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GROWTH AND DEVELOPMENT OF THE MULBERRY SILKWORM, *BOMBYX MORI* L. ON VITAMIN B AND C SUPPLEMENTED DIET

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Abstract: The effect of vitamin B and vitamin C on the growth and development of *Bombyx mori* L. has been studied. Oral supplementation of different concentrations of vitamin B and C to the 3rd, 4th and 5th instar larvae of multivoltine silkworm variety, BSR-95/14(M) resulted significant increase in larval and pupal characters. It was observed that the development of larvae and pupae took place up to a particular dose. There was also a significant increment in the various growth indices following vitamin supplements with the mulberry leaves.

Key words: Growth, development, vitamin, silkworm, *Bombyx mori* L.

INTRODUCTION

Feeding of nutritionally enriched leaves showed better growth and development of silkworms as well as improve the economic value of cocoons (Krishnaswami *et al.* 1971). The influence of micronutrients on larval development and cocoon characters of silkworm were also studied (Vishwanath and Krishnamurthy 1983). Fortification of mulberry leaves with supplementary compounds to increase the larval growth and development has been carried out. These include vitamins (Saha and Khan 1996, Nirwani and Kaliwal 1996, Nirwani *et al.* 1998, Etebari *et al.* 2004, Rahmathulla *et al.* 2007); hormones (Magadum *et al.* 1992, Trivedy *et al.* 1993, Saha and Khan 1997); amino acid (Khan and Faruki 1990, Qadar *et al.* 1994, Saha *et al.* 1994, Saha and Khan 1997); and minerals (Magadum *et al.* 1992, Islam and Khan 1993, Khan and Saha 1995). Nutritional background of the larval stage significantly influences the status of the resulting larva, pupa and fibre (Ahmed *et al.* 1998, Rahmathulla *et al.* 2002, Khan and Saha 2003, Etebari and Matindoost 2004). Although the mulberry leaves are the complete diet for silkworm, sometimes dietary deficiencies occur due to different factors.

Vitamins are a group of unrelated organic compounds needed only in minute quantities in the diet that are essential for specific metabolic reactions within the cell and necessary for normal growth and maintenance of health. It prevents associated deficiency diseases. Dosages of vitamins are very determinative for normal growth of silkworm. Etebari *et al.* (2004) reported yield decrease when

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the ascorbic acid concentration was enhanced in the silkworm diet. Similar effects of multivitamins on the silkworm have been confirmed by Saha and Khan (1996), and Etebari and Matindoost (2004). However, it has been reported that dietary supplementation of vitamins and minerals produced a significant increment in the growth and development of *B. mori* (Saha and Khan 1996).

The following investigation was undertaken to determine the effects of vitamin B and C supplementation with the diet on the growth and development of silkworm, *B. mori.*

MATERIAL AND METHODS

Experimental animal and rearing: The eggs of silkworm variety, BSR 95/14(M) were obtained from the Germplasm Bank, Bangladesh Sericulture Research and Training Institute, Rajshahi and reared in the Sericulture Laboratory, Department of Zoology, University of Rajshahi, following the scientific silkworm rearing techniques (Krishnaswami 1978, Rahman 1983). After disinfection and incubation of eggs; the larvae that hatched out were reared up to the second instar on fresh mulberry leaves (*Morus alba* L.). Third instar larvae were divided into seventeen experimental groups including the control. Each group consisted of three replications, each of 70 worms. The experiment was conducted at 28 ± 2°C and a relative humidity of 75.00 ± 6%.

Treatments: Vitamin B (Opsovit®) and Vitamin C (Ceevit) were purchased from local market and various concentrations were prepared by mixing the requisite amounts of vitamins in distilled water.

The concentrations of the vitamins used were as follows: T_0 = Control, T_1 = 2.9% B + 25% C, T_2 = 5.8% B + 25% C, T_3 = 8.7% B + 25% C, T_4 = 11.6% B + 25% C, T_5 = 2.9% B +12.5% C, T_6 = 5.8% B +12.5% C, T_7 = 8.7% B +12.5% C, T_8 = 11.6% B +12.5% C, T_9 = 2.9% B + 6.25% C, T_{10} = 5.8% B + 6.25% C, T_{11} = 8.7% B + 6.25% C, T_{12} = 11.6% B + 6.25% C, T_{13} = 2.9% B + 3.125% C, T_{14} = 5.8% B + 3.125% C, T_{15} = 8.7% B + 3.125% C, T_{16} = 11.6% B + 3.125% C. Fresh, whole mulberry leaves were treated by dipping in a particular type of these solutions and then were dried by fanning. The treated leaves were fed to the worms from 3rd to 5th instars, 4 times in a day. The control insects were reared on mulberry leaves dipped in distilled water only.

Parameters: The growth of larvae was measured after attaining maturity, i.e., one day before spining. Fifty larvae were taken randomly from each rearing tray and then weighed individually on an electric balance. After the formation of cocoons they were harvested and cut very carefully at one end obliquely with a sharp blade to record pupal growth. The pupae were separated according to sexes. They were then individually weighed on an electric balance. The larval

and pupal periods of the worms of various treatment groups were carefully recorded. The percentage of adult emergence was calculated by using the following formula:

$$\text{Emergence (\%)} = \frac{\text{No. of adults emerged}}{\text{No. of larvae used}} \times 100$$

The Effective Rates of Rearing (%) (ERRs%) of *B. mori* from different concentrations of vitamins were calculated by using the following formula:

$$\text{ERR (\%)} = \frac{\text{No. of cocoon obtained}}{\text{No. of larvae taken at 3rd instar}} \times 100$$

Statistical procedures: The data were subjected to analyses of a variance. The mean values were analyzed following Tukey's multiple comparison test (1953). The larval, pupal and larval-pupal indices were calculated following the formulae of Joshi (1985) and Prasad and Bhattacharya (1975).

RESULTS AND DISCUSSION

Larval parameter: The maximum larval weight was observed at treatment T_2 (8.7% B+12.5% C dose combination) (Table 1). In this dose combination moderate % of vitamin B and C was used. Many researchers found that the larval characters of *B. mori* was improved by different nutrients such as ascorbic acid, folic acid, thiamin, vitamin B complex, etc. (Saha and Khan 1996, Nirwani and Kaliwal 1996, Etebari *et al.* 2004, Rahmathulla *et al.* 2007, El-Karaksy and Idris 2009).

It has been reported that Thyroxin and vitamin B_{12} supplemented mulberry leaves significantly increased the body weight of silkworm larvae (Majumder and Medda 1975). Saha and Khan (1997) reported that the growth *B. mori* larvae were significantly influenced at lower doses when the worms were reared on Sinafort®-B supplemented diet. The oral supplementation of riboflavin resulted in a significant increase in larval and silkgland weight (Nirwani *et al.* 1996).

El-Karaksy and Idris (2009) observed that ascorbic acid significantly increased the weight of *B. mori* larvae and pupae. Gomaa *et al.* (1977) also reported that ascorbic acid significantly increased the weight of *B. mori* larvae. Several authors suggested that the enhancement in larval weight was related to phagostimulation of ascorbic acid (Singh and Reddy 1981, El-Karaksy and Idris 2009)

In higher doses of vitamin B or C in silkworm diet, the larval weight was considerably decreased. Saha and Khan (1996) also obtained similar results

with cocoon characteristics. Etebari *et al.* (2004) demonstrated that feeding of high concentrations of ascorbic acid decreased the silkworm larval weight due to hypervitaminosis.

Table 1. Effect of vitamins B and C on the weight of different developmental stages of *B. mori* L.(gm)

Concentration of vitamin(B%+C%)	Mature larval weight	Female pupal weight	Male pupal weight
	Mean ± SD	Mean ± SD	Mean ± SD
T_0 = Control	3.19 ± 0.046 ^{b}	1.61 ± 0.059 ^{bac}	1.26 ± 0.040 ^{a}
T_1 = 2.9+25	2.86 ± 0.095 ^{b}	1.62 ± 0.015 ^{ba}	1.18 ± 0.031 ^{bac}
T_2 = 5.8+25	3.15 ± 0.060 ^{b}	1.42 ± 0.038 ^{f}	1.26 ± 0.045 ^{ba}
T_3 = 8.7 +25	3.31 ± 0.180 ^{b}	1.53 ± 0.057 ^{bdec}	1.18 ± 0.031 ^{bac}
T_4 = 11.6+25	3.35 ± 0.051 ^{ba}	1.53 ± 0.021 ^{bdec}	1.17 ± 0.012 ^{bac}
T_5 = 2.9+12.5	3.42 ± 0.035 ^{ba}	1.57 ± 0.030 ^{bdac}	1.22 ± 0.025 ^{bac}
T_6 = 5.8+12.5	3.43 ± 0.042 ^{ba}	1.58 ± 0.042 ^{bdac}	1.18 ± 0.055 ^{bac}
T_7 = 8.7+12.5	3.54 ± 0.040 ^{a}	1.64 ± 0.010 ^{a}	1.16 ± 0.065 ^{c}
T_8 = 11.6+12.5	3.47 ± 0.045 ^{ba}	1.63 ± 0.042 ^{a}	1.18 ± 0.015 ^{bac}
T_9 = 2.9+6.25	3.49 ± 0.044 ^{ba}	1.53 ± 0.015 ^{bdec}	1.15 ± 0.006 ^{c}
T_{10} = 5.8+6.25	3.40 ± 0.030^{ba}	1.53 ± 0.012 ^{bdec}	1.18 ± 0.012 ^{bac}
T_{11} = 8.7+6.25	3.38 ± 0.038 ^{ba}	1.61 ± 0.017 ^{ba}	1.18 ± 0.026 ^{bac}
T_{12} = 11.6+6.25	3.36 ± 0.026 ^{ba}	1.58 ± 0.006 ^{bdac}	1.17 ± 0.010 ^{bc}
T_{13} = 2.9+3.125	3.37 ± 0.053 ^{ba}	1.51 ± 0.021 ^{dec}	1.18 ± 0.006 ^{bac}
T_{14} = 5.8+3.125	3.32 ± 0.047 ^{b}	1.56 ± 0.021 ^{bdac}	1.18 ± 0.010 ^{bac}
T_{15} = 8.7+3.125	3.26 ± 0.046 ^{b}	1.51 ± 0.012 ^{fde}	1.17 ± 0.006 ^{bac}
T_{16} = 11.6+3.125	3.29 ± 0.045 ^{b}	1.46 ± 0.046 ^{fe}	1.16 ± 0.006 ^{c}
F-ratio	17.113 (P<0.01)	11.033 (P<0.01)	3.551 (P<0.01)

Note: Means followed by the same letters in a column are not significantly different by Tukey's multiple comparison test (1953).

Pupal parameter: The effect of vitamin supplementation on pupal growth of *B. mori* is shown in Table 1. The male and female pupal weights in different concentrations were significantly increased. Saha and Khan (1996) found that vitamins and minerals significantly increased the growth of pupae compared to the untreated controls. Citric acid supplementation increased the growth of *B. mori* pupae at lower concentrations (Khan and Saha 1996b). Khan and Faruki (1990) observed that para-amino benzoic acid supplementation had significant effects on pupal weight, length, and breadth. Fe-PLUS® supplementation significantly increased the weight of pupae when compared with the untreated controls (Khan and Saha 1996a). Thianomin® enriched mulberry leaves significantly increased the pupal weight and length in comparison to controls (Faruki 1998).

Developmental period: The results of the supplementation of vitamins on larval and pupal periods (days) obtained in the present experiments are

presented in Table 2. It was observed that vitamin supplementation slightly reduced the larval periods in *B. mori* at lower concentrations as compared to the controls. It was also found that pupal periods significantly reduced following the supplementation. On the other hand, at some higher dose combinations slightly lengthened larval and pupal durations were obtained. It has been observed that feeding of minerals to the silkworms shortened the development periods in *B. mori* in comparison to the untreated controls (Saha and Khan 1996). Khan and Saha (2003) observed that low concentrations of thiamine significantly reduced the developmental periods of silkworm. Fe-PLUS® slightly decreased the larval and pupal periods in the treated worms but the developmental periods were increased at the higher concentrations (Khan and Saha 1996a). Nirwani *et al.* (1998) observed that dietary supplementation of riboflavin significantly decreased the larval period in all the treated groups.

Table 2. Effect of vitamins B and C on the larval, pupal and total developmental periods (days) of *B. mori* L.

Concentration of vitamin(B%+C%)	Larval period	Pupal period	Total Developmental period
	Mean ± SD (No.)*	Mean ± SD (No.)*	
T_0 = Control	23.45 ± 0.105 (180)	9.62 ± 0.040 (175)	33.07
T_1 = 2.9+25	23.48 ± 0.100 (185)	9.61 ± 0.050 (182)	33.09
T_2 = 5.8+25	23.42 ± 0.100 (178)	9.71 ± 0.010 (172)	33.13
T_3 = 8.7 +25	23.29 ± 0.100 (185)	9.61 ± 0.050 (180)	32.9
T_4 = 11.6+25	23.46 ± 0.100 (184)	9.62 ± 0.042 (180)	33.08
T_5 = 2.9+12.5	23.42 ± 0.100 (186)	9.52 ± 0.100 (185)	32.94
T_6 = 5.8+12.5	23.36 ± 0.100 (180)	9.63 ± 0.040 (176)	32.99
T_7 = 8.7+12.5	23.38 ± 0.100 (178)	9.70 ± 0.015 (174)	33.08
T_8 = 11.6+12.5	23.29 ± 0.100 (180)	9.66 ± 0.100 (172)	32.95
T_9 = 2.9+6.25	23.32 ± 0.100 (180)	9.65 ± 0.071 (172)	32.97
T_{10} = 5.8+6.25	23.44 ± 0.100 (182)	9.69 ± 0.061 (178)	33.13
T_{11} = 8.7+6.25	23.42 ± 0.100 (186)	9.68 ± 0.100 (183)	33.1
T_{12} = 11.6+6.25	23.45 ± 0.100 (188)	9.55 ± 0.050 (185)	33
T_{13} = 2.9+3.125	23.45 ± 0.100 (188)	9.56 ± 0.051 (185)	33.01
T_{14} = 5.8+3.125	23.48 ± 0.100 (182)	9.59 ± 0.055 (178)	33.07
T_{15} = 8.7+3.125	23.35 ± 0.100 (185)	9.55 ± 0.035 (180)	32.9
T_{16} = 11.6+3.125	23.35 ± 0.100 (185)	9.56 ± 0.045 (180)	32.91
F-ratio	1.200	2.868 (P<0.01)	

*Note: No. of insects sampled.

Effective Rates of Rearing (ERRs) and adult emergence: The results of the supplementation of vitamins on the percentage of effective rates of rearing and percentage of adult emergence are shown in Table 3. The treatments (T_2, T_3 and T_4) resulted significant increase as compared to the controls. Khan and Faruki (1990) observed that low concentrations of para-amino benzoic acid produced

detrimental effects on pupation and moth emergence. Saha and Khan (1996) observed that the effect of dietary supplementation of vitamins and minerals was to increase the ERR (%) of *B. mori*. Similar results have also been recorded by Pai *et al.* (1988).

Table 3. Effect of vitamins B and C on effective rate of rearing (ERR) and adult emergence (%) of *B. mori* L.

Concentration of vitamin(B%+C%)	ERR (%)	Adult emergence (%)	*d-value	Level of significance
	94.74	91.05	--	--
T_0 = Control	97.37	94.74	-1.403	NS
T_1 = 2.9+25	93.68	89.47	0.519	NS
T_2 = 5.8+25	97.37	98.42	-3.261	P<.05
T_3 = 8.7 +25	96.84	98.42	-3.261	P<.05
T_4 = 11.6+25	97.89	96.32	-2.124	P<.05
T_5 = 2.9+12.5	94.74	90.53	0.175	NS
T_6 = 5.8+12.5	93.68	90.53	0.175	NS
T_7 = 8.7+12.5	94.74	89.47	0.519	NS
T_8 = 11.6+12.5	94.74	89.47	0.519	NS
T_9 = 2.9+6.25	95.79	92.11	-0.372	NS
T_{10} = 5.8+6.25	97.89	94.74	-1.403	NS
T_{11} = 8.7+6.25	98. 95	95.79	-1.871	NS
T_{12} = 11.6+6.25	98.95	95.79	-1.871	NS
T_{13} = 2.9+3.125	95.79	92.11	-0.372	NS
T_{14} = 5.8+3.125	97.37	93.16	-0.763	NS
T_{15} = 8.7+3.125	97.37	93.68	-0.966	NS

Note: *d= Standardized normal deviate.

Table 4. The growth indices of *B. mori* from various concentrations of vitamin-enriched diet

Concentration of vitamin(B%+C%)	Mature larval weight index	Female pupal weight index	Male pupal weight index	Larval – pupal index
T_0 = Control	0.896552	1.006211	0.936508	1.000605
T_1 = 2.9+25	0.987461	0.881988	1	1.001814
T_2 = 5.8+25	1.037618	0.950311	0.936508	0.994859
T_3 = 8.7 +25	1.050157	0.950311	0.928571	1.000302
T_4 = 11.6+25	1.0721	0.975155	0.968254	0.996069
T_5 = 2.9+12.5	1.075235	0.981366	0.936508	0.997581
T_6 = 5.8+12.5	1.109718	1.018634	0.920635	1.000302
T_7 = 8.7+12.5	1.087774	1.012422	0.936508	0.996371
T_8 = 11.6+12.5	1.094044	0.950311	0.912698	0.996976
T_9 = 2.9+6.25	1.065831	0.950311	0.936508	1.001814
T_{10} = 5.8+6.25	1.059561	1	0.936508	1.000907
T_{11} = 8.7+6.25	1.053292	0.981366	0.928571	0.997883
T_{12} = 11.6+6.25	1.056426	0.937888	0.936508	0.998186
T_{13} = 2.9+3.125	1.040752	0.968944	0.936508	1
T_{14} = 5.8+3.125	1.021944	0.937888	0.928571	0.994859
T_{15} = 8.7+3.125	1.031348	0.906832	0.920635	0.995162

The present findings demonstrated that vitamin B and C supplementation at lower concentrations is of importance in improving growth and development of *B. mori*. This would contribute to sericulture through improvement of economic traits of *B. mori.* However, comprehensive studies with various dose combinations are required in future works.

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