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COMPARATIVE STUDIES ON THE GROWTH AND REPRODUCTIVE PERFORMANCES OF *RHODNIUS PROLIXUS* REARED ON DIFFERENT BLOOD SOURCES

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Host blood source was found to affect both the development and the reproductive performance of Rhodnius prolixus. *The insects were reared on citrated human, rabbit, chicken, sheep and horse blood sources, through a membrane feeder, during an entire life cycle, from eggs to adults. Development and reproduction in terms of the number of unfed insects, number of moulting, mortality intermoulting period, number of egg/female, conversion of blood into egg (mg meal/egg) and percentage of hatch as effective physilogical parameters were investigated. Our results showed that human or rabbit blood meals were more nutritionally efficient than the other blood samples used because (i) the insects developed faster, presented low mortality and about 80% of them reached the adult stage; and (ii) females oviposited an average of at least 100% more eggs. The inefficiency of chicken and horse blood sources as diets for* R. prolixus *was manisfested in (i) a decrease of the amount of ingested blood and (ii) only a reasonable nutritional quality. The inadequacy of sheep blood was observed by a mortality extremely high, poor moulting response and drastic reduction in egg production.*

Key words: *Rhodnius prolixus* – development – reproduction

The blood-sucking bug *Rhodnius prolixus* and other triatomines have been reared and maintained successfully in the laboratory for many years (for review see Núñez & Segura, 1987). Presently, the mass-rearing of *R. prolixus* is largely dependent on the availability of a suitable source of blood for the insect. Many workers described the use of either living animals such as rabbits, sheep or chicken as hosts (Buxton, 1930; Correa, 1954; Ryckman & Ryckman, 1967; Gardiner & Maddrell, 1972; Hill & Campbell, 1975) or the presentation of blood through appropiate types of membranes (Gardiner & Maddrell, 1972; McGuire et al., 1973; Garcia et al., 1975a, b; 1984; Pimley & Pimley, 1978). Moreover, Gardiner & Maddrell (1972) fed all larval instars of *R. prolixus* through artificial membranes on defibrinated sheep blood. However, deleterious effects of this blood showed a mortality unacceptably high, and a poor moulting response in 5th-instar. Differently, McGuire et al. (1973) pointed out that *Rhodnius* can be reared from generation to generation using a diet of heparinised human blood. Additionally, Pimley & Langley (1978) described the success of rearing *R. prolixus* for three generations on a diet of defibrinated pig blood fed through membrane. Recently, it has been reported that in adult females of *R. prolixus* the autogeny and egg production are dependent on the blood source. For example, a colony fed on human blood presents a reproductive capacity higher as compared to that of insects fed on sheep blood (Valle et al., 1987). The latter insects presented autogeny whereas the insects fed blood on sheep did not.

Nevertheles, in spire of these scattered data, only few comparative studies have been conducted to determine whether or not *R. prolixus* can be mantained on different blood sources with the same efficiency. If the insect could be successfully maintained on membrane feeding techniques, studies on the effect of different blood meal sources could be investigated, especially for large-scale rearing and nutritional requirements of this insect.

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TABLE I

Development, rejection to feed and mortality of *Rhodnius prolixus* reared from 1st- to 5th-instar larvae on different feeding sources

Instar-larvae	Blood source	Number of insects	Number of unfed insects (n)	(%)	Number of moulting (n)	(%)	Mortality (n)	(%)
1st-	Human	200	15	7.5	183	99	2	1.0
	Rabbit	200	10	5.0	188	99	2	1.0
	Chicken	200	10	5.0	187	98	3	1.5
	Sheep	200	5	2.5	121	63	5	2.5
	Horse	200	45	25.0	155	77	3	1.5
2nd-	Human	183	3	1.6	178	99	2	1.1
	Rabbit	188	6	3.1	179	98	3	1.6
	Chicken	187	6	3.2	178	98	3	1.6
	Sheep	121	6	4.9	72	63	9	7.8
	Horse	155	22	16.0	105	88	6	5.0
3rd-	Human	178	7	3.9	170	99	1	0.5
	Rabbit	179	7	3.9	169	98	3	1.7
	Chicken	178	8	4.4	163	96	3	1.7
	Sheep	72	7	9.7	42	65	12	18.4
	Horse	105	27	25.7	66	84	8	10.2
4th-	Human	170	5	2.9	165	100	0	0
	Rabbit	169	5	2.9	164	100	0	0
	Chicken	163	3	1.8	145	91	2	1.2
	Sheep	42	3	7.1	22	56	15	35.7
	Horse	66	12	18.1	21	76	8	14.8
5th-	Human	165	6	3.6	159	100	0	0
	Rabbit	164	7	4.2	157	100	0	0
	Chicken	145	6	4.1	96	69	4	2.8
	Sheep	22	1	4.5	5	25	10	47.6
	Horse	41	10	24.3	16	52	6	19.3

To show that the efficiency or reluctance to feed on a given blood meal is not due to the animal but rather to the palatability and nutritional value of the blood, we described herein the results of feeding, through a membrane feeder, using distinct blood source for *R. prolixus* during an entire life cycle, from eggs to adults. Comparison on the differences in engorgement, moulting period of time, numbers of moulting, egg production or in the efficiency of ingested blood conversion into eggs in insects fed on different blood sources may indicate the blood utilization and explain the variable fecundity and development of this insect when fed on different blood meal sources. Information about such changes may be useful for laboratories that apply *R. prolixus* as an insect model for studying biochemistry, physiology and control of triatomine vectors.

MATERIALS AND METHODS

Insects – Newly emerged first-instar larvae of *R. prolixus* used in these studies were obtained from a colony routinely maintained at our laboratory since 1965 using citrated human blood. Details of the standard rearing condition at 28 ^{o}C and handling procedures have been previously described (Garcia et al., 1975a, b; 1984).

Blood sources – Citrated blood samples from human, rabbit, chicken, sheep and horse were prepared as previously described (Garcia et al., 1984) and maintained for few hours at 5 ^{o}C were used throughout the experiments.

Experimental protocol – Five experimental groups (A-E) were formed out of 200 1st-instar

TABLE II

Comparative effects of different feeding sources on the body weight, intake of ingested blood and intermoult period between feeding to ecdysis of all instar larvae of *Rhodnius prolixus*

Instar-larvae	Blood source	Body weights (mg)	Ingested blood (mg)	Intermoult period (range in days)	
				after feeding	after hatching (cumulative)
1st-	Human	0.45 ± 0.04	3.5 ± 0.32	8 – 12	18 – 22
	Rabbit	0.45 ± 0.04	4.6 ± 0.38	7 – 10	17 – 20
	Chicken	0.45 ± 0.04	3.9 ± 0.36	8 – 12	18 – 22
	Sheep	0.45 ± 0.04	4.0 ± 0.42	15 – 18	25 – 29
	Horse	0.45 ± 0.04	3.4 ± 0.38	12 – 15	22 – 25
2nd-	Human	1.80 ± 0.15	8.2 ± 0.72	10 – 12	42 – 44
	Rabbit	1.30 ± 0.16	13.5 ± 1.40	10 – 14	40 – 44
	Chicken	1.40 ± 0.16	11.1 ± 1.06	11 – 15	43 – 47
	Sheep	1.20 ± 0.13	10.9 ± 0.97	14 – 20	53 – 59
	Horse	1.75 ± 0.19	6.1 ± 0.75	12 – 19	47 – 54
3rd-	Human	4.55 ± 0.38	33.7 ± 0.45	12 – 16	66 – 70
	Rabbit	5.25 ± 0.48	35.3 ± 0.40	11 – 16	65 – 70
	Chicken	4.90 ± 0.42	21.8 ± 0.27	14 – 22	71 – 79
	Sheep	4.05 ± 0.39	31.0 ± 0.37	15 – 25	84 – 94
	Horse	3.95 ± 0.45	14.0 ± 0.22	15 – 18	79 – 82
4th-	Human	12.80 ± 1.15	105.0 ± 9.8	13 – 17	93 – 97
	Rabbit	13.65 ± 1.55	111.9 ± 10.8	12 – 14	92 – 94
	Chicken	11.57 ± 1.30	69.0 ± 7.9	14 – 24	103 – 113
	Sheep	9.25 ± 1.25	101.0 ± 9.9	16 – 25	120 – 129
	Horse	8.40 ± 0.99	51.6 ± 6.8	13 – 25	105 – 117
5th-	Human	42.86 ± 4.85	257.2 ± 19.8	20 – 23	127 – 130
	Rabbit	30.57 ± 3.35	273.9 ± 25.9	18 – 24	122 – 128
	Chicken	32.33 ± 3.10	173.1 ± 19.4	21 – 30	144 – 153
	Sheep	25.52 ± 2.82	154.5 ± 16.9	24 – 31	163 – 170
	Horse	22.05 ± 3.55	118.0 ± 15.7	21 – 30	158 – 167

larvae each, and then allowed to feed 10 days after hatching, on either human (A), rabbit (B), chicken (C), sheep (D) and horse blood (E). We then followed the development of these groups until the imaginal stage. Larvae from the 2nd- to 5th-instar as well as adult individuals were allowed to feed on the respective blood meals each 10 days after the last moulting of each instar of development. The insects were allowed to feed on a membrane feeder apparatus (Garcia et al., 1984) for 30 min, after which the feeding response (number of bugs engorged) was recorded. The insects were weighed before and immediately after feeding and the intake of meal ingested was estimated by subtraction. Any bugs which fed less than one third of the blood weight ingested by fully gorged insects were rejected and those that did not undergo ecdysis during 10 days after the last moulting of their groups were removed and discarded. The adult females were mated, the eggs produced collected, and the percentage hatch were recorded for each experimental group. Any dead insects were removed and their number recorded.

RESULTS AND DISCUSSION

Blood meal source and larval development – In a typical experiment we followed the development of the five experimental groups of insects from the 1st-instar larvae to adult stage (Table I). As can be seen of the 200 1st-instar larvae of each group, 159 (79.5%), 157 (77.0%), 96 (48.0%), 5 (2.5%) and 16 (8.0%) insects, for human, rabbit, chicken, sheep and horse blood meals, respectively, reached the adult stage.

The small number of moulting in the group fed on sheep and horse blood was observed from the 2nd-instar to adult stage. Horse blood meal also presented the higher percentage of meal rejection. A significant mortality rate occurred among the insects of these two latter groups from the 2nd-instar larvae to adult stage but the mortality for bugs reared on sheep blood was about 50% higher than the group fed on horse blood. The groups fed on human, rabbit and chicken blood meals presented only background mortality (Table I).

Table II shows that insects fed on human and rabbit blood meals always presented shorter intermoult periods during the entire life cycle than the other groups reared on chicken, sheep and horse blood. The last three groups experienced a progressive ecdysis delayed during the evolution of the developmental cycle when compared with insects of the other two groups. The 5th-instar larvae of the groups fed on chicken, sheep and horse blood meals, for example, had very protracted intermoult period that extended from 21 even to 31 days after feeding, whereas insects of the group reared on human and rabbit blood meals underwent moulting in a period of 18-24 days.

The results on the body weights of the different instars together with the blood meal intakes are also demonstrated in Table II clearly showing that the mean blood meal sizes were reduced in the groups fed on chicken, sheep or horse blood.

Finally, under our experimental conditions the entire life cycle from the eclosion of eggs to adult stage ranged of 127-130, 122-128, 144-153, 163-170 and 158-167 days, respectively, for insects reared on human, rabbit, chicken, sheep and horse blood meals (Table II).

The results on the development of the larval instar reared with human or rabbit blood meals were very similar to those data obtained on *Rhodnius* fed on living hosts (Buxton, 1930; Gardiner & Maddrell, 1972) or reared on membrane feeder with rabbit or pig blood samples (Baines, 1956; Lake & Friend, 1968; Pimley & Langley, 1978).

Adult feeding and egg production – Table III shows the body weights of adult females *R. prolixus*, blood meal intakes, number of eggs produced, percentage of eclosion and conversion of mg of blood ingested to eggs, during two oviposition cycle, i.e., two feeding as adults.

Distinct diets differently changed adult female body weights. Insects reared on chicken, sheep and horse blood meals were 78.1-79.6%, 61.8-63.0% and 53.0-54.0% of the size of females reared on rabbit and human blood meals in the two reproductive cycles (Table III). They were weighed at 10 days post-moulting, when proportional differences remained the same. Since adult females fed on human and rabbit blood meals were larger than those reared on chicken, sheep and horse blood meals, they took heavier blood meals as adults and laid more eggs. However, the most interesting aspect of Table III is that the blood amount associated with the production of one egg is fairly constant (ranging from 4.8 to 5.4 mg ingested blood/egg) in females reared on human, rabbit, chicken and horse blood meals, although females reared on chicken and horse blood meals fed approximately 55% less blood than the other two groups. However, the quantify associated with the production of one egg for females fed on sheep blood surprisingly was twice larger than their former groups following the two feeding sessions (about 10 mg ingested blood/egg) (Table III).

Besides the results detailed above we could also observe that no significant mortality occurred among the adults over a period of 20 days after feeding. Actually, the mean hatch over 15 days of egg production exceeded 97% in all experimental groups.

Take together, our results correlated differences between distinct blood meal sources with the development and reproductive performances of *R. prolixus*. They clearly indicated that human and rabbit blood meals were more efficient nutritionally than the other blood samples used mainly because (i) the insects developed faster and about 80% of them reached the adult stage; and (ii) females oviposited an average of at least 100% more eggs than the other blood sources.

The success of rearing *Rhodnius* on human and rabbit blood meals seems to be dependent on properties of themselves which in same way lack in chicken, sheep and horse blood meals.

TABLE III

Comparative effects of different feeding sources on the body weight, intake of ingested blood, number of eggs and conversion of ingested blood to eggs of adult females of *Rhodnius prolixus*

Blood source	Number of females	Body weight (mg)	Ingested blood (mg)	Number of[a] eggs/female	Conversion of blood to eggs (mg meal/egg)	Hatch (%)
			1st-oviposition cycle			
Human	80	55.52 ± 6.90	155.9 ± 16.3	29.5 ± 3.7	5.3	98
Rabbit	78	56.67 ± 6.05	161.4 ± 15.8	30.0 ± 3.2	5.4	99
Chicken	47	44.24 ± 5.20	76.6 ± 9.8	14.5 ± 2.5	5.3	98
Sheep	20[b]	35.05 ± 4.43	66.4 ± 9.3	6.4 ± 1.2	10.4	97
Horse	20[b]	30.25 ± 4.87	72.4 ± 9.5	15.0 ± 3.4	4.8	100
			2nd-oviposition cycle			
Human	69	65.57 ± 7.25	158.6 ± 17.6	30.5 ± 4.2	5.2	99
Rabbit	68	67.84 ± 6.69	169.6 ± 16.9	32.0 ± 4.5	5.3	98
Chicken	40	50.05 ± 6.05	103.3 ± 9.7	19.5 ± 2.7	5.3	97
Sheep	18	42.70 ± 5.68	114.5 ± 13.9	11.8 ± 3.0	9.7	100
Horse	19	36.56 ± 4.79	104.0 ± 12.7	20.0 ± 4.1	5.2	98

a: each oviposition cycle was observed during 20 days.

b: since sheep and horse blood meals produced only a small number of adults (see Table I) we completed these groups of females from other experimental groups reared on the same feeding diets.

Some of these properties basing on the development and reproductive performances as well as on the intake of ingested blood were considered as the nutritional quality and the palatability of the meals. The human and rabbit blood samples therefore presented an excellent nutritional value and were acceptable as regards the taste for the insects; chicken and horse blood diets had a reasonable nutritional quality but a low palatability; finally, sheep blood meal was only partially rejected by the insects but the nutritional value was very poor for *R. prolixus*. The special feeding behavior of this insect makes it an useful model to investigate nutritional and palatability parameters of blood meals to hematophagus insects.

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REFERENCES

BAINES, S., 1956. Role of symbiotic bacteria in nutrition of *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol., 33:* 535-541.

BUXTON, P. A., 1930. The biology of a blood-sucking bug, *Rhodnius prolixus* Stal. *Trans. R. Ent. Soc. Lond., 78:* 227-239.

CORREA, R. R., 1954. Alguns dados sobre a criação de Triatomíneos em laboratório. *Folia Clin. Biol., 22:* 51-56.

GARCIA, E. S.; AZAMBUJA, P. & CONTRERAS, V. T., 1984. Large-scale rearing of *Rhodnius prolixus* and preparation of metacyclic trypomastigotes of *Trypanosoma cruzi*, p. 43-46. In C. M. Morel (ed.), *Genes and Antigens of parasites, A Laboratory Manual.* Fundação Oswaldo Cruz/FINEP/CNPq/WHO.

GARCIA, E. S.; GARCIA, M. L. M.; MACARINI, J. D. & UBATUBA, F. B., 1975a. Alimentação de *Rhodnius prolixus* no laboratório. *An. Acad. Brasil. Ciênc., 47:* 537-545.

GARCIA, E. S.; GARCIA, M. L. M.; MACARINI, J. D. & UBATUBA, F. B., 1975b. Influência do número de hemácias no volume de sangue sugado por larvas de *Rhodnius prolixus*. *Rev. Bras. Biol., 35:* 207-210.

GARDINER, B. O. C. & MADDRELL, S. H. P., 1972. Techniques for routine and large-scale rearing of *Rhodnius prolixus* Stal (Hem., Reduviidae). *Bull. ent. Res., 61:* 505-515.

HILL, P. & CAMPBELL, J. A., 1975. Improved rearing of symbiont-free and infected *Rhodnius prolixus* using rabbit-ear warmers and simple microbiological isolators. *Laboratory Practice, 24:* 746-747.

LAKE, P. & FRIEND, W. G., 1968. The use of artificial diets to determine of the effects of *Nocardia rhodnii* on the development of *Rhodnius prolixus*. *J. Insect Physiol., 14:* 543-562.

MACGUIRE, E. J.; HABOWSKY, J. E. J.; LUMB, G. & DE LA IGLESIA, F. A., 1973. An experimental approach to the study of drugs in invertebrate systems. I. Mass feeding of *Rhodnius prolixus* Stal (Hemiptera, Reduviidae). *Can. J. Zool., 51:* 315-318.

NÚÑES, J. A. & SEGURA. E. L., 1987. Rearing of Triatominae, p. 31-40. In R. R. Brenner & A. M. Stoka (eds), *Chagas' Disease Vectors; Anatomic and Physiological Aspects*. CRC Press, Boca Raton, Florida, USA.

PIMLEY, R. W. & LANGLEY, P. A., 1978. Rearing triatomine bugs in the absence of a live host and some effects of diet on reproduction in *Rhodnius prolixus* Stal (Hemiptera, Reduviidae). *Bull. ent. Res., 68:* 243-250.

RYCKMAN, R. E. & RYCKMAN, A. E., 1967. Reduviid bugs, p. 183-200. In C. N. Smith (ed.), *Insect colonization and mass production*. Academic Press, New York.

VALLE, D.; GOMES, J. E. P. L.; GOLDENBERG, S. & GARCIA, E. S., 1987. *Rhodnius prolixus* vitellogenesis: dependence upon the blood source. *J. Insect Physiol., 33:* 249-254.