ISJ 4: 86-91, 2007 ISSN 1824-307X

#### **MINIREVIEW**

A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm Bombyx mori L.

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Accepted August 02, 2007

## Abstract

Sericulture depends on rearing of silkworm on mulberry leaves; for this reason, silk production has direct relationship with larval growth on mulberry. The quality and quantity of mulberry leaves change due to climatic conditions and field practices. One of the alternative ways of improvement of larval feeding is enrichment of mulberry leaves with supplementary nutrients such as vitamins. Many studies are accomplished on the effects of mulberry leaves enrichment with vitamin on economic traits and biological parameters, and this review is the first trial for organization of all available data related to vitamins for elucidation of this topic of science.

Key words: silkworm; mulberry leaves; enrichment; vitamin; economic traits

# Introduction

Nutrition plays an important role in improving the growth and development of the silkworm, Bombyx mori L. like other organisms. Legay (1958) stated that silk production is dependent on the larval nutrition and nutritive value of mulberry leaves plays a very effective role in producing good quality cocoons. Seki and Oshikane (1959) observed better growth and development of silkworm larvae as well as good quality cocoons when fed on nutritionally enriched leaves. Silkworm obtains its entire nutritional requirement from mulberry leaves because this insect is monophagous and can complete the life cycle on mulberry leaves exclusively. Studies of Ito (1978) determined that generally vitamins present in the mulberry leaves satisfy minimum needs of silkworm but the amount of vitamins present in mulberry leaves varies on the basis of environmental conditions, usage of fertilizers in field and mulberry varieties and other field practices. Sengupta et al. (1972) showed that B. mori requires specific essential sugars, amino acids, proteins and vitamins for its normal growth,

Water-soluble vitamins consist of members of the vitamin B complex and the vitamin C.

survival and also for the silkgland growth. Akhtar

and Asghar (1972) found that vitamins and mineral

salts played an important role in the nutrition of

silkworm. The effect of vitamin supplementation on

the growth of B. mori have been investigated by

many researchers (Majumdar and Medda, 1975; Bhattacharyya and Medda, 1981; Das and Medda,

1988; Faruki, 1990, 1998, 2005; Babu et al., 1992;

Faruki et al., 1992; Khan and Saha, 1996; Nirwani and Kaliwal, 1996, 1998; Mosallanejad, 2002;

Etebari et al., 2004; Rajabi et al., 2006a,b). Keeping

the importance of vitamins on silkworm nutrition in

mind, following review was accomplished in order to

determinate enrichment efficacy of mulberry leaves

by vitamins. A list of vitamin quoted in international

research papers are showed in Table 1.

Ascorbic acid (vitamin C)

Water soluble vitamins

Ascorbic acid has many important functions in the animal body. It is a powerful antioxidant, protecting against oxidative damage to DNA, membrane lipids and proteins. Antioxidant activity of

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**Table 1** List of used vitamins and its derivation associated with related location.

Vitamin	Derivative	Location	References
Vitamin C		Iran	Etebari <i>et al.</i> (2004)
		Bangladesh	Sarkar <i>et al.</i> (1995)
		Egypt	El-karaksy and Idriss (1990)
		India	Chauhan and Singh (1992) Babu <i>et al.</i> (1992) Gomma <i>et al.</i> (1977)
Cyanocobalmin (B12)		India	Das and Medda (1998) Majumdar and Medda (1975) Bhattacharya and Medda (1981)
Pyridoxine (B6)		Iran	Rajabi <i>et al.</i> (2006b)
		Bangladesh	Faruki (2005)
Riboflavin (B2)		Iran	Rajabi <i>et al.</i> (2006a)
Thiamine (B1)		India	Nirwani and Kaliwal (1998) Khan and Saha (2003)
	Thianomin	Bangladesh	Faruki (1998)
Folic acid (B9)		India	Nirwani and Kaliwal (1996)
	PABA	Bangladesh	Faruki and Khan (1992) Khan and Faruki (1990)
	Fe-Plus	Bangladesh	Khan and Saha (1996)
Niacin (B3)		Iran	Etebari and Matindoost (2004) Saha and Khan (1996)
Multi-vitamin compounds		Iran	Etebari and Matindoost (2005)
		India	Muniandy <i>et al.</i> (1995)
		Bangladesh	Saha and Khan (1996)
		Brazil	Evangelista <i>et al.</i> (1997)
B-complex		Bangladesh	Sarkar <i>et al.</i> (1995)
Vitamin E		Iran	Mosallanejad (2002)

ascorbic acid decreases reactive oxygen species and oxidative pressure, and, as a result, the absorption of nutritious substances in the midgut would increase (Felton and Summers, 1993). Ascorbic acid shows a particular behaviour as it is very susceptible to degradation, especially when in solution, and/or exposed to light, oxygen, and free radicals. Ito (1961) recorded relationship of ascorbic acid supplementation and growth of silkworm. The absence of ascorbic acid in the diet of first and second instar larvae postponed growth and development of silkworm. There is enough Vitamin C in mulberry leaves and ascorbic acid content of growing larvae is dependent on amount of this vitamin in diet. Supplementation of mulberry leaves more than any other vitamin ascorbic acid has been used (Etebari et al., 2004). Several research demonstrated phagostimulatory effect of ascorbic acid for insects (Ito, 1978; Dobzhenok, 1974). In silkworm a gustatory stimulating activity have been observed to some extent (Ito, 1961). Gomma et al. (1977) observed that ascorbic acid significantly increased the weight of silkworm larvae. Babu et al.

(1992) observed that the first and second instar larvae reared on 1.5 % ascorbic acid enriched mulberry leaves resulted in higher silk filament length, weight and denier values. Sengupta et al. (1972) reported that silk production increased with 1% ascorbic acid in the diet of silkworm. Etebari et al. (2004) demonstrated that feeding on mulberry leaves enriched with ascorbic acid at 3% concentration decreased larval weight due to hypervitaminosis. Chauhan and Singh (1992) showed that 1% concentration of ascorbic acid could increase the number of eggs in the silkworm. Although its lower concentration the leaves in the first and second generation also did not have positive effects on the fecundity in the silkworm.

# Vitamin B Complex

The vitamin B complex is traditionally made up of 10 members (listed below) that differ in their biological actions, although many participate in energy production from carbohydrates and fats. The optimal levels of essential vitamins such as biotin,

**Table 2** Comparison of quantitative requirements for B- vitamins of the silkworm with the amounts in mulberry leaves (From Ito, 1978)

Vitamin	Minimum amount required mg/g of dry diet	Amount in mulberry leaves mg/g of dry matter
Biotin(B8)	1	0.2-0.8
Choline	750	930-1550
Inositol	1000	4000
Nicotinic acid(B3)	20	69-99
Pantothenic acid(B5)	20	16-35
Pyridoxine(B6)	5	43-50
Riboflavin(B2)	5	13-21
Thiamine(B1)	0.5	6.7

choline, pyridoxine, panthotinate, inositol, riboflavin, thiamine, nicotinic acid have been determined by Horie and Ito (1963, 1965) (Table 2).

### Riboflavin (B2)

Riboflavin is important in promoting the release of energy from carbohydrates, fats and proteins "i.e. in the metabolic pathway for ATP production". The enrichment of mulberry leaves with riboflavin at 77 ppm enhanced certain economic characters of silkworm, and improved silk production in north climatic conditions of Iran (Rajabi et al., 2006a). Male cocoon weight (1.195 g) was greater at 77 ppm while female cocoon weight (1.622 g) was greater at 127ppm. Maximum male pupal weight was recorded at 37 ppm (0.895 g) compared to 127 ppm for the female (1.169 g). Male and female shell weight (0.311 and 0.318 g) had significant increase at 77 ppm compared to control (0.276 and 0.277 g). Male and female cocoon shell percentage reached their maximum at 77 ppm treatment, which were 26.06 % and 21.46 % respectively. Average of 50 egg weight, number of eggs for every female and hatchability percentage did not show significant difference among the treatments and the control. Similar improvements were not obtained in Natanz, in the center of Iran, place which has dry climatic conditions.

# Folic acid (B9)

Folic acid plays a major role in cellular metabolism including the synthesis of some of the components of DNA and pigment precursor (National Research Council (U.S), 1987, Chapman, 1998). Yosuhiro and Sholchi (1971) noted that the silkworm growth decreased when folic acid was eliminated from artificial diet. Nirwani and Kaliwal (1996) determined that folic acid has phagostimulatory effects with significant increase in female and male cocoon weight and shell weight.

Para-amino benzoic acid (PABA) is a growth regulator and represents one of the forms of folic acid. PABA is one of the substances belonging to the vitamin B-complex group and supports vital function in insects and especially in silkworm (Pai *et al.*, 1988). PABA supplementation has no significant effects on adult weight whereas it caused deleterious effects on their length (P<0.001) and wing-span (P<0.001) (Faruki and Khan, 1992). Fe-

PLUS® (ferrus fumarate+folic acid) supplementation significantly increased larval, pupal and adult weight in comparison with controls with lowest and highest growths obtained at the concentrations of 0.32 and 0.64 %, respectively (Khan and Saha, 1996). Larval and pupal periods decreased at lower doses (0.08 and 0.16 %) while they increased after exposure to higher doses (0.32 and 0.64 %). Fertility increased significantly in all treatments when compared to control except for 0.64 % concentration (Khan and Saha, 1996).

# Pyridoxine (B6)

Pyridoxine is necessary for the proper functioning of over 60 enzymes that participate in amino acid metabolism. It is also involved in carbohydrate and fat metabolism. pyridoxine or its derivatives no larva reached the third instar under aseptic condition. Pyridoxine is important in protein metabolism and its deficiency in mammals results in decrease in phosphorylases (National Research Council (U.S), 1987). Faruki (2005) reported that mulberry leaf fortification by pyrol<sup>®</sup> (Pyridoxine hydrochloride HCl.25) in various concentrations from the third instar significantly reduced the fecundity. In this experiment it was found the lowest number of eggs was produced at the lowest concentration (10 µg/ml) and at higher concentrations it remains lower than in the control. Banerjee and Khan (1992) observed that vitamin B6 enhances the oviposition of the silkworm infected by Bacillus thuringiensis var. kurstaki but the rate was lower than the control. Faruki (2005) reported that higher concentration of vitamins reduced the fecundity and fertility of silkworm. Further, the percent reproduction control (PRC) in lower concentrations was more than higher concentrations (Faruki, 2005). The reproductive success in lower concentrations was prominent with compared with the higher concentrations (Faruki, 2005; Etebari and Matindoost, 2005). Rajabi et al (2006b) showed that in north climatic conditions of Iran, larval weight reached a maximum 2.601 g at the end of 5<sup>th</sup> instar. Effective rate of rearing (ERR) was higher (75.33 %) at 100 ppm concentration compared with other concentrations (10, 500 and 1000 ppm). Larval and silk indices reached their maximum at 100 ppm concentration while pupal and adult indices reached their maximum at 1000 ppm concentration in male and female. Larval duration was longer (622.5 h) in treatments against control (604.5 h). Treatment differences were recorded also in respect of other economic characteristics (Rajabi *et al.*, 2006b).

## Nicotinic acid (B3, Niacin)

Niacin is important for the release of energy from carbohydrates and fats, the metabolism of proteins and production of several hormones (National Research Council (U.S), 1987). Horie and Ito (1965) showed the required level of niacin for silkworm is highly regulated to the most appropriate level of 33  $\mu$ g/l of dry weight and the increase of niacin reduced the larval weight. Horie (1995) showed a reduction of requirement pattern with increasing larval weight. Niacin caused significant deleterious effects on larval growth. Cocoon parameters such as cocoon weight, pupal weight and cocoon shell weight also showed significant decrease in all treatments (Etebari and Matindoost, 2004). Furthermore, mulberry leaf enrichment with nicotinamide (10, 20 and 30 g/l) from first instar caused intensive mortality in the larval growth and only 1.2% larvae could reach the 5<sup>th</sup> instar. High doses (20 and 30 g/l) of nicotinamide killed all larvae before entering the 5th instar (Etebari and Matindoost, 2004). Horie and Ito (1965) reported that the analogues of niacin, 4-acetyl pyridine interrupts the larval growth when added to mulberry leaves and acted as an antimetabolite. Niacin with 0.5 g/l acted as an antifeedant for silkworm larvae and decreased their metabolism (Etebari and Matindoost, 2004).

## Thiamine (B1)

Thiamine is important for energy metabolism (National Research Council (U.S), 1987). Nirwani and Kaliwal (1998) reported that the weight of larvae and silk glands in all the thiamine fed groups had not shown any significant changes. On the other hand, larval duration, cocoon weight, shell weight and fecundity increased significantly. In opposition with what observed for ascorbic acid and folic acid, it has been found that thiamine has no phagostimulatory effect on silkworm (Horie and Ito, 1963; Nirwani and Kaliwal, 1996). Faruki (1998) reported that the thiamine derivative thianomin enhanced the growth of silkworm larvae, pupae and adults at all concentrations used (50, 100, 500 and 1000 ppm). Mulberry leaf enrichment with thianomin increased the growth indices such as larval, pupal and adult weight. Silk index increased too in all treatments. Thianomin increased cocoon characters such as cocoon weight, shell weight, cocoon shell length and breadth significantly (P< 0.05).

# Cyanocobalamin (B12, Cobalamin)

Cyanocobalamin plays important role in silkworm because it is a co-factor of propionate metabolism which is important substrate for biosynthesis of juvenile hormone in insects (Halarnkar and Blomquist, 1989). Vitamin B12 does not occur in mulberry leaves but considerable amount of this vitamin was observed in larvae and pupae. It is believed that actinomycetes in the gut

lumen produce vitamin B12. Das and Medda (1998) reported that supplementation of mulberry leaves with B12 vitamin could increase the synthesis of nucleic acid and proteins in the silkglands of the silkworm.

#### Pantothenic acid (B5)

Pantothenic acid is the precursor of coenzyme A that is vital for the metabolism of carbohydrates, the synthesis and degradation of fats, the synthesis of sterols and the resultant steroid hormones, and the synthesis of many other important compounds (National Research Council (U.S), 1987).

## Biotin (B8, Vitamin H)

Biotin has an important role in carbohydrate and fat metabolism. It has been showed that biotin is one of the essential vitamins for the silkworm *B. mori* (Horie *et al.*, 1966). It has important role in the synthesis of fatty acids in the silkworm and it is confirmed that minimal optimal level of biotin for growth and survival of the silkworm was much lower than those of other vitamins including nicotinic acid, pantothenic acid and pyridoxine (Horie and Ito, 1965). It is identical with the minimal threshold for alteration of fatty acid composition (Horie and Nakasone, 1968). However, to the best of our knowledge, research reports on enrichment mulberry leaves with biotin are not available.

#### Choline and inositol

Choline is traditionally not a vitamin; however it was identified as part of vitamin B complex. Inositol is an important part of signaling mechanism that transmits information from outside to the inside of cells. It is generally considered dispensable in insect diets. Choline and inositol are required by silkworm in higher level because they are lipogen substrate, also involved in the production of cell membranes. However, research reports on enrichment mulberry leaves with choline and inositol do not seem to be available.

## Multi-vitamin compounds

Saha and Khan (1996) described the extensive effects of multi-vitamin compounds as diet factors on growth interruption and the decrease of cocoon economical characteristics. It is showed that multivitamin and mineral compounds could increase the food intake, growth and conversion efficiency of silkworm (Muniandy et al., 1995). Evangelista et al. (1997) reported that the larval and cocoon weight increase under multi-vitamin compound treatment, but did not have any positive effects on cocoon shell weight. Feeding with multi-vitamins in the larval stage adversely affected the hatchability of eggs. Multi-vitamins even though could increase some biological characteristics in silkworm, did not influence the economical and yield contributing parameters. Etebari and Matindoost (2005) reported that feeding of silkworm on mulberry leaves enriched by with multi-vitamins from 4<sup>th</sup> instar increased female cocoon shell weight in 2.5%

concentration, while female pupal weight increased in 1% concentration. Male and female shell ratio did not increase compared to controls.

#### **Fat-soluble vitamins**

Fat-soluble vitamins consist of the A, D, E and K vitamins. Among these, enrichment of mulberry leaves with vitamin A, D or K for silkworm do not seem to have been studied.

#### Vitamin E

 $\alpha$ -tocopherol is slightly effective in increasing the number of eggs laid by moths and  $\beta$ -carotene has also some growth-promoting effect (Ito, 1978). Enrichment of mulberry leaves with E vitamin did not have significant effect on food consumption in silkworm larvae (Mosallanejad, 2002).

#### **Concluding remarks**

This review summarizes data showing that enrichment of mulberry leaves with various vitamins have different effects on economic traits and biological parameters of the silkworm. However, reported effects depend also on weather condition, larval stage treated, type of vitamin, varieties of mulberry and silkworm race studied. This possibly indicates the need for elaborating comprehensive studies on the subject. It is advisable that we keep in mind the negative effects of enrichment beside its positive effects on economic traits and biological parameters. Moreover, each intervention on the natural content of silkworm food should take into account also costs, environmental safety and large scale feasibility.

# Acknowledgements

Our studies cited in this paper were supported by the Biotechnology Institute and Iran Silkworm Research Center.

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