli) were quantified, those with access to fructose or sucrose solutions in the laboratory had higher levels than those supplied with labeled glucose. Caloric assays can be used to evaluate larval and adult diets (Magnarelli and Modi 1988).

The sand fly *L. ovallesi* (Ortiz) is the principal vector of *Leishmania braziliensis* in western and central Venezuela (Bonfante-Garrido *et al*. 1991a; 1991b; Feliciangeli 1991) and one of the most important vectors in the Venezuelan Andes (Añez *et al*. 1988). The purpose of the present study was to determine caloric contents of *L. ovallesi* fed with vertebrate blood from different sources, under laboratory conditions.

Materials and methods

Sand flies. Sand flies of the species *L. ovallesi* were reared in a closed laboratory colony and only females were used in the experiments. The colony originated from specimens collected at 1360 masl at El Arenal (8 º 35'' N, 71º 9' W), Ejido, in the Venezuelan state of Mérida. The colony was maintained in an incubator at 25°C ± 1° and RH 80% ± 10% and provided with saccharose solution *ad libitum*, in the Experimental Parasitology Laboratory of the University of Los Andes, Mérida, using the methods of Killick-Kendrick *et al*. (1977).

* Laboratorio de Parasitología Experimental LAPEX, Departamento de Biología, Facultad de Ciencias. Universidad de Los Andes. La Hechicera, Mérida-EDO-Mérida, 5101. Venezuela. FAX: 00 58 02 74 2401286, e-mail:nevelsa@ula.ve

Bloodmeal sources. Blood was collected in heparinized tubes from humans and healthy animals horse *Equus caballus*, chicken *Gallus domesticus*, pig *Sus scrofa domestica*, cow *Bos taurus*, goat *Capra hircus* and dog *Canis familiaris*. It was used when fresh and at least six replicate samples were taken from each species.

Artificial feeding. Two day-old females of *L. ovallesi* (n = 811) were allowed to take blood from an artificial feeding apparatus across a chick-skin membrane, with water circulating at a temperature of 39 ºC. Females were separated into batches of 100 in plastic containers (5.5 cm. per 2.0 cm.) and fed on blood from different vertebrate sources. The flies were allowed to feed through a chick-skin membrane fitted to a glass feeding apparatus with a well into which blood was introduced. Only fully engorged females were used in the analyses. These insects were maintained individually in glass tubes within an incubator at 25 ± 1°C, RH 80 ± 10% and 12:12 light/dark cycle. As a dietary supplement they were provided with saccharose solution *ad libitum*, which was renewed daily. The control group was fed with saccharose solution alone.

Caloric content. Caloric contents were calculated for all groups of females fed on different sources of vertebrate blood under laboratory conditions. Results are presented as calories per female, the mean being calculated using between 84 (human) and 108 (goat) blood-fed females. Values were calculated after bloodmeals had been fully digested, based on microscopic examination of sand fly guts. A solution of sodium dichromate in sulfuric acid was used to determine caloric reserves in individual insects, as described by Van Handel (1972). This involved homogenizing each female in 1.2 ml sodium dichromate solution in sulfuric acid within a glass test tube and boiling for 20 min. After heating, 1.8 ml of distilled water was added to each preparation. Color changes in the test solutions were then measured using a Milton Roy Spectronic 20D spectrophotometer. Optical density (OD) values of test solutions were compared with a standard curve for densities of various saccharose concentrations to convert readings into calories. All assays included saccharose standards as references, the color produced by 1 mg of saccharose (0.1 ml of 1% solution) with an optical density of 0.38 being equivalent to 4 cal. One cal is equivalent to an optical density of 0.095.

Statistical analysis. The data from optical density value of the sand flies were analysed by means of one-way ANOVA and statistical analyses for significance were based on the Tukey´s test for different values of n. All statistical analyses were carried out using the MINITAB computer program (version 10) and the program Statistics version 6.0.

Results

Caloric contents of *L. ovallesi* fed on blood from each of seven vertebrate species are shown in Figure 1. The highest caloric content (cal/female) was obtained from insects fed on human (x = 0.33; range 0.18-0.47), and the lowest from those fed on horse blood (x = 0.26; 0.15-0.39). In decreasing order of magnitude the caloric content for *L. ovallesi* fed on different types of blood was as follows: control < horse < goat < chicken < cow < pig < dog < human. Significant differences ($P \leq 0.05$) were seen for the following comparisons: cow vs dog, human vs control; pig vs horse, goat, chicken and control; dog vs cow, horse, goat, chicken blood and control; horse vs pig, dog, human and control; and human vs cow, horse, goat, chicken, and control (Table 1).

Discussion and Conclusions

The enormous reproductive potential of hematophagous insects is largely due to the female's success in locating a host, approaching it to feed, utilizing the blood to mature an optimal number of eggs and then finding a suitable site for oviposition. This pattern of behavior also means that females can transmit pathogens between hosts (Briegel 1990).

Ingestion of blood swells the epithelial cells, causing reversible phenomena such as secretion of the peritrophic matrix and liberation of proteolytic enzymes. The main products of blood digestion are amino acids. When blood is digested, the final product is excreted as ammonium urate (Rudin and Hecker 1982; Magnarelli and Burger 1984).

Dichromate solution oxidizes the insect completely, with proteins, carbohydrates, lipids and chitin being converted into carbon dioxide (Van Handel 1984; Magnarelli and Modi 1988). This technique is both rapid and sensitive and can be used to determine the nutritional state of females in a laboratory colony or assess the value of blood from different vertebrate hosts. Based on our results, all the blood sources provided energy for *L. ovallesi* although the caloric value of sand flies fed on horse blood was not significantly greater than that of unfed flies. These values obtained do not necessarily reflect the total caloric reserves available to the females, since they were gravid; a large proportion of the caloric reserves is used in egg production,

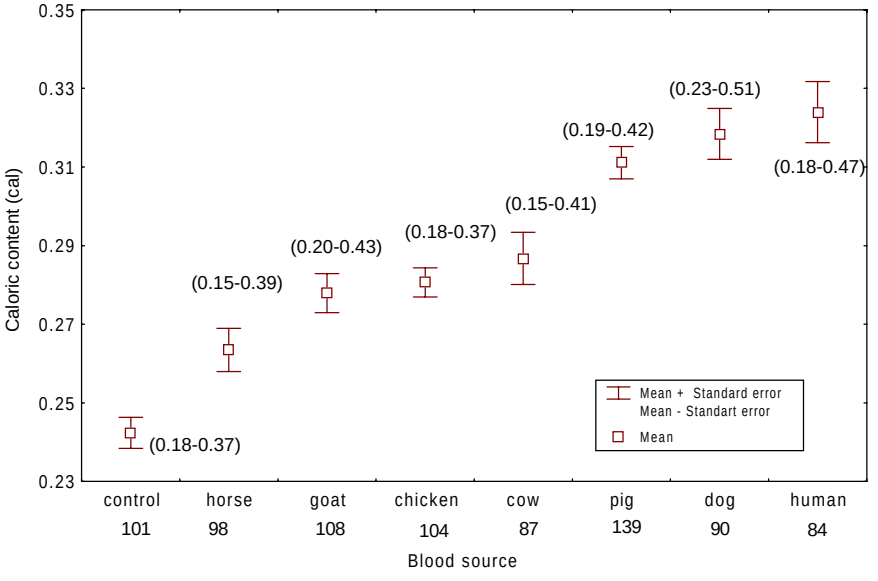

Figure 1. Caloric contents of sand flies *L. ovallesi* fed on blood from seven vertebrate hosts. Numbers in parentheses are ranges and numbers given below the blood source are values of n.

Table 1. Statistical analyses of caloric contents of *L. ovallesi* fed on blood of seven vertebrate hosts

A: One-way analysis of variance

Source	Sum of squares	Degrees of Freedom	Mean Squared	F	P
Effect	0.552435	7	0.078919	27.72645	0.000
Error	2.285623	803	0.002846		

B: Tukey's HSD test for different values of n

	Control	Horse	Goat	Chicken	Cow	Pig	Dog	Human
Control		0.102884	0.000088*	0.00004*	0.000033*	0.000032*	0.000032*	0,00003*
Horse	0.102884		0.556179	0.318605	0.076632	0.000032*	0.000032*	0.000032*
Goat	0.000088*	0.556179		0.999952	0.957644	0.000153*	0.00004*	0.000033*
Chicken	0.00004*	0.318605	0.999952		0.99522	0.00102*	0.000083*	0.000035*
Cow	0.000033*	0.076632	0.957644	0.99522		0.053253	0.002292*	0.00019*
Pig	0.000032*	0,000032*	0.000153*	0.00102*	0.053253		0.983949	0.772795
Dog	0.000032*	0.000032*	0.00004*	0.000083*	0.002292*	0.983949		0.997722
Human	0.000032*	0.000032*	0.000033*	0.000035*	0.00019*	0.772795	0.997722	

P*, Values for which the means corresponding to the blood sources compared are significantly different

with very little used for female nutrition (Rudin and Hecker 1982; Magnarelli and Burger 1984).

Nasci (1986) reported that large females of the mosquito species *Aedes aegypti* possess large energy reserves at eclosion, providing them with great flight potential and the ability to contact more hosts and transmit pathogens. However, Landry *et al*. (1988) found that significant seasonal differences in the body size of *Ae. triseriatus* had no effect on flight potential or life-span. Harre *et al.* (2001) found that *P. papatasi* fed on blood from eight species of mammals and detected no appreciable difference between these hosts with respect to sand fly mortality rates after 24h, number of eggs laid per blood-fed female or egg viability. Laboratory-reared males and females of both *L. longipalpis* and *P. papatasi* which had access to fructose or sucrose solutions had greater mean available energy reserves (x = 1.3 cal/insect) than individuals provided with glucose solution (x= 0.55). Available caloric reserves were low in natural populations of *P. papatasi* and these insects probably must feed repeatedly on vertebrate hosts and sugar sources to obtain sufficient nutrients for survival and reproduction (Magnarelli and Modi 1988).

Although, a high number of sand fly species have been successfully colonized during the last decade, the factors limiting their productivity and fecundity in the laboratory are unknown (Montoya *et al.* 1998; Luitgards-Moura *et al*. 2000). Knowledge about the physiological events taking place in the vector is important in understanding vector-parasite interactions necessary for disease transmission. Nutritional quality of blood varies between host species and may influence egg productivity, reduces development rates, longevity, and fecundity of the insects (Alexander *et al*. 2002). For an understanding the role of blood meal sources on sandfly biology, physiology, and *Leishmania* transmission both more field observations and laboratory studies comparing egg productivity of sandflies fed on different hosts, are necessary (Alexander *et al*. 2002; Hurd 2003).

The compatibility of the sand fly and its specific *Leishmania* parasite depends on the choice of host animals available, it could be an important factor in the distribution of leishmaniasis (Schlein *et al*. 1983). The proteins from the blood meal are digested by the sand fly gut. It appears that the enzymatic processes in the sand fly gut, functions differently when triggered by different types of meals, and the blood meal from distinct animal sources can be lethal to *Leishmania* (Adler 1964). *L. tropica* infection was inhibited in *P. papatasi* fed on turkey blood because a relatively high DNAase activity level was induced in the sand fly gut by nucleated erythrocytes (Schlein *et al*. 1983). However, the blood meals from different species of vertebrates have no deleterious effect on the development of either *L. braziliensis* and *L. amazonensis* in the gut of *L. migonei*; also, parasite development was compatible with digestion, independent of the blood meal source (Nieves and Pimenta 2002). The development of *L. infantum* infection was associated with suppression of blood protein digestion by sand flies fed on human or dog blood (Schlein *et al*. 1983; Daba *et al*. 1997). It also was demonstrated that the rate of blood meal digestion in *P. langeroni* varied according to the source of the vertebrate blood and *Leishmania* species involved (Daba *et al*. 1997).

Very little is known about how these nutrients are used during adulthood. Sand fly reproduction depends on the availability of blood meal sources such as domestic animals and synanthropic species. In endemic areas where some species of domestic animals are sources of blood meals, a higher number of sand fly vectors with more parasites occur. This fact provides a selective advantage to the vector competence in transmitting *Leishmania* to vertebrates. This was possible due to the relatively high isoleucine content in rodent blood, as opposed to its role as a limiting factor for oogenesis with human blood. Important role of isoleucine explained the results of several previous reports that showed variable mosquito fecundity with different host

(Briegel 1990). Similar physiological mechanisms may play a role in the sand flies. Although, feeding on blood from rodents was superior to that from humans with respect to fecundity in *Ae. aegypti*, it may be sub-optimal energetically (Briegel 1990). *L. braziliensis* has been found in domestic animals as dogs and equines as well as in wild mammals such as rodents, edentata and opossums (Aguilar *et al.* 1984; Grimaldi and Tesh 1993). *L. ovallesi* feeds upon a variety of vertebrate hosts, and could be considered as an opportunistic species (Añez *et al.* 1988; Nieves *et al.* 2004). Based on the results of the present study, there are significant differences in the caloric contents of female *L. ovallesi* fed on blood from different sources, with human, dog, and pig blood providing most energy. It might therefore, benefit females of this species to feed preferentially on these hosts. Values for females fed on horse blood were as low as those in the control group, which had been fed only in sugar. Further studies are required to determine how certain dietary factors affect vector potential and their consequences for *Leishmania* transmission. This information may enable health authorities to adopt policies concerning the presence of domestic animals in endemic areas and may comprise factor risk for *Leishmania* transmission.

Acknowledgments

We thank Carlos Araque for assistance with the laboratory colony; Luis Chavez for his invaluable help and cooperation; Efrain Entralgo, Guillermo Bianchi and Paolo Ramoni for guidance in statistical analysis; Leyda Quintero for help in providing blood samples; and the CDCHT-ULA (C-1278-04-03-B) and CONICIT (S1-2000000818) for providing financial support for this work.

References cited

ADLER, S. 1964. *Leishmania*. Advances in Parasitology 2: 35-96.

ALEXANDER, B.; CARVALHO, R.L; MCCALLUM, H.; PEREIRA, M.H. 2002. Role of the domestic chicken (*Gallus gallus*) in the epidemiology of urban visceral leishmaniasis in Brazil. Emerging Infectious Diseases 8 (12) :1480-1485.

AGUILAR, C.M.; FERNANDEZ, E.; DE FERNANDEZ, R.; DEANE, L.M. 1984 Study of an outbreak of cutaneous leishmaniasis in Venezuela. The role of domestic animals. Memórias do Instituto Oswaldo Cruz 79 (2): 185-195.

AÑEZ, N.; CAZORLA, D.; NIEVES, E.; CHATAING, B.; CASTRO, M.; DE YARBUH, A. L. 1988. Epidemiología de la leishmaniasis tegumentaria en Mérida, Venezuela. I. Diversidad y dispersión de especies flebotominas en tres pisos altitudinales y su posible role en la transmisión de la enfermedad. Memórias do Instituto Oswaldo Cruz 83 (4): 455-463.

BONFANTE-GARRIDO, R.; URDANETA, R.; URDANETA, I.; ALVARADO J. 1991a. Natural infection of *Lutzomyia ovallesi* (Diptera: Psychodidae) with leishmaniasis in Duaca, Lara State, Venezuela. Transactions of the Royal Society of Tropical Medicine and Hygiene 85 (1): 61.

BONFANTE-GARRIDO, R.; SPINETTI, H.; CUPILLO, E.; MOMEN, H.; GRIMALDI, G.1991b. *Lutzomyia ovallesi* (Diptera: Psychodidae) as a vector of cutaneous leishmaniasis in Venezuela. Parasitologia 33 (suppl): 99-104.

BRIEGEL, H. 1990 Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. Journal of Insect Physiology. 36 (3):165-172.

BRIEGEL, H.; WALTERT, A.; KUHN A, R. 2001. Reproductive physiology of *Aedes (Aedimorphus) vexans* (Diptera: Culicidae) in relation to flight potential. Journal of Medical Entomology 38 (4): 557-565.

DABA, S.; MANSOUR, N. S.; YOUSSEF, F. G.; SHANBAKY, N. M.; SHEHATA, M. G.; SAWAF, B. M. 1997. Vector-host-parasite inter-relationships in leishmaniasis. III. Impact of blood meal from natural vertebrate host on survival and the development of *Leishmania infantum* and *L. major* in *Phlebotomus langeroni* (Diptera: Psychodidae). Journal of Egypt Society of Parasitology. 27(3):781-794

FELICIANGELI, M. D. 1991.Vector of leishmaniases in Venezuela. Parasitologia 33 (suppl): 229-236.

GRIMALDI, G.; TESH, R. B. 1993. Leishmaniasis of the New World: current concepts and implications for future research. Clinical Microbiology Reviews 6 (3): 230-250

HARRE, J. G.; DORSEY, K. M.; ARMSTRONG, K. L.; BURGE, J. R,; KINNAMON, K. E. 2001. Comparative fecundity and survival rates of *Phlebotomus papatasi* sandflies membrane fed on blood from eight mammal species. Medical and Veterinary Entomology 15 (2): 189-196.

HURD, H. 2003. Manipulation of medically important insect vectors by their parasites. Annual Review of Entomology. 48 (4): 141-161.

KILLICK-KENDRICK, R.; LEANEY, A. J.; READY, P. D. 1977. The establishment, maintenance and productivity of a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). Journal of Medical Entomology. 13 (4-5): 429-440.

LANDRY, S. V.; DEFOLIART, G. R.; HOGG, D. B. 1988. Adult body size and survivorship in a field population of *Aedes triseriatus*. Journal of the American Mosquito Control Association. 4 (2): 121-128.

LUITGARDS-MOURA, J.F.; CASTELLON, E.G.; ROSA-FREITAS, M.G. 2000. Aspects related to productivity for four generations of a *Lutzomyia longipalpis* laboratory colony. Memórias do Instituto Oswaldo Cruz 95 (2):251-257.

MAGNARELLI, L. A.; BURGER, J. F. 1984. Caloric reserves in natural populations of blackfly, *Simulius decorum* (Diptera: Simuliidae), and deerfly, *Chrysops ater* (Diptera: Tabanidae). Canadian Journal of Zoology. 62 (12): 2589-2593.

MAGNARELLI, L. A.; MODI, G. B. 1988. Caloric determinations of phlebotomine sand flies (Diptera: Psychodidae). Journal of Medical Entomology. 25(2): 127-130.

MONTOYA, J.; CADENA , H.; JARAMILLO, C. 1998. Rearing and colonization of *Lutzomyia evansi* (Diptera: Psychodidae), a vector of visceral leishmaniasis in Colombia. Memórias do Instituto Oswaldo Cruz 93 (2): 263-268.

MOSTOWY, W. M.; FOSTER, W. A. 2004. Antagonistic effects of energy status on meal size and egg-batch size of *Aedes aegypti* (Diptera: Culicidae). Journal of Vector Ecology 29 (1): 89-93.

NASCI, R.S. 1986. The relationship between adult mosquito body size and parity in field populations. Environmental Entomology 15 (4): 874-876.

NIEVES, E.; PIMENTA, P. F. 2002. Influence of vertebrate blood meals on the development of *Leishmania* (*Viannia*) *braziliensis* and *Leishmania* (*Leishmania*) *amazonensis* in the sand fly *Lutzomyia migonei* (Diptera: Psychodidae). American Journal Tropical Medicine and Hygiene 67 (6): 640-647.

NIEVES, E.; DÁVILA-VERA, D.; PALACIOS-PRÜ, E. 2004. Daño ultraestructural del intestino medio abdominal de *Lutzomyia ovallesi* (Ortiz) (Diptera: Psychodidae) ocasionado por *Leishmania* (*Leishmania*) *amazonensis*. Parasitologia Latinoamericana 59 (3-4): 115-122.

RUDIN, W.; HECKER, H. 1982. Functional morphology of the midgut of sandfly as compared to other hematophagous nematocera. Tissue & Cell 14 (4): 751-758.

SCHLEIN, Y.; WARBURG, A.; SCHNUR, L.F.; SHLOMAI, J. 1983. Vector compatibility of *Phlebotomus papatasi* dependent on differentially induced digestion. Acta Tropica 40 (1): 65-70.

SCHLEIN, Y.; JACOBSON, R. L. 1998. Resistance of *Phlebotomus papatasi* to infection with *Leishmania donovani* is modulated by components of the infective bloodmeal. Parasitology. 117 (5): 467-473.

VAN HANDEL, E. 1972. Simple biological and chemical methods to determine the caloric reserves of mosquitoes. Mosquito News. 32 (4): 589-591.

VAN HANDEL, E. 1984. Metabolism nutrients in the adult mosquito. Mosquito News. 44 (4): 573-579.

Recibido: 12-oct-05 • Aceptado: 26-dic-05