Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Impact of folic acid as dietary supplementation on biological and economic characteristics of mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

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ARTICLE INFO
Article History
Received: 30/10 /2022
Accepted:29/12 /2022

Keywords

Growth, development, vitamin, folic acid, silkworm and *Bombyx mori* .

Abstract

The current study demonstrates the effect of different levels of the folic acid (Vitamin B 9) fortified on silkworm larvae 4th and 5th instars *Bombyx mori* L. (Lepidoptera: Bombycidae) was evaluated by rearing (Under laboratory condition) at silkworm laboratory on Entomology Department, Faculty of Agriculture, Mansoura University. Larvae were fed on mulberry leaves supplemented with different concentrations of folic acid T_1=5, T_2=7.5, T_3=10 and T_4= 12.5mg/ml. Results showed a significantly increased in all parameters, especially concentration T_4 = 12.5mg/ml the weight of larvae (1.0 and 2.83g in T_4 compared to 0.77 and 2.55 g in control, during 4th and 5th instars), respectively. Larval food consumption recorded during 4th and 5th instars have significantly higher mean values in T4 (3.25 and 4.45g) compared to control (2.62 and 3.75g) respectively. Also, the supplementation effected cocoon weight (1.98g/ cocoon), cocoon shell weight (0.498 g) and silk ratio% (25.15%) in T_4 comparison with control (1.55 ,0.369 g and 23.81%). The study highlighted the importance of dietary supplementation with folic acid in mulberry leaves for rearing larvae of mulberry silkworm *B. mori.*

Introduction

The silkworm Bombyx mori L. (Lepidoptera: Bombycidae) is a monophagous insect and feeds only on mulberry leaves during the larval stage of its life cycle (Singhal *et al*., 1999). Silkworms should be fed with good quality mulberry leaves for the successful production of good quality of cocoons (Vijaya *et al*., 2009). Many researches have been done on the dietary supplementation of mulberry leaf feed to silkworms. This supplementation includes vitamins, such as ascorbic acid, thiamin, miacin, multi-vitamins and folic acid (Nirwani and Kaliwal , 1996; Etebari,2002 and Etebari and Matindoost, 2004).

Folic acid known as (Vitamin B9) is a part of the B group vitamins and water soluble and is widely distributed naturally (Anonymous, 1998). Horie *et al.*, 1966; Sengupta *et al.*, 1972; Saha and Khan, 1996 and Faruki,1998 who found that the vitamins of B group, sugars, proteins, amino acids and minerals are in charge of the growth and development of the silkworm, *B. mori*. Folic acid was affected with a significant increase in female and male cocoon weight and shell weight by Nirwani and Kaliwal (1996).

The present study is aimed to determine the effect of folic acid on several biological and economical parameters in the mulberry silkworm *B. mori* .

Materials and methods

This study was carried out during the spring season, 2021 in silkworm laboratory in Entomology Department, Faculty, of Agriculture, Mansoura, University.

1. Silkworm *Bombyx mori* hybrids:

The eggs of silkworm (Local hybrids) were obtained from Sericulture Research Department, Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt.

2. Concentration of the folic acid:

Various concentrations were prepared by dissolving tablets of folic acid 5gm (Table 1) supplied by Nile Co. in distilled water by (1, 1.5, 2 and 2.5 tablet/100ml distilled water) (Gad *et al.*,2007).

Table (1): Different concentration of folic acid.

Concentration	Description
T_0	Control mulberry leaves without any supplementation
T_1	Mulberry leaves dipped on 5gm/ml folic acid solution
T_2	Mulberry Leaves dipped on7.5gm/ml folic acid solution
T_3	Mulberry Leaves dipped on 10gm/ml folic acid solution
T_4	Mulberry Leaves dipped on 12.5gm/ml folic acid solution

3. Preparation for rearing:

Before rearing, the rearing room, stands and tools used must be washed and treated with formalin solution 3% and left to dry before use.

4. Rearing technique:

After egg hatching larvae were reared in the laboratory condition at (At 25 ±2^{o} C and 75-80% humidity according to the rearing techniques (Krishnaswami, 1978). The larvae 1st, 2nd and 3rd instars feed on fresh and clean mulberry leaves connected every morning four times daily.

5. Experimental design:

Fourth instar, which is the stage was used in the present work. Larvae were divided into five groups including the control. Each group consisted of three replications, each one with fifty worms. Mulberry leaves were used dipped in folic acid solutions at 15 minutes and left leaves for mulberry drying by fanning. The treated leaves were given to the larvae of silkworm *B. mori* from 4th to 5th instars, 4 times a day. The control larvae were fed on mulberry leaves dipped in distilled water only. The biological and economical characteristics were measured.

6. Biological and economical parameters:

Some biological properties of the 4th and 5th instars of silkworm *B. mori* feed on mulberry leaves treated with various concentrations of folic acid supplementary under investigation were studied as; larval weight, food consumption, cocoon weight, cocoon shell weight and silk ratio %.

* Silk shell ratio% = Cocoon shell weight (g) /Cocoon weight (g)

* Cocoon shell weight (g)=

cocoon weight with pupae (g) - Cocoon weight without pupae(g) .

food consumption=*

Dry weight of offered leaves (g) – Dry weight of residual leaves (g)

7. Statistical analysis:

Data were collected and subjected to statistical analysis of different treatments to find out the low significant difference between the criteria of control and treated groups. CoHort Software (2004) was used in all experiments. Data were statistically analyzed by way ANOVA according to Duncan's Multiple Range Test.

Results and discussion

The impact of various concentrations of folic acid on the

growth of silkworm larvae and cocoons was investigated in the current study. The data on larvae weight and food consumption was recorded during 4th and 5th instars, also cocoon characteristics were recorded. All weights were documented with the help of Electronic Balance (Hussain *et al*., 2011). Data reporting on larval weight and food consumption were accomplished on the completion of 4th and 5th instars before molting.

1. Larval weight 4th instars:

Weight of the end 4th instars showed significant variations in larval weight i.e., 0.77, 0.83, 0.90, 0.96 and 1.0g in T_0, T_1, T_2, T_3 and T_4, respectively (Table 2). The present finds were in conformity with the findings of *Singaravelu et al*. (2001); Gad *et al*. (2007) and Balasundaram *et al*. (2013), who reported that the supplementation with different concentration of folic acid maximum increase larval weight 4th instar of *B mori*.

2. Food consumption 4th instars:

Data in (Table 2) showed that the end of 4th instar have significant difference in mean food consumption (g) i.e. 2.62,2.99,3.14,3.21and 3.25 (g) in, T_0,T_1,T_2,T_3 and T_4 respectively. The same results also observed on some other vitamins, minerals, folic acid and para-amino benzoic acid (Rahmathulla *et al*., 2007; Ashan *et al*., 2013; Balasundaram *et al*., 2013 and Sehrish *et al*. ,2017)**.**

3. Larval weight 5th instars:

Larval weight (g) was recorded at the end of 5th instars before spinning. Results gave significant differences in larval weight i.e., 2.52, 2.57, 2.65, 2.75and 2.83 g in T_0, T_1, T_2, T_3 and T_4 respectively (Table 2). The present study finds were in conformity with the findings of Gad *et al.* (2007) and Ravi-Kumara and Anil-Kumra, 2016, who reported that silkworm fed on mulberry leaves supplemented with various concentration of folic acid expressed in maximum larval weight over control. Similar results were observed on some other vitamins by Singaravelu *et al*., (2001).

4. Food consumption 5th instar:

Data recorded in Table (2) the food consumption yielded significant differences in mean food consumption, i.e. 3.75, 3.85, 4.30, 4.36 and 4.45 (g) in, T_0, T_1, T_2, T_3, T_4, respectively. These results are agreement in with the findings of (Balasundaram *et al*., 2013 and Sehrish *et al*., 2017) who observed that increased food consumption when larvae of silkworm *B. mori* feed on mulberry leaves treated with different concentrations of folic acid, minerals and multivitamins.

Table (2): Effect of different concentration of folic acid on larval weight and food consumption (gm) during 4th and 5th instars of silkworm *Bombyx mori*.

Treatments	Larval weight (g)		Food consumption(g)	
	4th	5th	4th	5th
T_0	0.77e	2.52e	2.62 e	3.75 e
T_1	0.83 d	2.57d	2.99d	3.85 d
T_2	0.90c	2.65c	3.14 c	4.30 c
T_3	0.96b	2.75b	3.21b	4.36 b
T_4	1.0 a	2.83a	3.25 a	4.45 a

Small later in a column among treatments significantly differences at 0.05% level of probility.

5. Cocoon weight (gm):

Cocoon weight reported after the completion of cocoon spinning, obtained in (table3) gave significantly increased weight of cocoon, i.e., 1.55,1.66, 1.76, 1.85 and 1.98 (gm) in, T_0, T_1, T_2, T_3 and T_4, respectively. These results are in agreement with El-Karaksy and Idriss (1990), who found that silkworm hybrid (155x 156) reared on mulberry leaf supplemented with folic acid recorded significantly higher

weight as well as control. Similar trends were obtained by (Sulac and Emre, 2000; Raj *et al*., 2002; Gad *et al.* 2007; Tantray and Trivedy, 2011; Balasundaram *et al*., 2013 and Ravi-Kumara and Anil-Kumra, 2016).

6. Cocoon shell weight (gm):

Weight of the cocoon shell calculated after removing pupae showed highly significant differences in shell weight, i.e., 0.369, 0.405, 0.430, 458 and 0.498 (gm) in T_0, $T_{,1}T_2$, T_3 and T_4 respectively (Table 3). Results corroborate the earlier findings of (Gad *et al*., 2007) who obtained that fortified mulberry leaves with different concentrations of folic acid enhance cocoon shell weight. Similar effect was noticed by (Singaravelu *et al*.,2001; Raj *et al*., 2002; Rahmathulla *et al*.,2007; Tantray and Trivedy, 2011 and Ravi-Kumara and Anil-Kumar 2016).

7. Silk shell ratio %:

Silkworms reared on mulberry leaves fortified with folic acid exerted influence on silk ratio %. different concentration resulted explained in (Table 3) that a high significant difference was noticed among all treatments compared with control, i.e., 23.81, 24.39,24.43, 24.75 and 25.15% in, T_0, T_1, T_2, T_3 and T_4, respectively. These results are supported by the observation of Rahmathulla *et al*. (2007) who reported that the bivoltine administrated with folic acid at concentrations 100 and 150 ppm had significantly higher shell ratio %. Similarly administrated of folic acid at various concentration increases the shell ratio % (Balasundaram *et al*.,2013 and Ravi-Kumara and Anil-Kumar 2016).

Table (3): Effect of different concentration of folic acid on cocoon weight(gm), cocoon shell weight (gm) and silk ratio% of silkworm *Bombyx mori*.

Treatments	Cocoon Weight(g)	Cocoon shell weight(g)	Silk ratio%
T_0	1.55e	0.369e	23.81e
T_1	1.66 d	0.405 d	24.39d
T_2	1.76c	0.430c	24.43c
T_3	1.85b	0.458b	24.75b
T_4	1.98a	0.498a	25.15a

Small later in a column among treatments significantly differences at 0.05% level of probility.

The impact of different concentrations of the folic acid supplementation (5,7.5,10 and 12.5 mg/ml) solution was evaluated by rearing silkworm larvae under control condition 1st fourth instar. Weight of body larvae (g) and food consumption showed maximum increases when larvae of silkworm *B. mori L*. fed on mulberry leaves fortified with colic acid as compared to control. Also, results explained high significant increment of cocoon weight (g), cocoon shell weight (g) and silk ratio %. The study confirmed the use of folic acid as a nutritional supplement to give raspberry mulberry leaves the higher nutritional value for silkworm feeding to achieve significant economic benefits.

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