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Effects of methionine and tryptophan on some economic characters in the mulberry silkworm *Bombyx mori* L.

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Abstract: Methionine and tryptophan were supplemented with food at four concentrations *viz.* 250, 500, 750 and 1000 ppm to evaluate the effects of these amino acids on the larval and pupal weight and different cocoon characters in the mulberry silkworm, *Bombyx mori* L. Treatments significantly enhanced the performance of all the parameters in both parental and F_1 generations except the filament denier. Significant differences were also observed between the generations of all the parameters except the filament length. However, the highest concentration (1000 ppm) of both the compounds decreased the growth parameters in comparison to control.

Key words: *Bombyx mori*, methionine, tryptophan, growth, filament length

Introduction

Nutrition plays a pivotal role in sericulture by improving the commercial characters of silkworms. Silkworm is a monophagous, deriving almost all the constituents required for its growth from the mulberry leaf. Feeding of nutritionally enriched leaves provided better growth and development of silkworms as well as gain in economic characters of cocoons (Krishnaswami *et al.*, 1971). Effects of diets with different nutrients such as proteins, amino acids, carbohydrates, hormones, vitamins and minerals on the better production of cocoon crops have been reported earlier by Narasimhamurthy & Govindappa (1988), Bose & Majumder (1989), Islam & Khan (1993), Reddy *et al.* (1994), Saha & Khan (1997a,b), Faruki (1998), Nirwani *et al.* (1998) and Nakamura (2000).

The structural components and all the enzymes regulating biochemical transformations of living cells are proteins and hence amino acids constitute a primary class of nutrients (Ito, 1972). Proteins and especially free amino acids play important roles in insect development. Presence of high levels of amino acids in insects indicates that besides their role in protein synthesis, they have additional functions related to neural transmission, detoxification, synthesis of phospholipids, energy production and morphogenetic processes (Chen, 1985). Rodwell (1993) reported that 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. There are many natural nonprotein amino acids produced by higher plants or microorganisms, acting as antiaminoacids, while many have been synthesized in the laboratory by the structural modifications of normal amino acids. The origins, mode of action, physiological and biochemical functions as well as the biological effects of such substances have been reviewed elsewhere (Shive & Skinner, 1963; Fowden *et al*., 1967).

The requirements of amino acids in silkworm were confirmed by a number of experiments (Ito & Inokuchi, 1972; Bose & Majumder, 1989; Quader *et al.,* 1994; Khan & Saha, 1995). It has been determined that 10 amino acids *viz.,* arginine, histidine, isoleusine, leusine, glysine, methionine, phenylalanine, threonine, tryptophan and valine are essential for silkworm nutrition (Arai & Ito, 1964; Inokuchi *et al.,* 1967; Ito, 1967; Akter & Arghar, 1972). Bose *et al.* (1989) stated that amino acids are one of the most important constituents in silkworms that require 18 amino acids for their adequate nutrition. It is thus obvious that the study of larval growth is an important aspect of research for the insects that are economically important. Successful cocoon crops in sericulture depend mostly on a healthy larval growth. Keeping these in mind, the present experiment therefore was aimed at determining the effects of methionine and tryptophan on the larval and pupal weights, and different cocoon characters of the silkworm, *Bombyx mori* L.

Materials and Methods

The eggs of the multivoltine race of *B. mori viz*., BSRI-83/3 were collected from the Germplasm Bank of the Bangladesh Sericulture Research and Training Institute (BSRTI), Rajshahi, and were refrigerated at $5^0 \pm 1^0$ C for the synchronization of hatching in the Silkworm Research Laboratory, Department of Zoology, Rajshahi University. After hatching the larvae were brushed to wooden rearing trays and reared on fresh mulberry leaves up to second moult following the standard techniques as suggested by Krishnaswami *et al.* (1978). L-methionine) and L-tryptophan (Fluka Chemicals Ltd., Great Britain), were used at four concentrations, *viz.,* 250, 500, 750 and 1000 ppm. The chemical composition of methionine is α-amino-γ-methylmercaptobutyrate and that of tryptophan is α-amino-β-3-indolepropionate. Methionine is glittering white in colour, bitter in smell, water-soluble dust powder and tryptophan is whitish

yellow in colour and water-soluble dust powder. Different concentrations of the amino acids were prepared by adding the requisite amounts of the compound to distilled water. The leaves were treated by dipping in these solutions and dried by fanning. Treated leaves were then supplied to the *B. mori* larvae from the first day of 3rd instar and were continued up to spinning, and succeded by parental and F_1 generation. The larvae of a control batch were simultaneously reared on fresh mulberry leaves dipping in distilled water only. Feeding was supplied four times a day at six-hourly intervals. Three replicates were made in each concentration of the amino acids and untreated control batch, each with 30 larvae. To determine the effect of amino acids on the experimental insects, the weight of mature larvae, pupae, shells, cocoons, shell-ratio (%), and shell weight, and the length and denier of filaments were recorded and analysed. The experiment was carried out at a mean room temperature of $28^0 \pm 1^0$ C and 85±5 % RH.

Results and Discussion

The effects of amino acids on the growth and economic characters of the parental and F_1 generations of *B. mori* are shown in Figs. 1 and 2. Both the amino acids enhanced the performance of all the parameters in the parental and F_1 generations. The highest and lowest performances were observed at 500 and 1000 ppm concentrations respectively for all the characters throughout the parental and F_1 generations except the filament denier of the parental generation. It has also been found that amino acids supplemented food has significant effects on the larval weight ($F_{4,24}$ = 45.41 and 85.30, P<0.001 respectively for methionine and tryptophan). A significant difference was also observed in between the generations for methionine ($F_{1,24}$ = 2205.29, P<0.001) and tryptophan ($F_{1,24}$ = 2385.16, P<0.001). Methionine concentrations significantly enhanced the pupal weight ($F_{4,24}$ = 5.85 and 5.93, P<0.001 for male and female, respectively), shell weight ($F_{4,24}$ = 7.40, P<0.001 for male and $F_{4,24}$ = 4.20, P<0.01 for female) and cocoon weight ($F_{4,24}$ = 10.77 and 10.49, P<0.001 respectively for male and female). Statistical analyses showed that tryptophan produced similar effects on male and female pupal weight ($F_{4,24}$ = 8.29 and 7.85, P<0.001), male and female shell weight ($F_{4,24}$ = 9.95, and 8.06, P<0.001) and cocoon weight ($F_{4,24}$ = 14.85 and 14.93, P<0.001 for male and female, respectively). It was further observed that the male was always lighter than their female counterparts throughout the parental and F_1 generations in both amino acid treatments. The shell-ratio (%) of male and female *B. mori* was increased due to amino acids supplementation; although methionine had no significant effects, tryptophan concentrations induced significant effects ($F_{4,24}$ = 2.87 and 2.40, P<0.05 for male and female, respectively).

The filament weight and length in *B. mori* were significantly increased when the larvae were reared on methionine ($F_{4,24}$ = 7.65 and 8.08, P <0.001) and tryptophan ($F_{4,24}$ = 11.82 and 12.29, P <0.001) supplemented food. Amino acids supplementation increased the filaments denier but did not produce significant effects. Compared to parental generation, methionine and tryptophan supplemented food vigorously increased the performances of all the characters in F_1 generation except filament length. Analyses of variance conducted on all such characters as larval, pupal, shell and cocoon weights, female shell-ratio (%) and filament weight and denier showed that significant differences existed between the parental and F_1 generations for both amino acid treatments (P<0.001). A significant difference was also noted between the generations for shell-ratio in male ($F_{1,24}$ = 4.37 and 5.86, P <0.05).

The present study has clearly demonstrated that low concentrations of methionine and tryptophan supplemented food increased the performance of the economic characters in *B. mori* that are similar to the findings of Harpar *et al*. (1970), Kade & Shepherd (1948), and McKittrick (1947) on other organisms. Improved growth and economic traits in silkworm following nutritionally enriched food with low concentrations of vitamin and minerals have been observed by several workers (Khan & Faruki, 1990; Faruki & Khan, 1992; Khan & Saha, 1996; Saha & Khan, 1996, 1997) that correlate with the present results. Tryptophan, the first reported amino acid was found to be nutritionally indispensable (Willcock & Hopkins, 1906), but Hick (1926) first suggested that it could be toxic when fed in excess to the rat. Krishnappa (1987) observed that the larval, pupal, cocoon and shell weights, filament length and denier were significantly increased due to amino acid supplementation in *B. mori*. Kabila *et al.* (1994) reported that aspartic and glutamic acids increased the larval growth in silkworm. Proline and leucine significantly increased the growth of larvae, pupae and cocoon characters of *B. mori* (Saha *et al.,* 1994) and cystine enhanced the cocoon and shell weights, which are closely related to the weight and length of the filament of *B. mori* (Saha & Khan, 1995). Khan & Saha (1995) also stated that alanine at 10 ppm, and glutamine at 10 and 100 ppm enhanced the growth and beneficial characters of silkworm. In relation with the present results Khan & Saha (1995) reported that higher concentrations of alanine and glutamine produced adverse effects on different parameters of the silkworm.

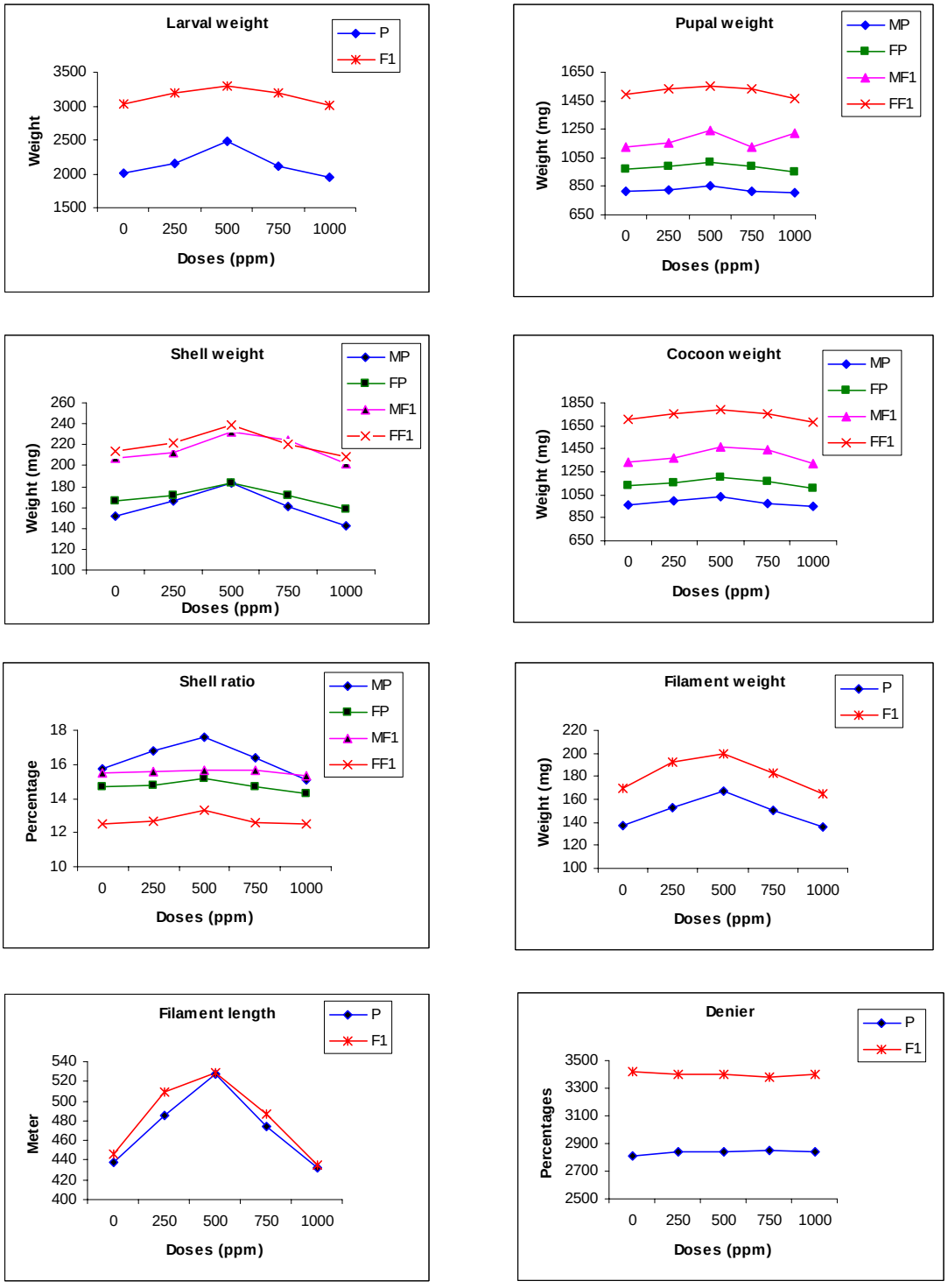

Fig. 1. Effect of methionine on larval and pupal weight, and cocoon characters in *B. mori* (P= parental generation; F_1= F_1 generation; MP= male parental; FP= female parental; MF_1= male F_1; and FF_1= female F_1)

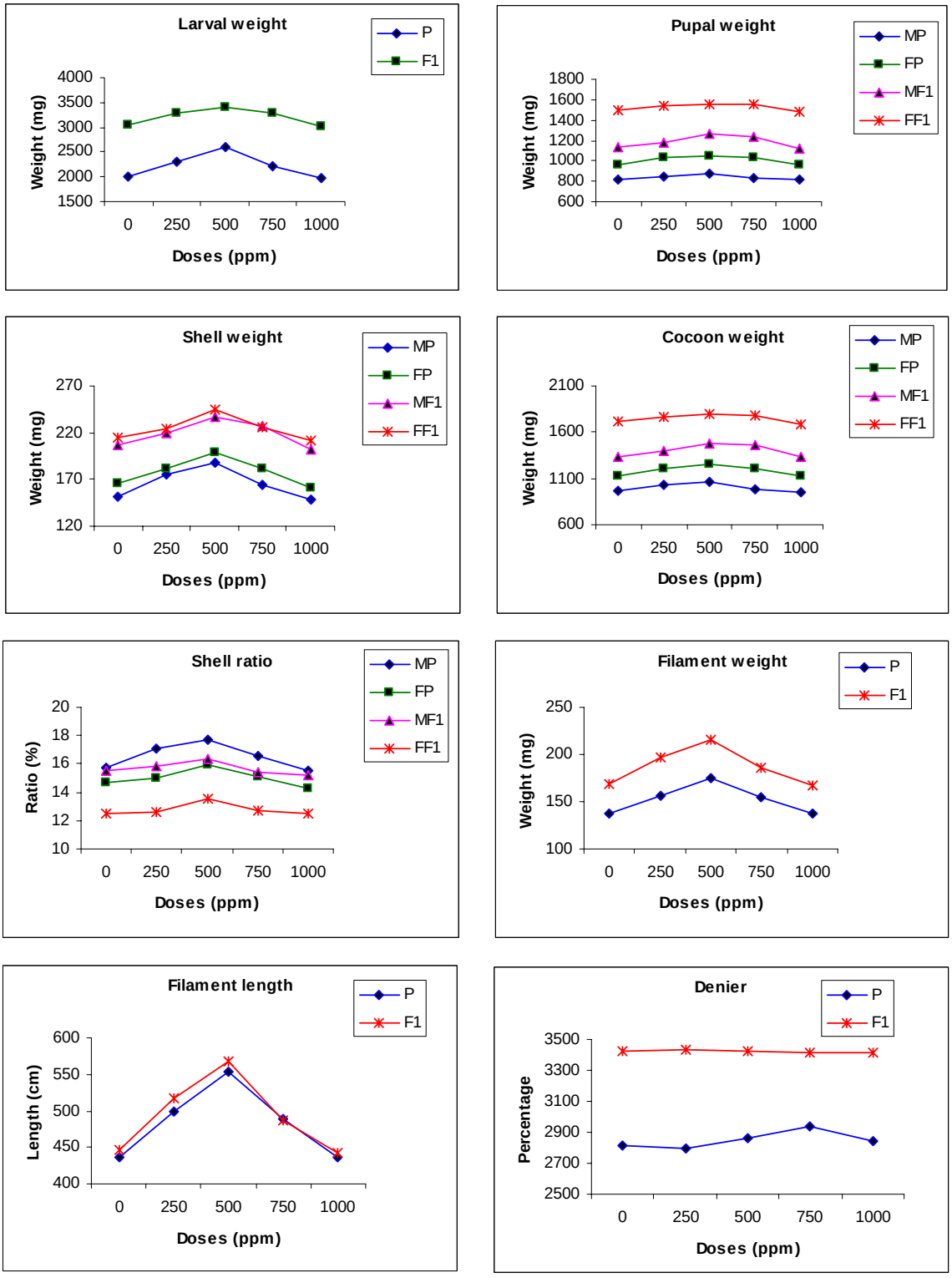

Fig. 2. Effect of tryptophan on larval and pupal weight, and cocoon characters in *B. mori* (P= parental generation; F_1= F_1 generation; MP= male parental; FP= female parental; MF_1= male F_1 and FF_1= female F_1)

Oral administration of antibiotics such as penicillin and streptomycin along with mulbery leaves increased the weight of silkworm meterage of reelable silk (Murthy *et al.*, 1951). Krishnappa (1987) also reported that post

cocoon parameter like filament length denier of silkworm was enhanced due to amino acid supplementation. According to Leonardi *et al.* (2001) nutrient absorption and its modulation are critical for animal growth. They demonstrated that leucine-methyl-ester (Leu-Ome) greatly increase the activity of the transport system responsible for absorption of most essential amino acids in the larval midgut of the silkworm *in vitro* culture, increasing larval growth, and produced cocoons which were 20% heavier than the cocoons of control. On the light of the present findings, more comprehensive works are very much solicited in this line with the array of concentrations. However, it may be recommended that low concentrations of methionine and tryptophan could be used to boost up the production of economic parameters in *B. mori*.

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