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<論 文>

Effect of Folic Acid on Economic Traits and the Change of Some Metabolic Substances of the Silkworm, *Bombyx mori* L.

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Abstract

Dietary supplementation of folic acid to silkworm larvae in different concentration (100, 200 and 300 μg/m*l*) to the 4th and the 5th instar resulted in a significant increase in economic parameters, such as female and male cocoon weights, shell weights, egg productivity and egg hatching percentage. However, larval duration was significantly decreased, as compared with that of carrier control. The increase in the glycogen and protein contents of the fat body was not significant, whereas the increase in trehalose content of haemolymph in all the treated groups and protein content of fat body and haemolymph in 100 μg/m*l* treated groups were significant. However, the haemolymph protein was significantly decreased in 300 μg/m*l* treated group.

Key words : Folic acid, fat body, haemolymph, glycogen, trehalose, protein, *Bombyx mori*

INTRODUCTION

The production of good quality of cocoons and silk depends on healthyness of larva and larval nutriation partially influenced by the nutritive value of mulberry leaf. The essential components of silkworm nutrition are proteins, lipids, carbohydrates, vitamins and minerals (Horie, 1976). The silkworm, *Bombyx mori* mainly depends on the mulberry leaves for its vitamins requirement since it is a monophagus insect. It is generally accepted that all insects require the water soluble vitamins viz., thiamine riboflavin, pyridoxine and pantothenic acid and in most cases folic acid, biotin and choline.

Akov (1967) showed that folic acid antagonists had inhibitory effect on larval development and egg production in *Aedes aegypti*. The dietary supplementation with ascorbic acid to silkworm larvae resulted in an significant increase in fecundity (Chauhan and Singh, 1992). Recently Sarkar, *et al.*, (1995) showed that improve the cocoon yield and filament length were improved by feeding with B-Complex and ascorbic acid to silkworm, *B. mori*.

The biochemical changes occured in the fat body and haemolymph are mainly depends on the nutritional quality and environmental condition in the silkworm. It has been reported that pyridoxine is involved in amino acid metabolism (Friedrich, 1988). Bhattacharyya (1981) has shown that vitamin 'B_{12}' stimulates protein and nucleic acid synthesis in the silkgland of nistari race of *B. mori*. It has been shown that the implication of folic acid as a co-factor for the conversion of phenylalanine to tyrosine in *Antheraea mylitta* (Wicker *et al.*, 1985). Therefore, the present investigation was undertaken to study the effect of folic acid on the economic traits; glycogen and protein contents of the fat body and trehalose and protein contents of the haemolymph of the silkworm *B. mori*.

MATERIALS AND METHODS

The eggs of biovoltine (NB_4D_2) silkworm were obtained from Karnatak State Sericulture Research and

Development Institute, Thalaghattapur, Bangalore, and reared in the laboratory by improved method of rearing techniques (Krishnaswamy, 1978 b, c). The fourth instar larvae were devided into five experimental groups including controls and each group consisting of 20 worms with five replications each.

The vitamin, folic acid (folvite) procured from, 'Cynamide' India Ltd., Gujarat, was individually dissolved in distilled water and diluted viz., 100, 200 and 300 μg/mg. Fresh mulberry leaves were soaked for 15 minutes in each concentration and then were dried and fed to silkworm at fourth and fifth instar. One of the four normal feeds per day was substituted with the vitamin treated leaves. The controls were fed with the leaves soaked in distilled water and normal leaf.

After the treatment the larval and post cocoon parameters and cocoon parameters were observed. The mean values of the result were shown in Tables 1 and 2 and each mean value was the observation of 10 worms. The experiments were conducted twice to conclude the results. The data collected were subjected to statistical analysis of variance test to find out the significance between the parameters of the distilled water control and treated groups (Snedecor and Cochran, 1967). The percent values for cocoon shell ratio and hatching percentage were transformed into sine angular values for statistical analysis. All the mean values of treated groups are compared to distilled water control.

1. Preparation of tissue

The fifth instar larvae were used for the estimation on sixth day. The larvae are dissected in *Bombyx* saline at the pH 6.5. Immediately the fat body was collected and used for the glycogen and protein estimation. The haemolymph collected in free chilled cenrifuged tube was used for the estimation of protein and trehalose.

Anthrone method (Sciefter *et al.*, 1950) was followed for the determination of glycogen content. Protein estimation was done according to Lowry *et al.*, (1951). Trehalose was estimated according to the method of Roe *et al.*, (1955). Anthrone positive carbohydrate in the haemolymph is considered as trehalose.

RESULTS AND DISCUSSION

The dietary supplementation of folic acid on the economic parameters and biochemical changes in the fat body and haemolymph of the silkworm are presented in the Tables 1 and 2 and Figs. 1 and 2.

1. Larval weight and silkgland weight

The larval and silkgland weights are significantly increased in all the treated groups (Table 1). A maximum increase of 24% larval weight in 100 μg and 200 μg and 23% silkgland weight in 100 μg treted groups were noted. Similar results have been reported with the supplementation of vitamin B and C to *B. mori* (Sarker *et al.*, 1995). The increased larval weight might be due to the folic acid acting as a feeding stimulant as in case of vitamin 'C' as reported by Thorsteinson (1958) and Ito (1961 a,b). Increase in silkgland weight might be due to stimulatory effect of folic acid for the synthetic activity of the silkgland.

2. Larval duration

The larval duration decreased significantly in all folic acid treated groups (Table 1). A maximum decreased of 12 hours was observed in 100 μg treated group. The shortening of larval duration was found to be an interesting observation. The decrease in larval duration might be due to the reduction in the light sensitivity as reported by Bruins *et al.*, (1991), where, the ascorbic acid. pyridoxine and riboflavin reduced the light sensitivity in *Drosophila melanogaster*. However, further investigation is essential to confirm the above findings.

3. Survival percentage

Feeding with all the there concentration of folic acid had no effect on the cocooning percentage, thereby indicating that the used concentrations are allowance limits and have not adversely affected the survival percentage (Table 1).

4. Cocoon weight, shell weight and shell ratio

Table 1. *Effect of folic acid on larval and post-cocoon parameters of the silkworm, B. mori.*

Groups	Dose (µg/ml)	Larval weight(gms)	Silkgland weight(gms)	Larval duration(hrs)	Survival(%)	Egg productivity (No.)	Hatching (%)
I	100	4.498*	2.042*	675*	91.03	835.00*	95.68
					72.60**		78.10**
		(124)	(123)	(98)	(100)	(123)	(99)
II	200	4.507*	1.971*	677*	90.17	829.00*	95.62
					71.11**		77.92**
		(124)	(119)	(98)	(99)	(122)	(99)
III	300	4.245*	2.030*	676*	89.96	834.00*	96.75
					71.53**		79.55**
		(117)	(122)	(98)	(99)	(123)	(100)
IV	Distilled water control	3.627	1.652	687	90.52	675.00	96.12
					72.06**		78.60**
		(100)	(100)	(100)	(100)	(100)	(100)
V	Untreated control	3.489	1.586	686	89.75	659.00	95.95
					71.04**		78.30**
		(96)	(96)	(99)	(99)	(97)	(99)
F-test		S	S	S	NS	S	NS
S.Em+		0.113	0.056	1.005	1.05	19.65	0.61
C.D. at 5%		0.320	0.156	3.050	3.12	56.16	1.76

* - Significant increase/decrease at 5%.
** - Angular transformed values
S.Em+ - Standard error mean.
C.D. - Critical difference.
S. - Significant.
N.S. - Non significant.
Percent increase/decrease over that of the distilled water control in parentheses.

$$\% \text{ Survival} = \frac{\text{Number of cocoon formed}}{\text{Total number of larvae tested}} \times 100$$

$$\% \text{ Hatching} = \frac{\text{Total number of eggs hatched}}{\text{Total number of eggs laid}} \times 100$$

The female and male cocoon weights and their shell weights were increased significantly in all the treated groups (Table 1). The maximum increased in female cocoon weight (13%) and shell weight (25%) in 100 µg and male cocoon weight (20%) ans shell weight (20%) was made in 300 µg treated groups. It is interesting to note that increase in cocoon shell weights of female and male is preceeded by increased in the silkgland weight of the larvae, although the increase of the sexual difference was not found in the silkgland weight.

The increase in cocoon shell weight might be due to the protein conversion efficiency of the silkgland which might by resulted from the increased availability of the folic acid as reported by Hamano (1989) with the supplementation of vitamin B_6 to silkworm, *B. mori*. The femal cocoon shell ratio is significantly increased whereas male cocoon shell ratio is marginally decreased.

5. Egg productivity and egg hatching percentage

The egg productivity is significantly increaed in all the treated groups (Table 1). A maximum increase of 23% was observed in 100 µg and 300 µg treated groups, compared to distilled water control. Similar results have been reported in silkworm fed with vitamin C. (Ito and Arai, 1965; Chauvan and Singh, 1992). However, in the present study it suggests that

Table 2. *Effect of folic acid on the cocoon parameters of ths silkworm, B. mori.*

Groups	Dose(μg/m*l*)	Female cocoon weight(gms)	Female cocoon shell weight(gms)	Female cocoon shell ratio(%)	Male cocoon weight(gms)	Female cocoon shell weight(gms)	Female cocoon shell ratio(%)
I	100	2.395*	0.486*	20.51* 26.88**	1.679*	0.418*	24.99 29.76**
		(113)	(125)	(109)	(119)	(118)	(99)
II	200	2.264*	0.472*	20.89* 27.15**	1.686*	0.409*	24.22 29.42**
		(107)	(121)	(111)	(120)	(115)	(96)
III	300	2.307*	0.480*	20.94* 27.16**	1.693*	0.427*	24.78 29.23**
		(109)	(123)	(111)	(120)	(120)	(98)
IV	Distilled water control	2.101	0.388	18.80 25.66**	1.402	0.353	25.17 29.81**
		(100)	(100)	(100)	(100)	(100)	(100)
V	Untreated control	2.026	0.361	17.90 24.91**	1.337	0.321	24.16 29.38**
		(96)	(93)	(95)	(95)	(90)	(95)
F-test		S	S	S	S	S	S
S.Em+		0.048	0.010	0.51	0.040	0.0110	0.61
C.D. at 5%		0.136	0.028	1.45	0.114	0.0320	1.73

* - Significant increase/decrease at 5%.
** - Angular transformed values
S.Em+ - Standard error mean.
C.D. - Critical difference.
S. - Significant.
Percent increase/decrease over that of the distilled water control in parentheses.

$$\text{cocoon} - \text{shell ratio} = \frac{\text{shell weight}}{\text{cocoon weight}} \times 100$$

folic acid can be utilized to increase the egg productivity in silkworm.

Supplementation of folic acid in the larval stage has neither improved the hatchability of eggs nor adversely affected it.

6. Biochemical parameters

1) Fat body glycogen and haemolymph trehalose content

Dietary supplementation of folic acid to silkworm larvae did not significantly increase the glycogen content of the fat body, whereas the haemolymph trehalose content increased significantly in all the treated groups (Fig. 1). A maximum increase in trehalose content was observed in 100 μg treated group. This might possibly be due to the conversion of glycogen into trehalose and its subsequent release into the haemolymph by the fat body. The increase in trehalose and glycogen contents may possibly be due to the phagostimulatory effect of the vitamin, as reported for vitamin C in *B. mori* (Thorsteinson, 1958, Ito, 1961), which might have led to increasing consumption of the food.

2) Protein content of the fat body and haemolymph

It was observed that in each of the folic acid treated groups the protein content of the fat body is not significantly changed, whereas the protein content of the haemolymph was significantly increased in 100 μg and decreased in 300 μg treated groups (Fig. 2). Irrespective of changes in the protein content of the fat body and increase or decrease in the protein con-

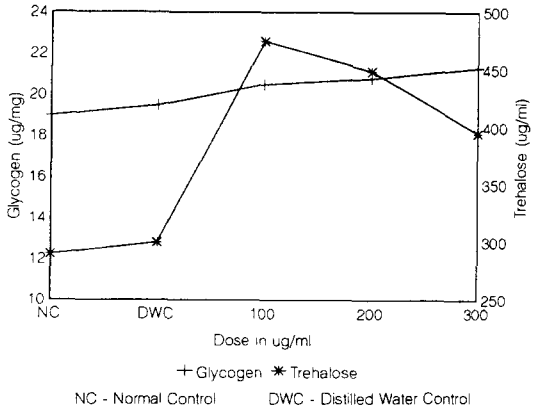

Fig. 1. *Effect of folic acid on the fat body glycogen. and haemolymph trehalose content of the silkworm, B. mori.*

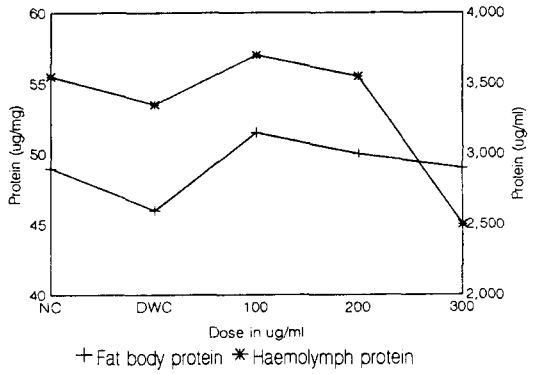

Fig. 2. *Effect of folic acid on the protein content of fat body and haemolymph of the silkworm, B. mori.*

tent of the haemolympy, there is a significant increase in the silkgland weight, cocoon shell weights of females and males in all these gropus (Tables, 1 and 2). These results suggest that the folic acid treatment in all the groups might have stimulated the synthesis and release of protein (without storing) by the fat body into the haemolymph and immediate sequestering of the haemolymph protein by silkgland. Since the silkgland weight is significantly increased in all these groups which subsequently resulted in a significant increase in female and male cocoon shell weights in all the groups (Tables, 1 and 2). This inference supports the views of earlier workers (Bhattacharyya, 1981; Wicker *et al.*, 1985) that vitamins (folic acid), stimulate the synthesis of protein in insects. The treatment with low dose favours the accumulation of proteins in the haemolymph before it is utilized by the silkgland.

In conclusion the vitamic, folic acid, may have soem effect on physiological metabolism as mentioned above and thereby the increase in the economic parameters of the silkworm, *B. mori.*

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적 요

엽산을 뽕잎에 각 농도별(100, 200 및 300 μg/m*l*)로 처리하여 4령 또는 5령 누에에 침식시킨 결과 전견중, 견층중, 산란성 및 부화율과 같은 실용형질이 향상되었으며 대조구에 비해 경과일수도 통계적인 유의성 있게 단축되었다. 지방체의 glycogen과 단백질 함량의 증가는 유의성이 없었으나 체액중 trehalose는 모든 처리구에서 증가되었으며, 지방체와 체액중의 단백질 함량은 100 μg/m*l* 구에서 유의적으로 높았다. 그러나 300 μg/m*l*구에서는 체액중 단백질 함량은 오히려 감소하였다.

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