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An artificial larval diet for blowfly, *Lucilia cuprina* (Diptera Calliphoridae)

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ABSTRACT

A simple artificial diet was devised for larvae of blowfly, *L. cuprica*. The artificial diet primarily composed of whole milk powder, bovine blood, chicken eggs and wheat bran. Growth and developmental parameters of blowfly reared on different composition of artificial diets were compared with those reared on Tilapia fish as control. No significant differences were observed in duration and mortality during the larval and pupal stages between larvae reared on artificial diets and those reared on Tilapia fish. Larval and pupal weights were found significantly greater on artificial Diet-A than those reared on other artificial diets and on natural diet. Adults reared on Diet-A were healthy, lived longer and laid significantly more eggs per female than those reared on Tilapia fish and all other artificial diets. Based on the results of the present experiment artificial larval diet composition Diet-A was considered to be the most suitable alternative to natural diet for blowfly rearing.

Keywords: Artificial diet, Blowfly, Lucilia cuprina, Development, Rearing.

1. Introduction

Australian sheep blowfly, *Lucilia cuprina* (Diptera:Calliphoridae) is a fly of medical and veterinary importance. In Bangladesh it is a major pest in sun-drying fish industry. Around 30,000 metric tons of marine fishes are seen dried in Bangladesh for both local consumption and export purposes. A considerable amount of fish and fishery products (about 30%) are either damaged or infested during the process of sun-drying in Bangladesh due to blowfly infestation [1, 2, 3, 4, 5]. To avoid losses, farmers use insecticides which are harmful to human health as well as to the environment. Therefore, it is important to develop environmentally safe and effective control measure.

Application of Sterile Insect Technique (SIT) against blowfly in the fish drying areas could be a better and safer alternative to chemical control method. The ability to regularly produce insects of consistent quality is a vital feature of the SIT. Sterile Insect Technique (SIT) depends upon inducing a high proportion of sterile mating in a natural population that reduces reproduction to a level below population maintenance. Therefore, the production of target insects, in sufficient number and of adequate quality to achieve this aim, is one of the principal requirements for the success of SIT [6].

Appropriate larval diet and optimum environmental conditions are very important for successful mass rearing. Natural host diet usually have unpleasant odor, limited by the availability and variable in quality. In contrast, artificial diets have pleasant odor, consistent quality and a good cost benefit relationship. Ingredients for artificial diets are available from commercial sources throughout the year and they are generally more economical than natural food materials [7, 8].

Several blowfly species such as *Lucillia sericata, Chrysomya megacephala* and *Phormia regina* have successfully been reared on different compositions of artificial diets [9, 10, 11, 12]. However, very few attempts have been taken to develop artificial larval diet for blow fly *L. cuprina*. In Bangladesh, scientist of Bangladesh Atomic Energy Commission (BAEC) had established laboratory rearing of blowfly using Hilsha fish and Bovine liver as larval diet. Later on, they established rearing of blowfly on relatively cheaper Tilapia fish.

But due to high cost and inconsistent production quality, the ongoing SIT program of BAEC appears to be infeasible [13]. The present study was thus undertaken to develop a cost effective stable artificial larval diet for blowfly utilizing locally available ingredients. We have developed an artificial larval diet for blowfly using wheat bran, whole milk powder, bovine blood and chicken egg. The newly developed diet is economical, easily accessible and nutritious compare to those of exiting natural larval diet.

2. Materials and Methods

2.1 Stock maintenance

Stock rearing of blowfly, *Lucilia cuprina*, was maintained at the blow fly rearing plant of Institute of Food and Radiation Biology, Savar, Dhaka, Bangladesh, in rectangular cages (142 cm×128 cm ×128 cm) covered with mosquito net made up of nylon at 25±5 ^{0}C average temperature, 70±10% relative humidity and 12 hr photoperiodicity. The adult flies were supplied with sugar and water soaked cotton as food substances. Fresh liver was subsequently supplied in adult cages as protein substitute necessary to initiate vitellogenesis.

2.2 Egg collection

For egg collection a small piece of liver was placed on a petri-dish which was then placed in the adult cage. The female laid eggs in mass or cluster. Eggs were gently separated from the substrate with the help of soft camel brush and forceps. It has been estimated that 0.5 gm egg contains about 6000 individual egg. These eggs were used for rearing.

2.3 Larval diet

2.3.1 Composition and preparation of artificial larval diet

To prepare artificial diet locally available ingredients were used. The ingredients were wheat bran, full cream milk powder (Fresh Brand), chicken egg, bovine blood (collected from the slaughter house) and water. Four different compositions of the ingredients were tested for their performance to support larval development of *Lucilia cuprina*. The compositions were named as Diet-A, Diet-B, Diet-C and Diet-D (Table 1). Diet A contained all three fundamental ingredients, i.e. whole milk powder, chicken egg and bovine blood, while one of these ingredients was omitted in diets B (bovine blood), C (Chicken egg) and D (whole milk powder). The ingredients for each diet were mixed well using a food mixture (Jaipan Brand) and then spread on a 14 inch aluminum plate (rearing plate) with equal thickness. Propionic acid was then added to the diet as a mold inhibitor. Wet cotton was placed along the ring of the rearing plate to retain moisture in the diet. Each rearing plate receives 0.5 gm of fresh blowfly eggs. In case of natural diet, the same amount of blowfly eggs (0.5 gm) was added in the rearing plate containing 1kg headless Tilapia fish.

Table 1: Composition of different artificial diets for *Lucilia cuprina*

Ingredients	Quantity			
	Diet-A	**Diet-B**	**Diet-C**	**Diet-D**
Wheat bran (g)	200	200	200	200
Whole milk powder (g)	50	50	50	-
Chicken egg (number)	3	3	-	3
Bovine blood (ml)	150	-	150	150
Water (ml)	150	150	150	150
Propionic acid (ml)	0.5ml	0.5 ml	0.5 ml	0.5ml

2.4 Experimental Procedures

All experiments were carried-out at 25±5 ^{0}C and 70±10% R.H. under a photoperiod of 12L-12D. Daily observation of the different life cycle stages was performed in order to evaluate the performance of artificial and natural diet. The rearing plates containing larval diets and blow fly eggs were placed into plastic bowls filled with sterile saw dust. Larvae hatched from the eggs passed from one instar to another. When the larvae reached the 4th instar they stopped feeding and dropped into the saw dust where pupation occurred and corresponding counting was carried out. Durations of developmental stages in different larval diets were recorded. Mature 4th instar larvae (drop-out larvae) and 3rd day old pupae of each diet were weighted. F1 adult emergence rate, adult longevity and fecundity were recorded and compared among the different diets tested. Fecundity of F1 females were performed by placing females individually in small cages where egg laying occurred. Eggs, laid by each female until its death, were counted daily. Mean generation time was recorded as time from the oviposition until female offspring were mature enough to oviposit their own eggs. Life cycle parameters on each diet were observed over three consecutive generations. One-way analyses of variance (ANOVA) subsequently the Tukey HSD test at $P<0.05$ was used for statistical analysis of mean values.

3. Results and Discussion

In the present study growth and developmental parameters of blowfly reared on antural and artificial diets were recorded and compared. The larval and pupal periods lasted almost the same time with all the diets tested (Table 2). The larval and pupal periods on artificial diets were slightly longer than those on natural diet but the differences of durations between natural and artificial diets were not statistically significant (Table 2, $p>0.05$). Egg to pupa transformation rate (63-68%) and adult emergence rate (97-98%) were found almost similar when larvae reared on natural diet and those reared on artificial diets (Table 2). These results suggest that the nutritional quality of artificial diets is comparable with those of natural diet. In another blow fly species, *L sericata*, larval and pupal mortality as well as pupal durations were not significantly different when larvae of *L sericata* reared on beef liver and on different artificial diets [12].

Table 2: Life history parameters of *L. cuprina* reared on Tilapia fish and artificial diets.

	Tilapia	Diet-A	Diet-B	Diet-C	Diet-D
Larval period (Day)	3.8±0[a]	4.5± 0.875[a]	3.95±0.29[a]	4.5± 0.87[a]	4.5±0.87[a]
Pupal period (Day)	4.99±1.15[a]	4.99±1.45[a]	5.66±1.45[a]	5.66±1.45[a]	4.11±0.19[a]
Pupariation rate (%)	64.06± 2.19[a]	68.61± 5.55[a]	62.64± 10.40[a]	65.49± 11.53[a]	63.02± 4.82[a]
Eemergence rate (%)	98.33± 0.58[a]	98.67± 0.57[a]	97.33± 2.08[a]	97±1.0[a]	98.43± 0.57[a]
Mean generation time(Day)	13.72[a]	14.70[a]	15.26[a]	15.31[a]	14.4[a]
Larval weight (mg)	34.58 ±1.80[b]	36.57± 3.75[a]	29.11± 8.07[e]	34.22± 4.02[b]	30.99± 3.86 [de]
Pupal weight (mg)	26.28± 3.29[b]	28.45± 0.61[a]	24.12± 0.84[c]	25.29± 0.41[bc]	24.35± 2.89[c]
Fecundity (number of eggs laid per female)	709.89±79.72[ab]	807±122.75[a]	607.67±14.51[bc]	593.44±44.37[bc]	597.33±17.85[bc]
Adult longevity (Day)	18.03± 0.56[b]	22.69± 1.49[a]	22.62± 1.31[a]	22.26±1.31[a]	22.67± 0.58[a]

Values are mean ± SD.

Values followed by same letter in each row were not significantly different at the 5% level

In the present study, the mean generation time, the time from the oviposition until female offspring were mature enough to oviposite their own eggs, were 13.72, 14.70, 15.26, 15.31 and 14.4 days when larvae reared on natural diet, Diet-A, B, C and D respectively (Table 2). Although the development of *L cuprina* on all artificial diets was a bit slower than on natural diet but the differences between natural and artificial diets were not statistically significant ($p>0.05$). In another experiment, the mean generation time of *L. cuprina* was reported as 19.8 days [14]. Geographic variation of the blow fly strain and different rearing conditions of the two experiments might have contributed to the differences of the generation time.

Although the larval and pupal mortality and developmental durations were not significantly different when larvae reared on natural and on artificial diets but the quality as expressed by larval and puapl weights as well as F1 adult longevity and fecundity were found significantly different among the diets (Table 2). Larval and pupal weights were found heaviest (Larvae 36.57±3.75, Pupa 29.11±8.07 mg) in Diet-A and lightest (Larvae 28.45±0.61, Pupa 24.12±0.84 mg) in Diet-B. Larval and pupal weights in natural diets were 34.58±1.80 mg and 26.28±3.29 mg respectively. The larval and pupal weights in Diet-A were significantly heaviest ($p>0.5$) than those in natural diet and other artificial diets (Table 2). Growth and development in Diet-B, C and D were not better than those in natural diet and in Diet-A. Diet-A consists of whole milk powder, chicken egg and bovine blood, while one of these ingredients was omitted in diets B (bovine blood), C (Chicken egg) and D (whole milk powder). Several studies on blow fly's growth factor requirements concluded that protein, cholesterol, vitamin B and albumin are essential growth factor for potential blow fly development [15, 16, 17, 18]. In our experiment we found best quality larvae and pupae from Diet-A which consists of eggs, bovine blood and whole milk powder. All these three ingredients are rich source of protein, cholesterol, vitamin B and albumin. These results suggest that *Lucilia cuprina* and in other blow flies protein, cholesterol, vitamin B and albumin are essential growth regulator.

The various artificial diets tested did not led to any significant differences in F1 adult's longevity, but the longevity differed considerably when compared with the control (Table 2). Adults emerged from heavy pupa reared on Diet-A lived longer life and laid significantly highest number of eggs per female than those from lighter ones (Table 2). In conclusion, among the diets tested, growth and development parameters of *L. cuprina* showed significantly high values with Diet-A. Thus Diet-A can be used for mass rearing of blowfly larvae for several basic and applied studies.

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