

Feeding on Lettuce and Mulberry Leaves Increases Silk Productivity and Biometrics of the Mulberry Silkworm *Bombyx Mori*

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Abstract

During the present study, two different host plants (lettuce and mulberry) were used for feeding 5th instar larvae of *Bombyx mori*. In the case of treatment (T₀), *B. mori* 5th instar larvae were daily fed on 4 meals of mulberry leaves (control treatment), while in treatment (T₁), larvae were fed on 10 meals of lettuce leaves: 4 meals at the 1st day, 3 meals at the 2nd day, 2 meals at the 3rd day and a meal at the 4th day; the rest of the fourth daily meals were mulberry leaves. (T₂), larvae were fed on 6 meals of lettuce leaves: 3 meals at the 1st day, 2 meals at the 2nd day and a meal at the 3rd day; the rest of the fourth daily meals were mulberry leaves. (T₃) larvae were fed on 3 meals of lettuce leaves: 2 meals at the 1st day and a meal at the 2nd day; the rest of the fourth daily meals were mulberry leaves. (T₄), larvae were fed on a meal of lettuce leaves at the 1st day: the rest of the fourth daily meals were mulberry leaves. (T₅), larvae were daily fed on a meal of lettuce leaves and 3 meals of mulberry leaves. (T₆), larvae were daily fed on 4 meals of lettuce leaves. The present results revealed that T₅ significantly increased the larval weight as compared with the other treatments (T₀, T₁, T₂, T₃, and T₄). While T₂ significantly increased the mean of pupal, cocoon, cocoon shell and silk gland weights by about 46.2 %, 44.6 %, 37.6 % and 55.7% respectively as compared with the T₀. The effect of feeding of *B. mori* 5th instar larvae on lettuce leaves on total and different haemocyte counts of *B. mori* last larval instar was also determined.

Keywords: Silkworm; *Lactuca Sativa*; Mulberry Leaves; Economic Parameters

Introduction

Silkworm, *Bombyx mori* L., as a monophagous insect get all required nutrients for its growth and development from mulberry leaves. In Egypt the climatical conditions and the efficiency of mulberry leaves allow only the rearing of monovoltine races of *Bombyx mori* L. The most important objective in the sericulture problems is to find out applicable methods to increase silk production. Thus, we have to continue our search to achieve an increase in the productivity of the silk yield of the mulberry silkworm. Nutrition plays a major role in sericulture field. It improves the silkworm growth, development, and feed consumption of silkworm which improving the silk yield.

Paul, et al. [1] noticed that mulberry leaf quality has direct effect on larval food consumption and growth.

Mulberry leaves varieties have significantly effect on shell and pupal weight. Perhaps, this could be because of more repeated contact with food, the time for conversion of assailants into body tissue is reduced such that more of the digested food is stored to be silk fibers. This is supported by the explanation of silk production in the silkworm by Narasimhana, et al. [2].

Adeduntan, et al. [3] found that feeding silkworm on varieties of mulberry leaves affected significantly on mortality of larva and cocooning, while its was not significant effect on good cocoon, flimsy cocoon, stained cocoons, cocoon shell weight and pupa weight, oviposition, egg fertility and hatchability.

Nasreen, et al. [4] fed the third instar larvae of *B. mori* on leaves of peepal (*Ficus religiosa*) and the larvae produced good cocoons as compared to those fed on mulberry.

The effect of some mulberry varieties on silk worms growth and silk production were studied in India [5].

Muthunagai, et al. [6] observed that feeding the 3rd larval instar of *B. mori* with *Ricinus communis* leaves shown wriggling movement and moved towards the corners of the tray. Also, Sign of annoyance and weakness was noticed.

Lettuce (*Lactuca sativa*) was first cultivated in ancient Egypt for the production of oil from its seeds. It was considered an important treatment for numerous diseases. Its nutritional contents were reported to contain

high quantities of vitamins, riboflavin, antioxidants and minerals [7,8].

Most of the research was concerned on rearing mulberry leaves. A few number of noticeable work was done on alternative hosts that could be used to get better silk quality and quantity. The main point of the present study was to find out new host plants for silkworm (*Bombyx mori* L.) larvae to increase the silk production. In the present work we chose lettuce leaves because it is abundant in Egypt and cheap in price.

Materials and Methods

Insect Material

The PM×CSR2 hybrid of the mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae), was procured from Sericulture and Agriculture Experiment Station, Vratza, Bulgaria. The silkworm larvae were reared according to Krishnaswami, et al.

Experimental Design

The present study was carried out on the 5th instar larvae of the mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae). It was designed to examine the effect of feeding regime of *B. mori* 5th instar larvae on lettuce and mulberry leaves. Freshly ecdysed *B. mori* 5th instar larvae were used for all experimental treatments. The experimented larvae were separated into seven groups. Each group contained three replicates, each replicate consists of 50 larvae. Experimental treatments were:

Treatment-0 (T₀):

Daily, larvae were fed on 4 meals of mulberry leaves.

Treatment-1 (T₁):

Day 1, larvae were fed on 4 meals of lettuce leaves.

Day 2, larvae were fed on 3 meals of lettuce leaves and 1 meal of mulberry leaves.

Day 3, larvae were fed on 2 meals of lettuce leaves and 2 meals of mulberry leaves.

Day 4, larvae were fed on 1 meal of lettuce leaves and 3 meals of mulberry leaves.

Day 5 to the end of last larval instar, larvae were fed on 4 meals of mulberry leaves.

Treatment-2 (T₂):

Day 1, larvae were fed on 3 meals of lettuce leaves and 1 meal of mulberry leaves.

Day 2, larvae were fed on 2 meals of lettuce leaves and 2 meals of mulberry leaves.

Day 3, larvae were fed on 1 meal of lettuce leaves and 3 meals of mulberry leaves.

Day 4 to the end of last larval instar, larvae were fed on 4 meals of mulberry leaves.

Treatment-3 (T₃):

Day 1, larvae were fed on 2 meals of lettuce leaves and 2 meals of mulberry leaves.

Day 2, larvae were fed on 1 meal of lettuce leaves and 3 meals of mulberry leaves.

Day 3, to the end of last larval instar, larvae were fed on 4 meals of mulberry leaves.

Treatment-4 (T₄):

Day 1, larvae were fed on 1 meal of lettuce leaves and 3 meals of mulberry leaves.

Day 2, to the end of last larval instar, larvae were fed on 4 meals of mulberry leaves.

Treatment-5 (T₅):

Daily, larvae were fed on 1 meal of lettuce leaves and 3 meals of mulberry leaves.

Treatment-6 (T₆):

Daily, larvae were fed on 4 meals of lettuce leaves.

Mortality was recorded daily for all the experimental groups. Biometrics such as larval weight, larval duration and cocoon parameters (cocoon weight, shell weight and pupal weight) were recorded.

Haemolymph Preparation

The haemolymph samples were taken from 5th instar larvae at mature larval stage. The total haemocyte count was calculated according to Jones, et al. [9]. To calculate the different haemocyte count, 100 cells were identified to their typical haemocyte type after staining a smear of haemolymph with Wright stain [10].

Statistical Analysis

All results were expressed as mean \pm standard errors. Statistical analyses were performed with one-way analysis of variance followed by unpaired two-tailed Student's *t*-test. A *p*-value < 0.05 was considered to be significant.

Results

Effect on Some Biological Parameters

The Analysis of Variance has indicated high significant differences (at *p*=0.05) between the treatment values in

respect of larval weight, pupal weight, cocoon weight, shell weight and, silk gland weight are shown in (Figure 1). *B. mori* larvae which fed on lettuce and mulberry leaves significantly increased the larval weight, cocoon weight, pupal weight, shell weight and shell ratio.

Feeding silkworms with lettuce leaves larvae (T₅) gave highest larval weight about 3.623 \pm 0.01g followed by (T₆) (larvae fed with lettuce leaves only) about 3.559 \pm 0.03g.

The average of cocoon weights were found to be highest at T₂ (1.362 \pm 0.03 g) followed by T₃ (1.276 \pm 0.03 g) then T₁ (1.269 \pm 0.06 g). No significant differences were observed between T₄ (1.193 \pm 0.01 g) and T₅ (1.135 \pm 0.01 g) while the mean weight T₀ was 0.942 \pm 0.02 and no cocoons were obtained at T₆.

As shown in Figure1 Significant differences in the mean weights of *B. mori* pupae were recorded. The mean pupal weights were 0.764 \pm 0.02, 1.040 \pm 0.01, 1.117 \pm 0.05, 1.044 \pm 0.04, 0.973 \pm 0.02 and 0.936 \pm 0.01 g for T₀, T₁, T₂, T₃, T₄ and T₅ respectively. No pupae were obtained at T₆. The highest means of pupal weights were observed at T₂ (by about 46.2% more than T₀) followed by T₃ (36.6%) then T₁ (36.1%).

Furthermore, the average of cocoon weights were found to be highest at T₂ (1.362 \pm 0.03 g) followed by T₃ (1.276 \pm 0.03 g) then T₁ (1.269 \pm 0.06 g). No significant differences were observed between T₄ (1.193 \pm 0.01 g) and T₅ (1.135 \pm 0.01 g) while the mean weight T₀ was 0.942 \pm 0.02 and no cocoons were obtained at T₆.

The average of silk gland weights were found to be highest at T₂ (1.247 \pm 0.03 g) followed by T₃ (1.204 \pm 0.03 g) then T₄ (1.186 \pm 0.01 g). No significant differences were observed between T₁ (1.105 \pm 0.02 g) and T₅ (1.053 \pm 0.05 g). Also, there were no significant differences between T₀ (0.801 \pm 0.04 g) and T₆ (0.773 \pm 0.01 g).

T₂ treatment significantly increased the average cocoon shell weights when compared with control T₀. The mean shell weights were 0.178 \pm 0.01, 0.229 \pm 0.01, 0.245 \pm 0.01, 0.232 \pm 0.03, 0.220 \pm 0.02 and 0.199 \pm 0.02 g for T₀, T₁, T₂, T₃, T₄ and T₅ respectively. The highest means of shell weights were observed at T₂ (by about 37.6% more than T₀) followed by T₃ (30.3%) then T₁ (28.7%) and T₄ (23.6%) Fig.,(1).

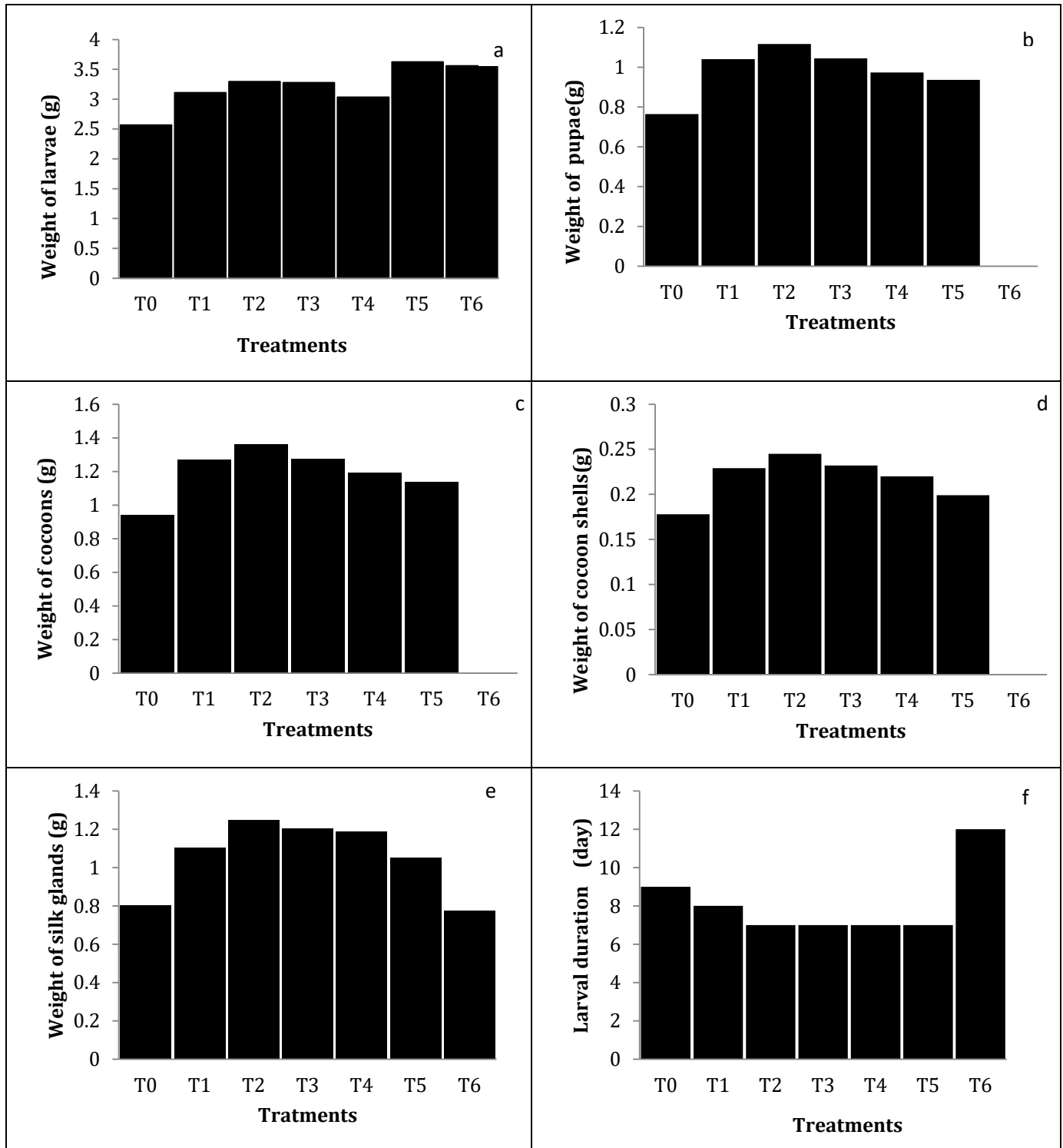


Figure 1: Effect of lettuce leaves feeding on some biological parameters.

- a. Larval weight b. Pupal weight c. Cocoon weight
d. Cocoon shell weight e. Silk gland weight f. Larval duration

Effect on Larval Duration

Feeding on lettuce leaves only (T₆) have exerted significant increase the total larval duration (12 days). The other treatments T₁, T₂, T₃, T₄ and T₅ recorded least larval duration (7 days) while T₂ was (8 days) and control was (9days) Figure 1.

Hemocyte Count

In this work the effect of nutrition on blood picture was studied, because haemocytes reflect the physiological status of larvae after feeding on lettuce leaves. Also, the toxic effect of some nutritional tested groups on haemocytes and accordingly, on the endocrine glands and hormones which affecting on the larval growth and development.

The light microscopic observations of the stained larval hemolymph revealed the presence of four morphologically distinct types of haemocytes.

Prohaemocytes (Pr), Granulocytes (Gr), Plasmatocytes (Pl) and Oenocytoides (Oe) were monitored. All tested groups clearly affected on the total and differential haemocytes counts of 5th instar larvae of *B. mori*.

With regard to lettuce and mulberry leaves feeding, a significant decrease in the percentage of the Pr was observed about -31.9 and -44.4 % after feeding as a group (T₂) and (T₃), respectively (Table 1). A significant increase was observed in the number of granulocyte after feeding as a group (T₂) and (T₃) about 31.06 and 22.65%, respectively.

Treatments	THC	Pr	Gr	Pl	Oe
Control	29600 d	4.5±0.4 ^a	25.6±0.9 ^c	23.6±1.1 ^d	9.1±0.6 ^d
T1	32000 ^c	3.7±0.3 ^b	27.5±1.2 ^b	24.3±1.2 ^c	13.6±1.2 ^b
T2	46600 ^a	3.0±0.3 ^c	35.6±1.2 ^a	28.5±0.6 ^a	15.5±0.6 ^a
T3	35250 ^b	2.5±0.4 ^d	33.1±0.7 ^a	27.2±1.6 ^a	15.2±1.6 ^a
T4	31000 ^c	2.2±0.8 ^d	32.3±1.3 ^a	25.5±1.2 ^b	14.3±1.2 ^b
T5	25750 ^d	1.6±0.2 ^e	23.6±0.7 ^b	22.5±1.2 ^c	12.5±1.2 ^c
T6	22452 ^e	1.2±0.5 ^f	21.5±0.8 ^c	20.7±0.8 ^d	11.7±0.8 ^c

Table 1: The effects of feeding *B. mori* 5th instar larvae on lettuce and mulberry leaves in total and different haemocyte count. Data are mean±SE. The statistical analyses were performed using unpaired two-tailed Student's t-test (*p<0.05).

Feeding the 5th larval instar as a group (T₂) and (T₃) increased the number of Pl about 20.7 and 15.25%, respectively. Also, the number of Oe significantly increased about 70.3 and 67.03%, respectively more than control.

On the other hand, the number of Pr, Gr and Pl markedly decreased after larvae fed as a group (T₆) about 22.8, 16.01 and 12.2, respectively less than control. While the number of Oe significantly increased about 28.5% more than control (Table 1).

Discussion

Lettuce (*Lactuca sativa*) was cultivated the first time in ancient Egypt for producing oil from its seeds. Lettuce leaves contain high amount of vitamins (A,C,K,B group), riboflavin, antioxidants (beta-carotene, Lutein-Zeaxanthin, flavonols, minerals (calcium, magnesium, iron, iodine, zinc, copper, manganese, selenium) [7,8].

Silkworm is commonly raised on leaves of different varieties of mulberry (*Morus sp.*) There had always been a

search for alternate host plants which can raise silkworm larvae to produce better silk in quality and quantity like *Morus nigr* [6]. Also, tried to feed silkworm larvae on mixed feeding. He noted that mixed feeding improved the insect growth as compared to feed on leaves of a single plant species [6].

In the present study feeding on lettuce and mulberry leaves together may have a useful effect on the growth of the silkworm *Bombyx mori* and also increased the larval weight, pupal weight, cocoon weight, shell ratio and silk gland weight by increase feeding efficacy than control whereas feeding on lettuce leaves only, the overall performance of silkworms was comparatively reduced and larvae don't develop and finally died. Many researchers have shown that the larval and cocoon characters improve by different concentration of plant extracts [5,11].

The improvement in silk productivity might be due to the useful biochemical constituents present in the lettuce leaves, better feeding and bioavailability of nutrients to the larvae through mulberry leaf. The results are in

agreement with the findings of Shahin, et al. [12] who also observed an increase in silk productivity when mulberry leaves were fortified with 0.10 %, lettuce oil. Murugesh, et al. [13] treating mulberry leaves with *T. procumbens*, *T. terrestris* and *P. hysterothorus* improved the silk production. Further, Sridevi, et al. [14] also found treated mulberry leaves with *W. somnifera* increased silk production in (6.68 cg per day) followed by *T. cordifolia* (6.21 cg per day) and *T. arjuna* (6.12 cg per day).

On the light of haemocytes study, four haemocyte types were observed in the haemolymph of *B. mori* larvae, namely Prs, Pls, Grs, and Oes. Similar results were found by Nakahara, et al. [15]. The number of haemocyte population might differ depending on the developmental stages and insect family [16]. Strand, et al. [17] reported, GR was the most abundant hemocyte type in Lepidoptera larvae. Also, It has been shown that Gr, Pl, and Oe play important roles in silkworm immunity [15]. Gr, Pl, and Oe are participating in phagocytosis, nodule formation, and PO activity, respectively. The effect of feeding larvae on lettuce leaves as a secondary host was study on haemocytes and defense responses of *B. mori* larvae. In this study, the THC, Gr, and Pl during feeding regime significantly increased, except at treatment (T5) and (T6). Reductions in THC, Prs, PLs and GRs of insects during feeding large amount of lettuce leaves as groups (T5) and (T6) treatment are reported to due to toxic effect of some botanical compound of lettuce leaf, inhibitory effects of these compounds on endocrine glands and their secretions and immunity response of tested *B. mori* larvae.

Tiwari, et al. [18] reported that ecdysone controlled the production, multiplication and differentiation of haemocytes. Moreover, the reduction in (Pr) number may be due to the inhibition of their mitotic division, their diversion to other types of haemocytes or the inhibition the response of hematopoietic organs for their production [19].

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