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# EVALUATION OF SOME DIETARY COMPONENTS ON CERTAIN BIOLOGICAL ASPECTS AND DIGESTIVE EFFICIENCY OF ERI SILKWORM. I. BIOLOGICAL STUDIES [66]

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## ABSTRACT

The different components of artificial diet offered the late larval instars of the Eri silk worm, *Philosamia ricini* (Boisd.) were evaluated to select the most appropriate components, which gave normal biological values, high amounts of natural silk and the maximum fecundity of mated female moths. The highest larval and pupal weights, the heaviest silk gland, the maximum weights of fresh and dry cocoons and cocoon shell as well as the high cocoon ratio were recorded after rearing the larvae on the artificial diet suggested by **Kaleemurrahman and Gouri (1982)** after replacing tapioca leaf powder with castor leaf powder and adding sucrose, ascorbic acid (D5), followed by the same diet after replacing soybean powder with bran powder (D1), and the same diet after adding casein, sucrose, and ascorbic acid (D6). Therefore the first diet (D5) was recommended as a simple artificial diet for rearing this beneficial insect to obtain the highest fecundity and silk production.


**Key words:** Eri silk worm, *Philosamia ricini*, Artificial diets, Biological aspects, Silk production, Fecundity.

## INTRODUCTION

The Eri silk worm, *Philosamia ricini* Boisd. ranks next to the mulberry silk worm, *Bombyx mori* L. for producing natural silk. It produces a very white silk known as eri silk; the cocoons are not reeled but spin as yarn.

According to **Martchenko (1960); Kafian (1969); Sidhu *et al* (1969) and El-Shaarawy *et al* (1975 a & b)**, the weight of cocoons and the raw silk yield produced by both *B. mori* and *P. ricini* depend upon the chemical composition of the mulberry leaves offered as food to the former species, and castor bean leaves offered the later species.

The aim of the present work is to evaluate the different components in the artificial diet on certain biological aspects and silk produced by the Eri silk worm for the purpose of finding out the most appropiate components for rearing this insect in the laboratory.

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## MATERIAL AND METHODS

The Eri silkworm cocoons, *Philosamia ricini* Boisd. were obtained from the institute of Genetic Engineering, Agricultural Research Center (ARC), Ministry of Agriculture, Dokki, Cairo.

The artificial diet recommended by **Kaleemurrahman and Gouri (1982)** which is referred to as D2 in Table (1) was chosen as control artificial diet after replacing tapioca leaf powder with castor leaf powder. According to **Hosny *et al* (1986)**, castor leaf powder was the most favourable source to be added to the semi-synthetic diet for rearing *P. ricini* larvae. This control diet was selected to study the effect of replacement of dietary component (D1) or addition of certain components (D3, D4, D5 and D6) on certain biological aspects, fecundity and silk production by feeding the late larval instars (fourth and fifth) on the different types of artificial diets. Such late larval instars are considered a good indicator for the following stages (**Gomaa *et al* 1977**).

The different types of tested artificial diets were prepared by weighing the exact weights of different ingredients of each diet (Table, 1). Each diet was prepared as follows:

The half amount of distilled water was heated up to 90°C. Agar agar was solved in the hot water by stirring in one direction and then cooled to 60°C. Glucose or sucrose, whenever used, was added, but castor bean leaves and soybean were dried on 60 °C and grinded before adding them. All other ingredients, except vitamin C and Chloramphenicol (antibiotics), were mixed together by using an electric whipper.

Table 1 Components of artificial diets for rearing *P. ricini* fourth and fifth instar larvae

| Components of artificial diets | Weight of diet components (g) | | | | | |
|---|---|---|---|---|---|---|
| | D1 | D2* Control | D3 | D4 | D5 | D6 |
| Dry castor bean leaf powder | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| Soybean powder | - | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Bran | 1 | - | - | - | - | - |
| Casein | - | - | - | - | - | 1.0 |
| Sucrose | - | - | - | 1.0 | 1.0 | 1.0 |
| Fructose | - | - | 1.0 | - | - | - |
| Ascorbic acid | - | - | - | - | 0.090 | 0.090 |
| Chloramphenicol | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 |
| Agar-agar | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Water | 25 | 25 | 25 | 25 | 25 | 25 |

*According to **Kaleemurrahman and Gouri (1982)**

The diet was packed into polypropylene bags, put into a sterilized stainless steel tray and cooked in an autoclave at 100°C for 50 minutes. Steaming of the diet mixture is a process in order to reduce the number of microorganisms present in the food, to affect softening and slight decomposition, and to improve the physical properties of the diet (**Miyoshi, 1975**). According to **Genova (1994)**, sterilization of an artificial diet was necessary for 20-30 minutes at 100°C, then poured into petri dishes and stored at 5°C. In the present work the diet was cooled under room temperature and the other components (Vitamin C and antibiotics) were added to the remaining quantity of water and the whole diet components were mixed together by using an electric whipper and kept at 5°C. According to **Eid *et al* (1989)** an addition of low doses of antibiotics to larval food increased the activity of transaminases in the silk glands of *P. ricini*, cocoon, silk and silk gland weights. Therefore, it is necessary to add an antibiotic compound to the larval diet. This diet was valid for larval feeding for 4 days.

Larvae of *P. ricini* were reared on fresh and clean castor bean leaves during the first three successive instars. At the beginning of the fourth instar, larvae were offered semi-synthetic diet till pupation and spinning the cocoons. Generally, the experimental larvae were reared in the laboratory under controlled conditions of 25 ± 2 °C, 70 ± 3 % R.H. and the daily photoperiodic regime of 16: 8, L: D. Larvae were reared in plastic dishes each measured 15 × 20 × 5 cm. (width, length and height, respectively) covered with muslin. The number of larvae per dish was 5 individuals and replicated 10 times for each instar and diet.

The durations of the fourth and fifth instar larvae as well as the mortality percentages of larvae reared on all diet types were recorded. Five larvae from each treatment were dissected and the silk glands were picked up, weighed and recorded. After pupation, the pupal durations and weights were recorded. Fresh and dry cocoons as well as cocoon shell were also recorded. In this case, thirty fresh cocoons and after their dryness for each treatment were weighed separately and the cocoon shell ratio was calculated according to the formulation of **Krishnaswami *et al* (1972)** as follows:

$$\textit{Cocoon shell ratio} = \frac{\textit{Weight of cocoon shell}}{\textit{Weight of fresh cocoon}} \; X \; 100$$

After emergence, the number of eggs laid by each mated female moth resulted from larvae fed on different diet types were recorded. The obtained data were statistically analysed.

## RESULTS AND DISCUSSION

### 1. Durations of different developmental stages

#### 1.1. Fourth and Fifth larval instars

As shown in Table (2), the mean durations of the fourth and fifth instar larvae of *P. ricini* fed on fresh castor bean leaves (natural food) were 10.12 and 13.20 days, respectively.

The mean durations of larvae fed on control artificial diet {D2 of **Kaleemurrahman and Gouri (1982)**} were 11.60 and 10.20 days for the fourth and fifth instars, respectively. These larval durations did not differ from those of fourth and fifth instar larvae fed on artificial diets D1, and D5.

Table 2. Mean durations of the fourth and fifth instar larvae of *P. ricini* fed on different types of diets and the accumulative mortality

| Type of diets | Duration (days) of the following larval instars | | | Corrected accumulated mortality |
|---|---|---|---|---|
| | Fourth | Fifth | Total | (%) |
| Fresh castor leaves | 10.12 ± 1.36 | 13.20 ± 1.59 | 23.20 ± 1.46 | - |
| D1 | 11.60 ± 1.45 | 12.00 ± 1.55 | 22.60 ± 1.50 | 6.93 |
| D2 (Control) | 11.60 ±1.32 | 10.20 ± 1.68 | 21.80 ± 2.25 | 7.90 |
| D3 | 9.80 ± 1.53 | 14.80 ± 1.71 | 24.60 ± 1.62 | 8.55 |
| D4 | 9.60 ± 1.61 | 14.00 ± 2.01 | 23.60 ± 1.81 | 8.61 |
| D5 | 11.00 ± 1.74 | 10.20 ± 1.98 | 21.20 ± 1.86 | 7.70 |
| D6 | 9.40 ± 1.51 | 13.20 ± 1.73 | 22.60 ± 1.62 | 5.21 |
| Mean of artificial diet | 10.33 ±1.78 | 12.40 ± 1.89 | 22.73 ± 1.78 | 7.32 |
| L.S.D. at 0.05 | 2.22 | 3.09 | 2.98 | 2.81 |

On the other hand, the shortest durations of fourth instar larvae and the longest ones of fifth instar larvae were obtained after feeding them on D3, D4 and D6. The L.S.D. values for both instars (2.22 and 3.09 days) emphasize the obtained results.

### 1.2. Pupal stage

The data given in Table (4) clearly show that the mean pupal duration was 15.50 days after feeding the larvae on fresh castor bean leaves. Feeding *P. ricini* late instar larvae of *P. ricini* on artificial diets prolonged the duration of the resulted pupae, being 22.21 days as an average. By applying the L.S.D. value (1.33 day) the pupal durations could be arranged into the following three ascending groups according to the type of artificial diets offered larvae:

First group: contained larvae fed on control diet or D2 (19.56 days).

Second group: Included larvae fed on D1, D5, and D6 (22.50, 22.07 and 21.22 days, respectively).

Third group: composed of larvae fed on D3 and D4 (24, 25 and 23.14 days, respectively).

### 1.3. Adult stage

The mean longevities of *P. ricini* male and female moths fed as larvae on castor bean leaves were 9.82 and 8.14 days, respectively. Female longevity was shorter than male. The difference between both longevities proved to be statistically significant (t = 0.7879). When larvae were fed on different types of artificial diets the life span of the resulted moths did not significantly differ from that of moths fed on fresh leaves. This was common for larvae fed on D1, D2, D4, D5, and D6 i.e., mean longevities of 10.14, 9.96, 10.02, 9.84 and 9.22 days for male moth and 8.81, 8.78, 8.45, 8.14 and 7.82 days for female moth were recorded after feeding the late larval instars on the preceding artificial diets, respectively. The longest life span of moths of both sexes was obtained when larvae were supplied with D1 and D3 (Table 6).

### 2. Mortality of larvae

The percentages of larval mortality after feeding the late instars of *P. ricini* larvae on different types of artificial diets were recorded and the obtained values were corrected and tabulated in Table (2). The data clearly show that no significant difference was noticed between larval mortalities after feeding them on D1, D2, D5, and D6, being 6.93, 7.90, 7.70 and 5.21 %, respectively. The highest mortality was, however, obtained after feeding the larvae on D3 and D4, where death occurred among 8.55 and 8.61% of larvae, respectively.

### 3. Weights of different developmental stages

### 3.1. Full grown larvae

As show in Table (3) the full-grown larva is considered as a good indicator for the following developmental stages of the experimental insect. The mean weight of mature larva fed on castor bean leaves was 5.03 g.

Table 3. Mean weights of silk gland of full-grown *P. ricini* larvae fed on different types of diets during the late instars

| Type of diets | Weights (g) | |
|---|---|---|
| | Full grown larvae | Silk gland |
| Fresh leaves | 5.0275 ± 0.4224 | 1.4492 ± 0.2881 |
| D1 | 4.7532 ± 0.4621 | 1.3995 ± 0.2995 |
| D2 (Control) | 4.0268 ± 0.3513 | 1.0107 ± 0.2743 |
| D3 | 3.5435 ± 0.3948 | 0.6431 ± 0.2594 |
| D4 | 3.7412 ± 0.4012 | 0.8506 ± 0.3012 |
| D5 | 4.8326 ± 0.3336 | 1.4142 ± 0.2841 |
| D6 | 4.5824 ± 0.4123 | 1.2058 ± 0.3123 |
| Mean of artificial diet | 4.2466 ± 0.3926 | 1.0873 ± 0.2885 |
| L.S.D. at 0.05 | 0.4204 | 0.2112 |

Feeding the late instar larvae of different types of artificial diets decreased the larval weight at different degrees depending on the type of diet offered them during the fourth and fifth instars. By applying the L.S.D value (0.4204 g), the larval weight could be arranged into the following three descending groups:

First group: Larvae offered D1, D5, and D6 (4.75, 4.83 and 4.58g, respectively.)

Second group: Larvae supplied with D2 (Control) 4.03 g.

Third group: Larvae fed on D3 and D4 (3.54 and 3.74 g, respectively.)

Generally, the maximum weight of full-grown larvae was obtained after feeding them on D5 followed by those fed on D1 and D6.

### 3.2. Pupae of both sexes

Female pupae were always heavier than males and their mean weights were 1.89 and 2.30 g for male and female ones, respectively after feeding them in the late larval instars on fresh castor bean leaves. The pupal weights of *P. ricini* fed as late instar larvae on different types of diets are given in Table (4).

Rearing the larvae on different types of artificial diets decreased the pupal weights of both sexes. However, this decrease was not significant in case of D1, D5 and D6 for both sexes. The lowest pupal weights were obtained after feeding the larvae on D3 and D4. The L.S.D. values emphasize the obtained results.

## 4. Silk production

### 4.1. Weight of silk gland

According **El-Shaarawy *et al* (1975 a & b)**, the weight of silk glands picked up from the full grown larvae of the Eri silk worms is positively correlated with the quantity of silk produced by these larvae. Table (4) indicates the mean weights of silk glands of larvae fed on different types of food. Feeding the larvae on fresh castor been leaves gave the heaviest silk gland (1.45 g). However, artificial diets caused an obvious decrease in silk gland weights at different degrees.

Table 4. Mean durations and weights of *P. ricini* pupae (g) fed as late instar larvae on different types of diets

| Type of diets | Mean duration of pupal stage (Days) | Mean weights (g) of Male pupa | Mean weights (g) of Female pupa |
|---|---|---|---|
| Fresh leaves | 15.50 ± 1.89 | 1.8856 ± 0.3214 | 2.2995 ± 0.4541 |
| D1 | 22.50 ± 3.68 | 1.8140 ± 0.3512 | 2.2672 ± 0.4124 |
| D2 (Control) | 19.56 ± 2.79 | 1.7452 ± 0.2914 | 2.1468 ± 0.5121 |
| D3 | 24.25 ± 3.15 | 1.3458 ± 0.3112 | 1.9419 ± 0.3992 |
| D4 | 23.14 ± 4.04 | 1.5221 ± 0.4006 | 2.0025 ± 0.2812 |
| D5 | 22.07 ± 3.72 | 1.8386 ± 0.3421 | 2.2764 ± 0.3462 |
| D6 | 21.22 ± 2.89 | 1.8002 ± 0.3356 | 2.2423 ± 0.4246 |
| Mean of artificial diet | 22.12 ± 3.38 | 1.67765 ± 0.3387 | 2.1462 ± 0.3960 |
| L.S.D. at 0.05 | 1.33 | 0.0452 | 0.0367 |

By applying the L.S.D. value (0.2112 g.) the weights of silk glands could be arranged into the following three descending groups according to the type of artificial diet offered to the late larval instars:

First group: larvae fed on D1, D5 and D6 (1.40, 1.41, and 1.21g. respectively).

Second group: Larvae supplied with D2 (Control), (1.01 g.)

Third group: Larvae reared on D3 and D4 (0.64 and 0.85g., respectively).

### 4.2. Weight of cocoons

The mean weight of fresh cocoon resulted from larvae fed on fresh castor bean leaves was 2.6452 g. Feeding the larvae on different types of artificial diets decreased the fresh cocoon weights to an average of 2.4156 g. The rate of decrement proved to be statistically significant. The high weight of cocoon was recorded when larvae were supplied with D5, D1 and D6 (2.5261, 2.4913 and 2.4846 g., respectively), with no significant difference between these means.

The same trend could be applied for the weight of dry cocoons. By applying the L.S.D. value (0.0211g), the weights of dry cocoons could be arranged into the following four descending groups according to the type of artificial diets offered the late larval instars:

First group: larvae fed on fresh leaves (902.3 mg.).

Second group: larvae fed on D5, D1 and D6 (872.3, 862.2 and 855.1 mg, respectively).

Third group: Larvae supplied with control diet, D2 (743.5 mg).

Fourth group: Larvae offered D4 and D3 (701.8 and 678.9 mg, respectively).

As in cases of fresh and dry cocoons, the weight of cocoon shell greatly affected by the type of food offered *P. ricini* larvae. As shown in Table (5), the heaviest cocoon shell (335.6 mg) was obtained when larvae were fed on fresh leaves. This weight was significantly higher than those obtained by larvae fed on different types of artificial diets. The later could be divided into the following three descending categories (L.S.D.= 20.2 mg):

First group: larvae fed on D5, D1 and D6 (313.7, 304.1 and 298.4 mg, respectively).

Second group: larvae fed on D2 (Control), and D4 (255.2 and 239.9 mg, respectively).

Third group: Larvae supplied with D3 (230.1 mg).

To ensure the effect of diet type on the quantity of natural silk produced by *P. ricini* larvae, the cocoon shell ratios in all cases were calculated and recorded in Table (5). As shown in this Table, the highest ratios were obtained when larvae were fed on natural food, D5, D1 and D6, being 12.69, 12.42, 12.21 and 12.00 %, respectively.

### 5. Fecundity of mated female moths

As shown in Table (6), the average number of eggs laid by mated female moth of *P. ricini* fed as larvae on fresh castor bean leaves was 289.74. When larvae were fed on D5, D1 and D6 the numbers of eggs produced by the resulted mated females were 291.03, 270.36 and 261.14 eggs/ Female, respectively, with no significant difference between them and that of females fed as larvae on castor leaves. The L.S.D. value (31.01 eggs) emphasizes the obtained results.

Table 5. Cocoon shell ratios and mean weights of fresh and dry cocoons and cocoon cortex (g) produced by *P. ricini* larvae fed during the late instars on different types of diets

| Type of diets | Mean weights (g) of | | | Cocoon shell ratio (%) |
|---|---|---|---|---|
| | Fresh cocoon | Dry cocoon | Cocoon shell | |
| Fresh leaves | 2.6452 ±0.43 | 0.9023 ± 0.1992 | 0.3356 ± 0.0869 | 12.69 |
| D1 | 2.4913 ±0.52 | 0.8622 ± 0.2612 | 0. 3041 ±0.0821 | 12.21 |
| D2 (Control) | 2.3622 ±0.47 | 0.7435 ± 0.1781 | 0.2552 ± 0.0783 | 10.80 |
| D3 | 2.3018 ±0.62 | 0.6789 ± 0.2212 | 0.2301 ± 0.0622 | 10.00 |
| D4 | 2.3275 ±0.41 | 0.7018 ± 0.1934 | 0.2399 ± 0.0771 | 10.31 |
| D5 | 2.5261± 0.36 | 0.8723 ± 0.2023 | 0.3137 ± 0.0599 | 12.42 |
| D6 | 2.4846 ±0.54 | 0. 8551±0.1853 | 0.2984 ± 0.0643 | 12.00 |
| Mean of artificial diet | 2.4156 ±0.49 | 0.7856 ± 0.2069 | 0.2736 ± 0.0707 | 11.29 |
| L.S.D. at 0.05 | 0.0522 | 0.0211 | 0.0202 | - |

Table 6: Life span of moths of both sexes and fecundity of *P. ricini* mated females fed as late instar larvae on different types of diets

| Type of diets | Life span of moth (days) | | No. of eggs / Mated female |
|---|---|---|---|
| | Male | Female | |
| Fresh leaves | 9.82 ± 1.73 | 8.14 ± 1.15 | 289.74 ± 23.25 |
| D1 | 10.14 ± 2.45 | 8.81 ± 1.85 | 270.36 ± 17.94 |
| D2 (Control) | 9.96 ± 1.98 | 8.78 ± 1.64 | 255.15 ± 26.18 |
| D3 | 12.05 ± 3.01 | 9.89 ± 1.41 | 222.34 ± 19.76 |
| D4 | 10.02 ± 2.49 | 8.45 ± 1.55 | 239.77 ± 24.11 |
| D5 | 9.84 ± 3.15 | 8.14 ± 1.42 | 291.03 ± 25.17 |
| D6 | 9.22 ± 2.26 | 7.82 ± 0.99 | 261.14 ± 21.44 |
| Mean of artificial diet | 10.21 ± 2.56 | 8.65 ± 1.48 | 256.63 ± 22.43 |
| L.S.D. at 0.05 | 0.94 | 1.03 | 31.01 |

From the aforementioned results it could be concluded that the artificial diet D5 followed by D6 and D1 are considered the most appropriate diets for rearing *P. ricini* larvae. Such diets produced the high amount of natural silk and fecundity. The obtained values are relatively near those obtained by insects fed as larvae on fresh leaves. Therefore, these artificial diets are recommended for rearing this insect species in places free from castor bean plants or at late larval instar (fourth and fifth) from the economic point of view to reduce the production cost of silk in case of mass rearing.

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# تقييم بعض المكونات الغذائية على بعض االنواحى البيولوجية و كفاءة الهضم فى دودة حرير الخروع

## ١- الدراسات البيولوجية

## [٦٦]

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تهدف هذه الدراسة إلى تقييم بعض مكونات البيئة الغذائية المصنعة لدودة حرير الخروع على بعض النواحى البيولوجية لها بغرض التوصل الى انسب بيئة غذائية يمكن ان تربى عليها الحشرة ،حيث درس كفاءة تغذية ديدان حرير الخروع فى العمرين الرابع و الخامس على اعتبار أن العمر اليرقى الأخير يعتبر دليل قوى على حيوية و نشاط الحشرة فى الأطوار التالية ، وقد تم تكوين خمسة بيئات غذائية مصنعة بالإضافة إلى البيئة المصنعة المأخوذة من المراجع كمقارنة و كذلك تغذية اليرقات على غذاء طبيعى (ورق نبات الخروع).

وقد أوضحت النتائج المتحصل عليها أن أعلى وزن لليرقات والعذارى وأثقل وزن لغدة الحرير وأعلى وزن لشرانق سواء كانت طازجة أو جافة ، قد تم تسجيله فى حالة اليرقات المرباة على البيئة الصناعية التى اقترحها **كليم الرحمن وجورى (١٩٨٢)** بعد استبدال أوراق التابيوكا الجافة و المطحونة بأوراق الخروع الجافة والمطحونة وإضافة سكروز وحمض الاسكوربيك اسيد (D5) ، يليها نفس البيئة مع اضافة فول الصويا و الردة (D1) ، ثم يليها البيئة التى تحتوى على كازين وسكروز وحمض الاسكوربيك.

وتوصى الدراسة باستخدام البيئة المصنعة (D5) فى تغذية الأعمار المتقدمة ليرقات دودة الخروع للحصول على أعلى كفاءة تناسلية وانتاج الحرير.

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