EVALUATION OF FIVE FORMULATIONS OF ORNAMENTAL FISH FEED USED IN LABORATORY REARING OF *ANOPHELES ALBITARSIS* LARVAE.

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SUMMARY: Five different types of fish feed formulations were compared as potential diets for laboratory rearing *Anopheles albitarsis* larvae. Four Brazilian formulations were compared to an imported formulation used world-widely in rearing mosquitoes. The criteria used to evaluate these diet formulations were mortality rate of larvae, number of pupae and yield of males and females. Analysis of the data (t test) showed that the imported diet formulation produced greater number of adults. However, one of the Brazilian manufactured diet formulations ("Tropifish") proved to be as effective as the standard formulation. This product had the additional advantage of being less expensive and locally manufactured.

KEY WORDS: Colony, *Anopheles albitarsis*, biology, fish feed formulations, laboratory rearing.

INTRODUCTION

Anopheles (Nyssorhynchus) albitarsis Lynch-Arribalzaga, 1878 is part of a polymorphic complex of species, distributed from Guatemala to Argentina. These species are found over the entire Brazilian territory and were confirmed as a human malaria vector in some parts of Brazil (RACHOU, 1958; FERREIRA, 1964).

In order to facilitate the studies related to the biology and control of these vectors, it is important to be able to dominate the techniques for rearing and maintaining mosquitoes in laboratory, particularly the standardization of feed formulations given to anopheline larvae, that constitutes one of the major problems in their maintenance in laboratory (COLUZZI, 1964).

Several authors have been able to rear *A. albitarsis* colonies in the laboratory (BARRETO & COUTINHO, 1943; GALVÃO & GRIECO, 1943; GALVÃO *et alii*, 1944). For several generations this specie was maintained through spontaneous copulation in cages. Some modifications in the larvae feed formulations were introduced as proposed by BOYD (1926) and BOYD *et alii* (1935). ARRUDA *et alii* (1982) fed *A. albitarsis* and *A. aquasalis* Curry, 1932 larvae with dog food, with satisfactory results. In recent studies, a member of the albitarsis complex (ROSA- FREITAS, 1989), KLEIN *et alii* (1990) used one part of ground wheat germ and two parts of ornamental fish formulations to feed *A. deaneorum* Rosa-Freitas, 1989.

While testing types of feed formulations used in domestic fish rearing to feed larval forms of *A. albitarsis*, the objectives of the current study were: (a) observe the development of immature forms, (b) evaluate the percentage of adult emergence and (c) standardize larval diet formulations.

MATERIALS AND METHODS

The current study was developed on the premises of the United States Army Medical Research Unit-Brazil Entomology Laboratory in the Instituto de Biologia do Exército, Rio de Janeiro, Brazil.

To analyze the data, Epi Info 6, Beta Test version, was used as recommended by the World Health Organization (WHO), through a 5% significance level.

Collection of *A. albitarsis*

Two hundred and fifty four *A. albitarsis* females were captured and identified on 13 Jan 93, from 5.30-09.00 pm, in the Massaranduba municipality, Santa Catarina State, Brazil. The peak of the biting period was at 08.30 pm, in a rice plantation located in a valley. The collection site was in an inhabited area, people serving as bait to attract mosquitoes, who were collected by an aspiration mechanism.

The females were placed in small cardboard cylinders topped by nylon screens and a synthetic rubber protection covering side openings. The next morning, the females were fed with human blood and the styrofoam boxes shipped to Rio de Janeiro by bus, a 12 hour jouney.

***Anopheles albitarsis* colony in laboratory**

After the female *A. albitarsis* arrived at the laboratory, they were fed on human volunteers. After a three days wait, the females were placed in chlorine-free water plastic basins. They were kept in containers with screened tops and open bottoms where the mosquitoes contacted the water surface. The sides of the basins were lined with filter paper to protect the eggs from dissecation.

Two or three days after oviposition, the 1st stage larvae hatching occurred. The larvae were separated in plastic basins (7.5 cm deep by 17.5 cm diameter) designated for the development of immature forms. To feed larvae, a mixture of 50% TetraMin® "E" ad 50% TetraMin® "L" was used. Both feed formulations were imported. Using a pipette, the pupae were removed daily and put into a plastic cup containing de-chlorinated water, and left in a cardboard cylinder topped with a nylon screen, closed by an elastic material on the lateral opening, that makes the handling of both the cups and the adult mosquitoes possible.

When adults emerged, 24-48 hours after the pupae, the female and male mosquitoes were separated through aspiration to different small cardboard boxes. The mosquitoes were fed through a 10% water and sugar soaked cotton. Induced copulation was used to maintain the mosquito colony. This specific technique was first described by MCDANIEL & HORSFALL (1957) for *Aedes vexans* and later adjusted to the anophelines by BAKER *et alii* (1962).

Laboratory conditions

The water used at the laboratory was filled into tanks which were permanently agitated by a small air compressor. The purpose of the water agitation was to evaporate the chlorine contents of the water. The water tanks also helped to maintain the high level of humidity (between 80 and 95%) in the room, where there was an air conditioner, that turned on automatically any time the temperature rises above 27°C. If the temperature fell below 27°C, this would automatically trigger the heater, so that the temperature inside the lab was 27°C ± 1°C.

Separation of larvae in basins

After the eclosion of the larvae as a result of the induced copulation, 30 plastic basins were filled up with 200 ml of de-chlorinated water each. A total of 1,500 larvae were transferred into the water, 50 larvae into each basin.

Every 6 basins (300 larvae) received a different type of feed formulation. Each basin had a tag on describing the species, type of feed formulation (codes from A-E), date when larvae were transferred, and the number of the basin (1-6).

The basins were displayed and numbered randomly. Data information concerning larval mortality was checked every day.

Feed formulations

Five different types of feed formulations were used for this study, all purchased in aquarium shops. Four of them were manufactured in Brazil, and the last one was the result of a mixture of 50% of two different feed formulations produced in Germany (Formulation D – Control). All comparisons were made in relation to the last formulation and in almost its totality in the replicates, as well.

The formulation composition and substitutes were obtained from the wrappings and the levels of guarantee were obtained through the Laboratório de Controle de Alimentos e Rações of Universidade Federal Fluminense/Departamento de Tecnologia de Alimentos/Laboratório de Controle de Alimentos e Rações/ RJ (Tables 1 and 2).

Standardization of the quantity given to larvae

In view of the different types of granulation, with the exception of the one manufactured in German, all feed formulations were ground in a blender. In order to feed the mosquitoes with a uniform amount of the formulations, a special measuring system was designed by cutting off the tip of five disposable pipettes and gluing it onto an applicator. Thus, the pipette tip was used as a measuring cup. In order to have a minimum variation of fish formulation amounts, a high precision scale was used to weigh 20 samples of each different type of fish formulations. The mean amount of ration given to the larvae in each standard sample was 5.5 mg.

Larval feeding procedure

The 1st stage larvae were fed one standard sample of fish formulation in the morning. From stage 2 phase, they were fed twice a day; one standard sample in the morning and one in the afternoon. The formulations were sprayed on the surface of water.

Cleaning the basins, temperature records and pH

While the immature forms of mosquitoes developed, the surface of the basins were cleaned up every morning by using tissue paper to remove the excess of the feed formulation and

the exuviae. On some occasions, a disposable pipette was used to remove dead larvae from the bottom of the basins.

There was no need to change the water either partially or totally for any reason, although it was required to replace the evaporated water. Five days after transferring 1st stage larvae, the water level of the basins fell due to evaporation. Therefore, 50 ml of de-chlorinated water was distributed in each basin on alternate days. An aquarium thermometer was used to keep the temperature of water even, whereas the pH control of the water was measured by using ColorpHast® strips on alternate days. The water temperature remained at 25°C and the pH remained at 6.0 in the entire experiment.

Pupae separation

As the pupae emerged, they were separated in lots according to the diet given in their previous stage. The pupae were transferred to water filled plastic tubes (7 cm high by 2.5 cm diameter) closed at extremity. Each plastic tube was contained a maximum of 4 pupae. The number of pupae was registered in the same data form used for the larvae.

Separation of the adult mosquito

As the adult mosquitoes emerged, they were separated in lots and numbered according to sex.

RESULTS

The statistical analysis regarding to fish formulation D used as standard demonstrated:

As for the number of male and female that emerged, there was no significant difference ($p < 0,05$) between the three replicates in both sexes. For either the females and the males, there was a significant difference ($p < 0,05$) between the fish

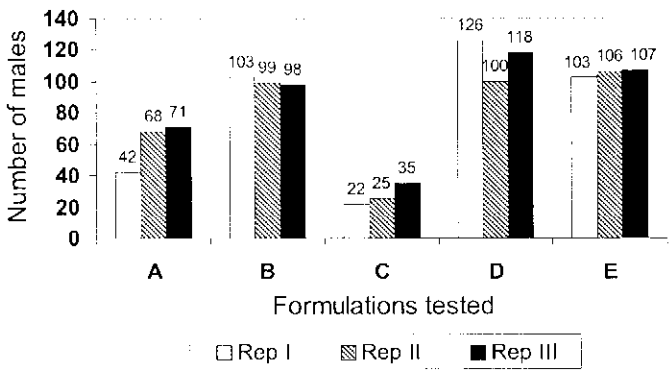

Fig. 1 - Number of *Anopheles (N.) albitarsis* males reared in laboratory in three replicates of five different types of fish food.

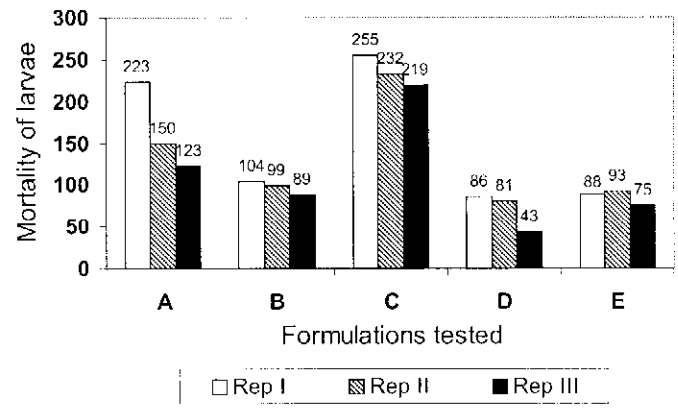

Fig. 3 - Mortality of *Anopheles (N.) albitarsis* larvae reared in laboratory in three replicates of five different types of fish food.

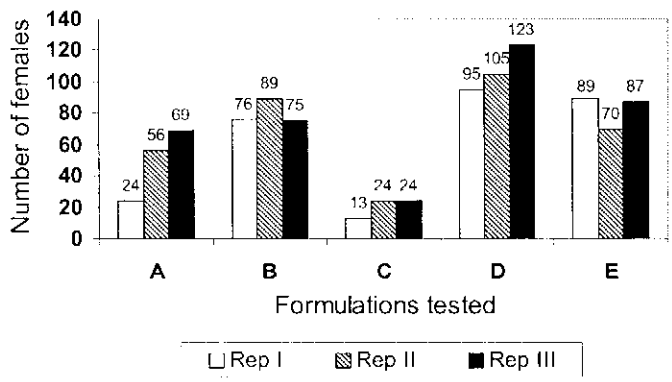

Fig. 2 - Number of *Anopheles (N.) albitarsis* females reared in laboratory in three replicates of five different types of fish food.

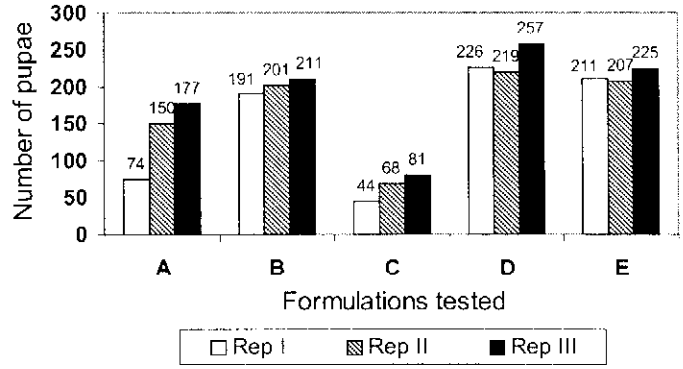

Fig. 4 - Number of *Anopheles (N.) albitarsis* pupae reared in laboratory in three replicates of five different types of fish food.

formulations A x D and C x D. Fish feed formulations B, E and D did not show significant difference between one another. Their superiority was evidenced over the formulations A and C (Figs. 1 and 2).

There was no significant difference ($p< 0,05$) in the death rate of the larvae in the three replicates. There was a significant difference ($p< 0,05$) between the formulations A x D and C x D, with a low efficacy of formulations A and C. Formulations B, E and D did not differ significantly from each other (Fig. 3).

As demonstrated statistically, concerning the number of emerging pupae, formulation D was superior than E, B, A and C. Formulation C presented the poorest results among them. (Fig. 4).

DISCUSSION

The method used to rear *A. albitarsis*, concerning the abiotic data, agrees with the literature and proves the need of an acid pH (ANDRADE, 1957) and water temperatures between 21°-27°C (BOYD, 1926; BOYD *et alii*, 1935; ROZEBOOM, 1936; GALVÃO *et alii*, 1944; GERBERG, 1970). The use of induced copulation seemed to be efficient to establish the colonies, once it ensured the embrionary development of the eggs. The efficiency of this method could be proved since a lineage of mosquitoes was successfully reared and maintained in laboratory conditions.

Considering that the insects used for the treatments and repetitions of this study were from the same origin, there would be no influence of lineage on the comparative results of the different formulations. Some difference in the lineage might occur in those species recently adjusted to captivity and others previously held in captivity. However, that is a subject for a further investigation.

The results, systematically analyzed, confirm the choice of the formulations at the beginning of the investigation to act as a standard model. Formulation D presented the best performance along the study period.

The standard formulation résulted in the smallest rate of larval mortality, produced the largest number of pupae and adults. Formulation C showed the worst results concerning the number of males and females (Figs. 1 and 2), a larger rate of larvae mortality and a smaller number of pupae (Figs. 3 and 4).

Based on the statistical analysis, formulation E showed good results in rearing *A. albitarsis*. It was observed that formulation E was the 2nd best in terms of the number of larval death, number of pupae and adults. These results demonstrate that the described composition seemed to be almost as efficient as the standard formulation.

Formulation E might be used routinely for rearing *A. albitarsis* in Brazil, specially if one considers the advantage of being an national product, when one considers all the difficulties and costs associated with importation.

Table 1 - Formulation composition information and the substitutes obtained for all formulations.

FORMULATION	COMPOSITION
Formulation A: AlconCURE® (Produced and packed by Ind. e Com. de Alimentos Desidratados Alcon Ltda.)	Fish flour, shrimp flour, beef flour, pre-hydrolyzed soy protein, corn gluten, algae, wheat flour, fish oil, soy oil, lecithin and salt. Substitutes: crab meat, corn gluten and alfalfa leaf flour. Enrichment by Kilo: vitamin A (10.000,00 UI), vitamin D_3 (2.000,00 UI), vitamin E (10,00 UI), vitamin K_3 (6,00 UI), vitamin B_1 (2,00 mg), vitamin B_2 (8,00 mg), vitamin B_{12} (20,00 mcg), calcium pantotenato (24,00 mg), niacin (60,00 mg), BHT anti-oxidant (0,10 gr), food coloring Indigota (1,40 gr) and oxitetracicline (2,50 gr).
Formulation B: AlconGUPPY® (Produced and packed by Ind. e Com. de Alimentos Desidratados Alcon Ltda.)	Fish flour, shrimp flour, algae flour, oat flour, alfalfa leaf flour, dehydrated yeast, lecithin and animal fat.
Formulation C: Nutral® (Manufactured and packed by Vitanutre Rações e Suplementos Ltda.)	Fish flour, shrimp flour, entrails flour, beef flour, wheat germ flour, fish oil (preserved with BHT), wheat flour, isolated soy protein, wheat germ flour, oat flour, dehydrated alfalfa flour, aniz, soy flour, pre-gelatinized corn, beetroot puree, tomato puree, animal fat (preserved with BHA),salt, irradiated yeast and soy lecythin. Substitutes: corn flour, blood flour and malt husks. Enrichment per KG: vitamin A (140.000,00 UI).
Formulation D: Control, 50% TetraMin® "E" + 50% TetraMin® "L" (Manufactured and packed by TetraWerke)	TetraMin® "E": Fish flour, dry yeast, shrimp flour, wheat flour, oats, ground wild rice, soy flour, soy extracted solvent, wheat germ, wheat germ oil, chlorophyll, carotene and lutein extracted from natural sources such as: leaves, dehydrated alfalfa and urucum seed extract.
	TetraMin® "L": Fish flour, wild rice, dry yeast, shrimp flour, potato protein, wheat gluten, oats, soy oil, hydrogenated sea animal oil, peeled soy food, sorbitol and algae flour. Enrichment per 450g: vitamin C (200mg).
Formulation E: TropiFish® (Manufactured and packed by Flora Bleher Ltda.)	Fish flour, liver, wheat germ, wheat flour, soy flour, barley flour and spinach.

Table 2 - Levels of guarantee obtained for six formulations.

Formulation	Comercial name	Humidity	Gross protein	Ethereal extact	Fibrous matter	Mineral matter	Ca	P
Formulation A	AlconCure®	5.54%	41.48%	5.79%	5.50%	12.93%	2.84%	1.24%
Formulation B	AlconGuppy®	6.27%	43.08%	8.91%	5.00%	11.95%	2.66%	1.26%
Formulation C	Nutral®	3.79%	48.96%	7.60%	4.00%	22.41%	7.59%	3.21%
Formulation D	TetraMin® "E"	4.34%	47.80%	8.13%	6.00%	Unevaluated	3.33%	1.16%
(CONTROL)	TetraMin® "L"	6.54%	49.23%	10.25%	2.00%	9.72%	3.22%	1.24%
Formulation E	TropiFish®	6.27%	24.82%	8.17%	2.50%	11.04%	4.08%	1.73%

The number of adult insects was 74.44% with the formulation D and 62,44% with the formulation E. Comparing these results against the literature, it could be concluded that the method was satisfactory, since the mean rate described BARRETO & COUTINHO (1943) was 72.0% and the discrepancy rate between those formulations was not above 10,0%.

The pupae and larval death rate was referred by BARRETO & COUTINHO (1943) as being 7.69% and 18.75% respectively. In the present study, however, the pupae and larval death rate were, respectively, 22.0% and 23.33% for formulation D, and 28.56% and 28.44% for formulation E. It might be possible that the differences between those percentages in this study were consequences of the method used.

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SUMÁRIO

Cinco formulações de rações para peixes ornamentais foram analisadas como potenciais dietas para larvas de *Anopheles albitarsis* criadas em laboratório. Quatro dessas formulações foram produzidas no Brasil e comparadas com uma ração importada usada internacionalmente para criação de mosquitos. O critério usado para a análise dessas rações incluíram: Taxa de mortalidade das larvas, número de pupas e número resultante de machos e fêmeas. A análise de dados (teste *t*) revelou que a ração importada proporcionou maior número de adultos, entretanto, uma das rações produzidas no Brasil, "Tropifish", provou ser de efetividade similar à ração padrão. Esse produto tem a vantagem adicional de possuir menor custo e ser produzido localmente.

PALAVRAS-CHAVE: Colônia, *Anopheles albitarsis*, biologia, ração para peixes, criação em laboratório.

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