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Effects of methionine and tryptophan on some quantitative traits of silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae)

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Abstract: The present experiment was carried out to determine the effects of methionine and tryptophan on some quantitative traits of silkworm, *Bombyx mori* L. Mulberry leaves were treated with 250,500,750 and 1000 ppm of amino acids and were provided to the worms as food. Compared to the controls amino acids (especially 500 and 750 ppm) significantly increased the fecundity, fertility, adult emergence, developmental periods and longevity of *B. mori* adults but decreased the mortality.

Key words: Amino acids, *Bombyx mori*, fecundity, mortality, longevity.

Introduction

Like other organisms, nutrition plays a pivotal role in silkworms by improving their commercial characters of silkworms. Silkworm is monophagous, so it requires constituents for its growth derived from the mulberry leaves and the quality of the leaves influences greatly the biology of the worms. According to Dow (1986), lepidopteran larvae are characterized by intense feeding activity and a rapid growth. The alimentary apparatus of these animals is formed by a vestigial foregut, a short hindgut and a wide long midgut. Santos *et al.* (1984) observed that the digestive and absorptive functions of the larval midgut epithelium are performed by the coordinated activity of two different cell types: columnar cells and goblet cells. Columnar cells are responsible for synthesis and secretion of digestive enzymes. Giordana *et al.* (1982) observed that columnar cells are responsible for the nutrients absorption. Silkworm requires certain essential sugars, proteins, amino acids and vitamins for its normal growth, survival and also for the growth of its silk glands (Sengupta *et al.*, 1972). Krishnaswami *et al.* (1971) showed that silkworms exhibit better growth and development due to nutritionally enriched leaves supplementation. Significant developments in the research on silkworm nutrition started with the formulation of artificial diets with different nutrients *i.e.*, proteins, amino acids, and carbohydrates, hormones, vitamins and minerals. Better production of cocoon crops was investigated by many workers like Bose & Majumder (1989), Reddy *et al.* (1994), Saha & Khan (1997a,b), Faruki (1998), Nirwani *et al.* (1998), Nakamura (2000), Narasimhamurthy & Govindappa (1988), Islam & Khan (1993).

The structural components and all the enzymes regulating biochemical transformation of living cells are proteins and hence amino acids suggest a primary class of nutrients. Amino acids are closely related to the biosynthesis of silk proteins as well as to the growth of silk glands of *B. mori* (Ito, 1972). It has been experimentally determined by Bose *et al.* (1989) that silkworms require 18 amino acids for their adequate nutrition. Amino acids and their derivatives participate in intracellular functions as diverse as nerve transmission, regulation of cell growth and the biosynthesis of various compounds in silkworm (Rodwell, 1993). The requirements of amino acids in *B. mori* are confirmed by different deletion experiments (Qadar *et al.,* 1994; Eid *et al.,* 1989; Khan & Saha, 1995 and Saha *et al.,*1994). Krishnappa (1987) reported that supplementation with amino acids reduces the total larval duration and survival percentage. According to Leonardi *et al.* (2001) nutrient absorption and its modulation are critical for animal growth. They demonstrated that leucine methyl ester (Leu-Ome) can greatly increase the activity of the transport system responsible for the absorption of most essential amino acids in larval midgut of the silkworm.

Considering the key role played by amino acid absorption in silkworm nutrition and development, attempts have been made here to assess the effects of methionine and tryptophan on the fecundity, fertility, mortality, adult emergence, longevity and developmental periods of the silkworm, *B. mori* L.

Materials and Methods

A high-yielding multivoltine breed of the silkworm, BSRI–83/3 was procured from the Silkworm Germplasm Bank of Bangladesh Sericulture

Research and Training Institute, Rajshahi, and reared in the Animal Genetics and Breeding Laboratory, Department of Zoology, University of Rajshahi. Standard rearing techniques were followed (Krishnaswami, 1979). After hatching, the larvae were reared up to 2nd instar on fresh mulberry leaves (*Morus alba*).

Water soluble amino acids L-methionine and L-tryptophan were collected from the Department and four different concentrations (250, 500, 750 and 1000 ppm) were prepared. The fresh mulberry leaves were dipped in different concentrations of of the amino acid solutions separately and dried up by fanning and chopped. Then the leaves were given to the silkworms on their 3rd instar first feeding. Control insects were reared simultaneously on untreated leaves. Three replications per concentration containing 30 healthy larvae were maintained. The treatments were continued up to spinning and succeeded by parental and F_1 generation. The data were collected and used for statistical analysis.

Results and Discussion

In case of fecundity, the maximum numbers of eggs laid at 750 ppm were 531.53+25.96 (605.73+49.72) in the parental and F_1 generation in methionine and 536.33+21.64 (615.87+60.39) in tryptophan supplementation. Analysis of variance showed highly significant differences in eggs between the generations only. The highest fertility was also found at 750 ppm in the parental and F_1 generation (96.73+1.60, 97.18+1.12 and 97.25+1.23, 97.83+0.91) in the methionine and tryptophan supplementation, respectively in fertility. Analysis of variance showed highly significant differences between the concentrations and generations (8.77 and 18.89, P<0.001 in methionine and 16.16 and 21.19, P<0.001 in tryptophan) (Table 1). The lowest larval and pupal mortalities was obtained at 500 ppm in parental and F_1 generation (Fig.1). Larval mortality showed significant difference between generations (3.658, P<0.05) in case of methionine and between concentrations (3.034, P<0.05) in tryptophan supplementation. But pupal mortality showed insignificant differences (0.375, 0.244, 1.026 and 0.798 respectively). Figure 2 demonstrates that the highest percentage of adult emerged at 500 ppm (95.56+3.85, 90.00+3.33 in methionine and 96.67+3.34, 92.22+1.92 in tryptophan) in the parental and F_1 generation. Analysis of variance also showed significant differences (9.114, 10.911, P<0.001 and 8.903, P<0.001, 4.834, P<0.05), between both concentrations and generations. Developmental periods i.e., larval and pupal period were significantly reduced in the parental and F_1 generations due to methionine (11.86, 2624.79, 27.23 and 405.30, P<0.001) and tryptophan (10.06, 2868.69, 36.49 and 424.67, P<0.001) supplementation (Table 1). It was observed that methionine and tryptophan enhanced the male and female longevity at 750 ppm when compared with control line. Analysis of variance exhibited highly significant differences between the concentrations (4.73 and 4.75, P<0.01 in methionine and 7.08 and 12.17, P<0.001 in tryptophan) and between the generations (4.15, P<0.01 and 460.13m P<0.001 in methionine and 16.38 and 390.15, P<0.001 in tryptophan respectively) (Table 3).

Krishnappa (1987) found that fecundity and fertility were well pronounced due to amino acid supplementation. He also reported that amino acids reduce the total larval duration and mortality of larvae and pupae. Similarly Saha *et al.* (1994) observed that proline and leucine enhanced the reproductive potentiality (i.e., fecundity, fertility etc.). Kabila *et al.* (1994) recorded the reduced larval duration in *B. mori* reared on feed supplemented with neutralized ascorbic acid. Khan & Saha (1995) also observed that alanine and glutamine significantly increased the growth and development and decreased the larval and pupal periods. According to Horie & Watanabe (1983), if growth depends on a large number of differentiated physiological activities, the functional properties of the intestine and its ability to absorb efficiently, amino acids can influence the development of the whole organism. In *B. mori,* in particular, up to 65% of digested nitrogen is utilized for silk production during the last instar and the level of dietary protein and limiting amino acids in the diet strongly affects larval growth and silk production. Leonardi *et al.* (2001) demonstrated that intestinal amino acid absorption is an important step in nitrogen metabolism and directs the biological development of the whole organism. They also showed that leucine-methyl-ester (Leu-Ome) can greatly increase the activity of the transport system responsible for absorption of most essential amino acids in the larval midgut of *B. mori.* They recorded the leucine uptake activation by Leu-Ome in brush border membrane vesicles and in the apical membrane of epithelial cells in the midgut incubated *in vitro* and observed that the addition of this strong activator of amino acid absorption to diet significantly affected the larval development.

Table 1. Developmental periods of *B. mori* feed on amino acid-treated mulberry leaves

Amino acids	Concentrations (ppm)	Larval period (Mean±SD)		Pupal period (Mean±SD)		F-values
		Parental	F_1	Parental	F_1	
Methionine	0 (Control)	19.61±0.69	17.64±0.64	9.78±0.42	9.18±0.42	$^{Aa}11.86^{***}$
	250	19.51±0.63	17.39±0.49	9.41±0.49	8.68±0.67	$^{Ab}2624.79^{***}$
	500	19.39±0.54	17.31±0.47	9.43±0.49	8.51±0.71	$^{Ba}27.23$***
	750	19.45±0.63	17.39±0.49	9.38±0.49	8.33±0.55	$^{Bb}405.30^{***}$
	1000	19.84±0.73	17.62±0.49	9.35±0.48	8.57±0.79	
Tryptophan	0 (Control)	19.61±0.69	17.64±0.64	9.77±0.42	9.18±0.42	$^{Aa}10.06^{***}$
	250	19.49±0.59	17.36±0.48	9.61±0.50	8.56±0.70	$^{Ab}2868.69^{***}$
	500	19.38±0.53	17.31±0.46	9.39±0.49	8.36±0.55	$^{Ba}36.49^{***}$
	750	19.40±0.54	17.34±0.48	9.30±0.46	8.19±0.39	$^{Bb}424.67^{***}$
	1000	19.71±0.65	17.53±0.40	9.23±0.50	8.82±0.92	

Note: A = Larval period, B = Pupal period; a = between concentrations, b = between generations; *** P < 0.001

Table 2. Effects of amino acids on the reproduction of *B. mori* females

Amino acids	Concentrations (ppm)	Fecundity (Mean±SD)		Fertility (%) (Mean±SD)		F-values
		Parental	F_1	Parental	F_1	
Methionine	0 (Control)	509.13±42.37	569.53±40.94	93.72±1.21	95.46±2.30	$^{Aa}2.36^{NS}$
	250	517.07±48.03	579.13±38.16	95.43±1.86	96.45±1.58	$^{Ab}78.32^{***}$
	500	528.93±39.36	590.93±54.24	96.02±1.48	97.11±1.34	$^{Ba}8.77$***
	750	531.53±25.96	605.73±49.72	96.73±1.60	97.18±1.12	$^{Bb}18.89^{***}$
	1000	513.87±34.03	571.47±59.88	94.84±2.21	96.52±1.76	
Tryptophan	0 (Control)	509.13±42.37	569.53±40.94	93.72±1.21	95.46±2.30	$^{Aa}2.74^{NS}$
	250	523.13±45.26	586.33±55.59	95.09±1.57	96.63±1.45	$^{Ab}68.89^{***}$
	500	532.47±34.58	598.33±51.92	96.75±1.86	97.45±1.62	$^{Ba}16.16^{***}$
	750	536.33±21.64	615.87±60.39	97.25±1.23	97.83±0.91	$^{Bb}21.19^{***}$
	1000	516.87±28.08	576.47±79.63	95.28±1.96	96.67±1.20	

Note: A = fecundity, B = fertility; a = between concentrations, b = between generations
*** P < 0.001, NS = non-significant

Table 3. Longevity of *B. mori* adults resulting from larvae feed on amino acid-treated mulberry leaves

Amino acids	Concentrations (ppm)	Longevity of males (Mean±SD)		Longevity of females (Mean±SD)		F-values
		Parental	F_1	Parental	F_1	
Methionine	0 (Control)	3.40±0.74	3.73±0.70	6.47±0.52	8.33±0.49	$^{Aa}4.73^{**}$
	250	3.60±0.51	3.87±0.83	6.60±0.63	8.60±0.83	$^{Ab}4.15^{**}$
	500	3.80±0.68	3.93±0.80	6.73±0.46	8.80±0.77	$^{Ba}4.75^{**}$
	750	3.93±0.70	3.93±0.46	6.87±0.35	8.93±0.83	$^{Bb}460.13^{***}$
	1000	3.13±0.46	3.47±0.52	6.33±0.49	8.40±0.51	
Tryptophan	0 (Control)	3.40±0.74	3.73±0.73	6.47±0.52	8.33±0.49	$^{Aa}7.08^{***}$
	250	3.53±0.52	4.00±0.65	6.93±0.46	8.93±0.70	$^{Ab}16.38^{***}$
	500	3.67±0.49	4.20±0.56	7.07±0.68	9.07±0.59	$^{Ba}12.17^{***}$
	750	4.07±0.70	4.33±0.49	7.20±0.70	9.27±0.70	$^{Bb}390.15^{***}$
	1000	3.33±0.48	3.67±0.49	6.53±0.52	8.27±0.59	

Note: A = Longevity of males, B = Longevity of females; a = between concentrations, b = between generations
** P < 0.01, *** P < 0.001

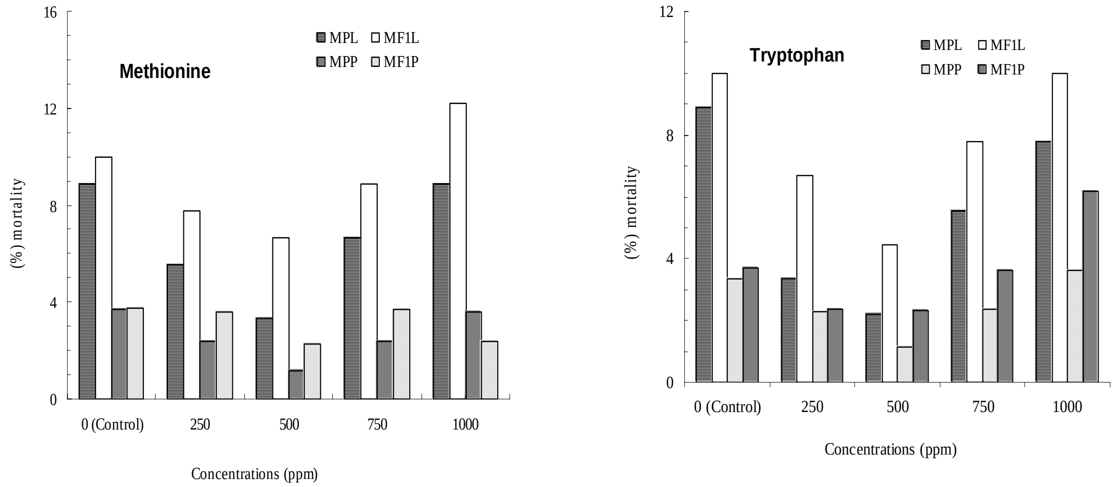

Fig. 1: Mortalities in larval and pupal stages in *B. mori* resulted from amino acid-treated leaves.
MPL = mortality of parental larvae, MF_1L = mortality of F_1 larvae
MPP = mortality of parental pupae, MF_1P = mortality of F_1 pupae

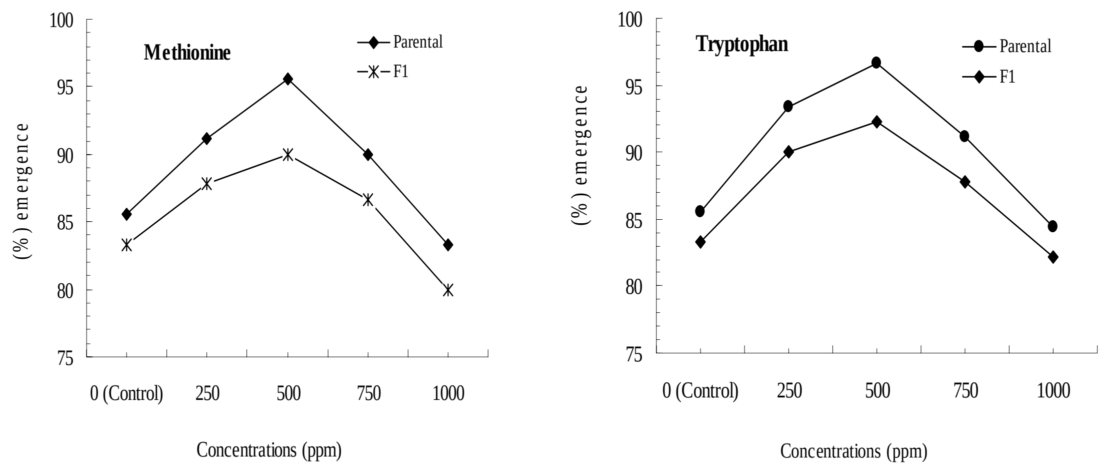

Fig. 2: Adult emergence (%) in *B. mori* from amino acid-treated leaves.

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