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[Review]

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Different Aspects of Mulberry Leaves Supplementation with Various Nutritional Compounds in Sericulture

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The supplementation of mulberry leaves, with the aim of yield, enhancement using vitamins, minerals and other compounds have generally been attended from 1990s and many researches have been conducted. More than 30 different compounds from complementary nutrients have been analyzed and in different regions where different climates various results have been achieved. This review has attempted to discuss the results of different works on enrichment of mulberry leaves with supplementary compound. Generally the most effects of these compounds are in the regions where productive parameters are low. In the areas that follow a normal pattern in economical parameters the enrichment of the leaves havent had significant economical effects.

Key words: Nutrition, Mulberry leaves, Supplementation, Silkworm

Introduction

Feeding of an organism supplies the energy for growth, development, reproduction and many of its other needs (Chapman, 1998). Most of the insect species have similar nutritional needs because of the similarities in the main chemical compounds and also metabolic pathways of their body. Amino acids, proteins, lipids, carbohydrates, nucleic acid, minerals, vitamins and water are the most important nutritional needs of the insects which they are able to make some of these nutrients by themselves and some of their needs has to be provided by eating foods or by symbiotic organisms which they harbour (Bursell, 1970; Chapman, 1998).

The silkworm nutritive requirements are very different and the most of it is supplied by feeding on mulberry leaves. Although the mulberry leaves are complete diet for silkworm it is possible that some deficiencies occur for different reasons. The supplementation of the leaves results higher yield because the production of good quality and quantity of silk depends on larval nutrition and healthiness of the larva, which are partially influenced by the nutritive value of mulberry leaves (Ito, 1978).

The metabolic pathways in the plant are often eliminated from the supplementation procedure and the substances directly enter the insect body affecting the metabolism. The supplementation, with the aim of yield enhancement using vitamins, minerals and other compounds have generally been attended from 1990s and many researches have been investigating them. More than 40 different compounds with supplementary nutritients have been analysed at different regions with different climates various results have been achieved.

About 55% of the researches on this topic have been done in India and the rest of it in Bangladesh, Iran, Pakistan, etc. The raw silk production per hectare of the land is about 52 kg in China, 40 kg in Japan and just 14 kg in India (Bajpeyi *et al.*, 1991). The low productivity is mainly attributed to low mulberry yield and poor quality of leaf (Chamundeswari and Radhakrishnaiah, 1994). Thus it appears that to boost up silk production, some supplements are essential to the silkworm in addition to their natural food depending upon the agroclimatic conditions. This review has attempted to discuss the results of different works on enrichment of mulberry leaves with supplementary compounds such as vitamins, amino acids, minerals, etc. Table 1 shows a list of different supple-

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Table 1. The list of some supplementary compounds utilized in sericulture

Supplementary compounds	Location	Author (s)
Vitamins		
Ascorbic Acid	Egypt	El-Karaksy and Idriss (1990)
	India	Babu *et al.* (1992); Chauhan and Singh (1992)
	Bangladesh	Sarker *et al.* (1995)
	Iran	Etebari (2002); Etebari *et al.* (2002); Talebi *et al.* (2002)
B-complex	Bangladesh	Sarker *et al.* (1995)
Cyanocobalamin (B 12)	India	Majumdar and Medda (1975)
		Bhattacharyya and Medda (1981) Das and Medda (1988)
Folic Acid	India	Nirwani and Kaliwal (1996)
Multi Vitamin	India	Muniandy *et al.* (1995)
	Bangladesh	Saha and Khan (1996)
	Berzail	Evangelista *et al.* (1997)
	Iran	Etebari *et al.* (2002)
Vitamin E	Iran	Mosalanejad *et al.* (2002)
Thiamine	India	Nirwani and Kaliwal (1998)
Minerals		
Ammonium Dihydrogen Phosphate	Pakistan	Ashfaq *et al.* (1996)
Cobalt Chloride	India	Chakraborthi and Medda (1978) Bhattacharyya and Medda (1981); Sailaja *et al.* (1997)
Calcium	Pakistan	Akram (1991)
Calcium lactate	Bangladesh	Khan and Saha (1997)
Copper sulphate	India	Magadum *et al.* (1992)
Ferrous fumarate	Bangladesh	Khan and Saha (1996)
Iron (?)	Pakistan	Sabir (1991)
Iodophor	India	Venkataramana *et al.* (2001)
Potassium Iodide	India	Chakraborthi and Medda (1978); Majumdar (1982)
		Magadum (1992)
	Bangladesh	Sarker *et al.* (1995), Qader *et al.* (1993)
Potassium Iodide + Nacl	Bangladesh	Qader *et al.* (1993)
Potassium permanganate	India	Gupta *et al.* (1990)
Potassium sulpahte	India	Nirwani and Kaliwal (1996)
Magnesium	Pakistan	Zaman *et al.* (1996)
Multi Mineral	Iran	Etebari and Fazilati (2003)
Nickle	India	Chamundeswari and Radhakrishnaiah (1994)
Zinc chloride	India	Hugar and Kaliwal (2002); Balamani *et al.* (1995)
		Hugar *et al.* (2002)
Ferrous sulphate	India	Nirwani and Kaliwal (1995)
Magnesium sulphate	India	Nirwani and Kaliwal (1995)
Nickle chloride	India	Hugar *et al.* (1997)
Potassium nitrate	India	Goudar and Kaliwal (2000)
$Mg(SO_4) + KNO_3$	India	Goudar and Kaliwal (2002)
Potassium permanganate	India	Bhattacharya and Kaliwal (2003)
Amino Acids And Nitrogenous Compounds		
Alanine	Bangladesh	Sarker *et al.* (1995); Khan and Saha (1995)
Aspartic acid	India	Kabila *et al.* (1994)
Glycine	Egypt	Moustafa and El-Karkasy (1988)
	Bangladesh	Sarker *et al.* (1995)
	Iran	Etebari (2002)
Glutamic Acid	Iran	Etebari (2002)

Table 1. Continued

Supplementary compounds	Location	Author(s)
Other compounds		
Antibiotics	Bangladesh	Saha *et al.* (1995)
Casitose	India	Ravikumar *et al.* (2002)
Gibberellic acid	India	Das *et al.*(1993)
P-soyatose (Hydrolyzed protein)	India	Krishnan *et al.* (1995); Vanisree *et al.* (1996)
Rain water	India	Thangavelu and Bania (1990)
Sugar (sucrose)	Bangladesh	Sarker *et al.* (1995)
Thyroxin	India	Majumdar and Medda (1975); Rajashekhargouda *et al.* (1998)
Vertebrate Sex Hormone	Bangladesh	Saha and Khan (1997); Khan *et al.* (2002)
Urea	Iran	Etebari (2002)

mentary compounds that were utilized along with the mulberry leaves in different region.

Vitamins

Vitamins are one of the organic compounds used by organisms and limited amount of them is essential for natural performance. Vitamins play different roles in insects lives. The lack of their presence interrupts enzymatic reactions because they often act as a co-enzymes. Some vitamins act in the immunity system of the insects. Nutrient needs of insects are very much depended on the sex and the stage of growth. During fifth instar of silkworm larvae, the enhancement of thiamin, riboflavin and pyridoxine vitamins in the haemolymph is observed which this is due to their essential need to these vitamins at this stage of larval life. Because fifth instar is the most important stage of silk production (Zang and Ma, 1991). It has been reported that riboflavin, niacin, biotin, coline, inositol, panthotonic acid, pyridoxine and thiamin from B vitamins are essential for silkworm (Ito, 1978; Horie, 1995). Studies of Ito (1978) determined that generally the vitamins present in the mulberry leaves satisfy minimum needs of silkworm but the amount of these vitamins in mulberry leaves depends on different climate, seasons, mulberry varieties and the use of fertilizers in the field.

Supplementation of mulberry leaves more than any other vitamin ascorbic acid has been used. Although folic acid, cyanocobalamine, niacin, vitamin E and multivitamins have also been studied (El-Karkasy and Idriss, 1992; Mosallanejad *et al.*, 2002). The functions of ascorbic acid in the insect is not fully understood but it is assumed that like mammalians, they are involved in tyrosine metabolism, collagen synthesis, steroids synthesis, carnithin synthesis, nervous balance, feeding stimulation and/or immunity system reactions, detoxification they also act as an important antioxidant (Navon, 1978; Dong and Zheng, 1984; Felton and Summers, 1993, 1995; Timmermann *et al.*, 1999).

Ascorbic acid has highly improved the silk yield and different results have been achieved from different researches (Sarker *et al.*, 1996; Etebari, 2002; Talebi *et al.*, 2002; Etebari *et al.*, 2002, 2004). Babu *et al.* (1991) and El-Karkasy and Idriss (1990) have reported that supplementation of mulberry leaves with 0.25 – 2% ascorbic acid can increase the larval weight, cocoon weight and cocoon shell weight. Chauhan and Singh (1992) have also showed that 1% concentration of ascorbic acid could increase the number of eggs in the silkworm. Although its lower concentrations did not have any effects on fecundity and supplementing the leaves in the first and second generation also did not have positive effects on the fecundity in the silkworms.

In conclusion it is clear that the larval stage and the type of the leaves used have the most effects on the obtained results and these can not easily represent in large scale.

Muniandy *et al.* (1995) have used the registered compound of filibon, which contained vitamins and minerals on the silkworm *B. mori* larvae. Consequently after the enhancement of absorption, the metabolism, growth the larvae and the length of larval stage decreased but the post cocoon parameters were considerably improved.

Nirwani and Kaliwal (1996a) have reported that the dietary supplementation of folic acid improved the economical characteristics of the silkworm by 20 – 30%. Folic acid as a co-factor is essential in the reaction of transforming phenyl alanine into tyrosine. It was also reported that dietary supplementation with folic acid to silkworm larvae did not significantly increase the glycogen content of the fat body, whereas the haemolymph trehalose content increase significantly in all the treated groups. 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. It was observed that the protein content of the haemolymph was also significantly increased in the folic acid treated groups. This also influences increase in the silkgland weight and cocoon shell weight. This inference supports the views of the earlier workers (Bhattacharyya, 1981; Wicker *et al.*, 1985). Nirwani and Kaliwal (1998) have also reported that oral supplementation with thiamine with different concentrations to silkworms larvae resulted in a significant increase in the larval duration, cocoon weight, shell weight and fecundity in the silkworm, *B. mori.* They have also showed a significant increase in the fat body glycogen and haemolymph trehalose contents, whereas, at the higher concentrartion the protein content of the fat body and haemolymph decreases. It suggests that the thiamine have stimulatory effect on silkworm larvae.

Supplementation of mulberry leaves with vitamin B_{12} which is not present in the mulberry leaves could increase the synthesis of nucleic acids and proteins in the silkgland of the silkworm, *B. mori* (Das and Medda, 1988). This vitamin and folic acid are essential for the synthesis con of nucleic acid and cyanocobalamine is another active form of vitamin B_{12} is a very important co-factor for the metabolism of propionate in insect. Propionate is an important substrate for biosynthesis of juvenile hormone (Halarnkar and Blomquist, 1989).

But we always have to keep in mind the effects of hypervitaminosis in the insects. The enhancement of vitamins to 8 times in the diet of peach green aphid *Mysus persicae* not only increases the performance but it also causes the death of nymphs and intensive reduction of female fecundity (Mitsuhashi and Saito, 1996). Etebari (2002) has also showed that hypervitaminosis does not only cause the enhancement of larval and cocoon weight but it also has many negative effects. Niacin with the concentrations of 0.5 g/lit when treated on larvae acts as an anti-feedant and decreases their metabolism. In these larvae high mortality was observed and due to the intensive decrease of cholesterol, which is a substrate for ecdyson synthesis, the ecdysis was disturbed and the length of larval stage increased 36 days more than control. In these larvae hyperuricemia has occurred which that itself causes the interruption of metabolism.

Etebari *et al.* (2002) have demonstrated that the 3% treatment of ascorbic acid when utilized from the beginning of the first instar not only did not decrease the cocoon parameters but it also decreased the egg number. McFarlane (1992) has suggested that the high levels of ascorbic acid can inhibit the spermatogenesis in some insects and decreases the viability of produced eggs. Kaur and Sirvastava (1995) have reported that the excessive supplementation with ascorbic acid could interrupt amounts of vitamin C in its life cycle *Dacus cucurbitae.* These negative effects are also reported from other insects too (Dobzhenok, 1974; Navon, 1986; Saha and Khan, 1996; Etebari and Fazilati, 2003).

In the supplementation of mulberry leaves often vitamin solution after being made until the consumption time would be kept under different conditions. It is assumed that environment temperature, light, humidity etc could have various effects on the obtainable results. For example, ascorbic acid in the aquatic solution can quickly transform into dehydroascorbic acid and this could cause the different results. In some references dehydroascorbic acid is mentioned as an anti-vitamin which its increase in the diet causes mortality of olive fly *Dacus oleae* (Tsiropoulos and Cavalloro, 1983; Tsiropoulos, 1985.)

Minerals

The requirement of different minerals in various insects has been investigated (Chapman, 1998; Ito, 1978). Minerals include almost 10% of mulberry leaves. It has been reported that 28% of the silkworm larval structure in different ages include the absorbed minerals (Ito, 1978). So the minerals are one of the most important components of the silkworm and other insects diet. Deficiencies in nitrogen, phosphorus and potassium affected the growth and nutrient content of the mulberry leaves that in turn affected the growth and economic characters of the silkworm. In particular, a deficiency in phosphorus affected the uptake of other elements by the mulberry leaves and had an adverse effect on the cocoon and silk characters (Radha *et al.*, 1988). Supplementation of mulberry leaves with mineral salts has the most effectiveness between the other complementary compounds and many researchers have studied this aspect of enrichment. Mineral salts include more than 40% of utilized compounds in these studies (Ito and Niminura, 1966a, b; Horie *et al.*, 1967; Viswanath and Krishnamurthy, 1982; Loknath *et al.*, 1986; Sabir, 1991; Hugar and Kaliwal, 2002).

Khan and Saha (1997) have showed that treatment with calcium lactate resulted in higher fecundity and egg viability in *B. mori.* The larvae were fed by mulberry leaves supplemented with multi minerals resulted that the larvae's weight at third day of fifth instar had significant difference with controls, but the weight of silkgland didn't show such difference. The total protein had significantly increased in all multi mineral treatments (Etebari and

Fazilati, 2003). Also it was suggested that protein metabolism had been activated in treated larvae with cobalt (Sailaja *et al.*, 1997).

Supplementation of mulberry leaves with potassium salts have been studied by many researchers (Majumdar, 1982; Gupta *et al.*, 1990; Magadum, 1992; Qader *et al.*, 1993; Nirwani and Kaliwal, 1996). Gupta *et al.* (1990) have reported that rearing *B. mori* on mulberry leaves dipped in 0.001% potassium permanganate as a disinfectant improved larval survival, larval and pupal weights, and cocoon quality and weight. This investigations is not limited to mulberry silkworm, the other wild types of silkworm have also been used for this study (Govindan *et al.*, 1989).

Zaman *et al.* (1996) have reported that supplementation of mulberry leaves with 0.15% Mg and 0.20% N solution can improved biological performance of the silkworm larvae. The maximum food (54.22 g/10 larvae) was consumed and cumulative coefficient of utilization was also significantly higher (55.90%) in the larvae fed with 0.15 % Mg and 0.20% N treated leaves. Body weight and body length were also higher in the same treatment ultimately resulting in increased silk yield. Mulberry leaves treated with different concentration of phosphorus alone and in combination with 0.1% nitrogen solution, when fed to silkworm larvae throughout its larval life, revealed that the leaves treated with 0.1% P and 0.1% N concentration yielded the best larval development on one hand and silk yield on the other (Ashfaq *et al.*, 1999).

Nirwani and Kaliwal (1995) have reported that supplementation of mulberry leaves with ferrous and magnesium sulphate increases the larval and adult biological performance. It has been reported that oral supplementation with nickel chloride to the bivoltine silkworm 4^{th} and 5^{th} instar larvae resulted in a significant increase of silkgland weight, female cocoon weight, male cocoon weight, shell weight and its ratio, filament length, filament weight and its denier and moth emergence percentage (Hugar *et al.*, 1997). Have been reported that magnesium, calcium, phosphorous, potassium, iron, manganese and zinc were essential elements required by the silkworm, *B. mori.* Feeding with calcium, magnesium, manganese, sulphate, iron, potassium, iodide and iodized salt, manganese and ferrous sulphate to silkworm, shown to increase the economic parameters and decreases the larval duration (Bajpeyi *et al.*, 1991; Islam and Khan, 1993; Qader, 1993; Hugar and Kaliwal, 1999; Gouder and Kaliwal, 2000c).

Gouder and Kaliwal (2001b) have also reported topical application with minerals falls of magnesium sulphate and potassium nitrate showed significantly decrease larval weight, silkgland weight with other decreased larval, cocoon and adult parameters. Gouder and Kaliwal (2001b) have also reported that supplementation with potassium nitrate significantly increased the fat body glycogen, haemolymph trehalose, protein and lipids in the silkworm, *B. mori.*

Recently it has been reported that oral supplementation with potassium permanganate to fifth instar larvae of the silkworm, *B. mori* resulted in a significant increase in the glycogen content of the fat body and haemolymph trehalose. The protein and the lipids content of the fat body and haemolymph was also significantly increased in all the potassium permanganate treated groups. It has also reported that supplementation with potassium permanganate increases the larval weight, silkgland weight along with other enhanced cocoon and adult parameters (Bhattacharya and Kaliwal, 2003).

As shown in Table 1 a lot of minerals can be used in mulberry field as fertilizer that moreover increased the silk yield, the production of good quality mulberry leaves were improved. Different investigators studied the effects of foliar spray of nitrogen, iron, zinc and other micronutrients on the silkworm and mulberry leaves production (Santhy and Sukumar, 1995; Sarker and Absar, 1995; Bose and Majumder, 1996).

Amino acids and nitrogenous compounds

Nitrogen, which is the main component of amino acids and proteins, is one of the essential elements for the growth and development of insects, which generally is obtained by feeding although that some insects are able to maintain their need in some other processes. Silkworm uses 65% of absorbed nitrogen through 5^{th} instar for silk production. Therefore, nitrogen sources present in the diet can have high effects on larval growth and cocoon production (Horie and Watanabe, 1983; Unni *et al.*, 2000).

Zaman *et al.* (1996) have demonstrated that mulberry leaves enrichment with 2% nitrogen causes the weight increase of the silkworm larvae. Karowe and Martin (1989) have reported that the relative growth of *Spodoptera eridania* is independed from the amount of nitrogen in the diet.

Main studies on mulberry leaves supplementation with nitrogenous compounds and amino acids and the evaluation of their effects on silkworm rearing have been conducted and different conclusions have been obtained (Sarker and Absar, 1995; Yeasmin *et al.*, 1995; Zaman *et al.*, 1996; Basit and Ashfaq, 1999; Etebari, 2002; Etebari and Fazilati, 2003). Amino acids have multiple metabolic functions in the living cells. Diversity in the amount of free amino acids of haemolymph is generally affected by diet. Variation in the type of amino acids occurs due to different reasons. Changes in these compounds cause different signs.

In host plant of silkworm at different years and regions, type of irrigation and soil fertilizers many fluctuations are observed in the amount of amino acid, which can affect the rearing efficiency (Sharma *et al.*, 1995). Silkworm absorbs 72 – 86% of amino acids from the mulberry leaves and in the females more than 60% of absorbed amount is consumed for silk production (Lu and Jiang, 1988). Intestine absorption of amino acids is one of the most important stages of nitrogen metabolism in insect body. Absorption of these compounds in the midgut of moth larvae especially in silkworm is an active mechanism depended to potassium ion and the alkaline pH of exogenous part of peritrophic membrane. The enrichment of leaves with amino acid could increase the efficiency relatively but it cannot be expected that always there is a positive correlation between the supplementation and biological efficiency.

Kabila *et al.* (1994) have described that adding aspartic acid with 1 and 2% concentrations to leaves increases the economic traits in silkworm. Sarker *et al.* (1995) have demonstrated that with utilizing alanine (0.5%) and glycine (0.5%) observed more than 14% weight increase in 5^{th} instar larvae. Etebari (2002) has demonstrated that 0.5% glycine if treated from beginning of 5^{th} instar larvae could cause maximum of 12.3% weight increase. Supplementation glutamic acid with 1% concentration was also able to increase the larval weight by 10% and from 4.21 in control it reaches to 4.64 g. The glutamic acid also causes the enhancement of food consumption in the larvae and increases the absorption percentage in a way that the amount of glucose, cholesterol and triacylglyserol of haemolymph of treated larvae is much more than the control. But considerable changes have not occurred in the amount of protein although it is assumed that with the increase of amino acid and nitrogenous compound in the diet the haemolymph protein increases. Khan and Saha (1995) have also suggested that supplementation of leaves with 100 and 1000 ppm alanine and 1000 ppm glutamine decrease many biological characteristics.

Urea, which has more than 40% nitrogen in its structure, has many uses as a chemical fertilizer in agriculture. Spraying mulberry leaves with 5% urea solution in the field increases the amount of nitrogen in leaves. Many chemicals fertilize and micronutrients are suggested for spraying in the field (Sarker and Absar, 1995; Yeasmin *et al.*, 1995). In this procedure, the plant absorbs the solution from cuticle and spiracles much faster and enters them into metabolic pathways.

In enrichment of leaves with supplementary compounds the metabolic pathway of plant is not considered and only the residue is important. Sarker and Absar (1995) have found that with spraying 0.5% urea on mulberry leaves in the field, the weight of silkworm larvae grown on then shows more than 11% increase which this value in two studied Indian strains were almost constant. In this group of larvae the cocoon weight increased by 2.4% and the length of silk fiber by 9%. Etebari (2002) suggested that if the same concentration would be treated on leaves separated from tree and are fed to the larvae, not only does not increase the biological performance of the insect but also significant decrease was observed. Although 0.01% concentration of urea could increase the weight of treated larvae by 10.6% but there was not considerable difference in cocoon characteristics. Urea residue on leaves and the urea in the plant after consumption by larvae are analyzed by urease and NH_3 produced in nitrogen metabolism and glutamic acid synthesis is entered (Inokuchi, 1992). While the urea treated in the farm has entered into plant metabolism and the other nitrogenous compounds that are essential for the plant are produced.

Supplementation of low concentrations of urea increased the food consumption in the silkworm, *B. mori* (Ashfagh *et al.*, 1996; Zaman *et al.*, 1996). Etebari (2002) has demonstrated the increase of consumption rate by 17 and 23% using 0.1and 0.01% concentrations of urea respectively although there were different reports showing the reduction of consumed food in the insects treated with nitrogenous compounds (Baker, 1975; Brodbeck *et al.*, 1999). Zaman *et al.* (1996) have reported that the cocoon shell weight reached to 0.236 g in the larvae that fed with 0.2% nitrogen which shows 52% weight increase when compared with that of controls. Also when larvae fed on leaves supplemented with 0.2% nitrogen and 0.15% magnesium the weight of cocoon shell reached to 0.301 g, which shows more than 94% growth that was very interesting. The point which is important in most of the analysis is that the most efficiency of mulberry leaves supplementation, is observed in the regions or conditions that the cocoon weight or as a whole the performance is low. Tables 2 and 3 respectively show the most efficiency of different compounds on mulberry leaves enrichment on pre and post cocoon parameters. The highest percentage of variations occurs in those that have the lowest performance and in those with appropriate growth supplementation could not have much effects on them.

The point, which must be taken in mind, is the procedure of nitrogen transformation is depended on cellular structure. Now this question comes in the mind that whether the larvae always enter into the metabolism of protein the enhancement of nitrogen which is added to their diet in different forms? Four stages could assumed for insects to plant proteins and their transformation to cellular structure:

1. The consumption of protein or nitrogenous com-

Table 2. The maximum effect of some supplementary compounds on pre-cocoon parameters of silkworm

Compounds and concentration	Larval weight (g)			Silkgland weight (g)			Larval duration (hr)			Reference
	Tre.	Con.		Tre.	Con.		Tre.	Con.		
Alanine (10 ppm)	1.426	1.385	(3)*[1]	-	-	-	463	503	(-8) ns	Khan and Saha (1995)
Ascorbic Acid (2%)	4.468	3.927	(13.7)*	-	-	-	-	-	-	Etebari (2002)
Copper Sulphate (30 ppm)	2.176	1.446	(50)*	0.456	0.329	(39)*	766	790	(-3) ns	Magadum *et al.* (1992)
Filibon® 3% (Multi vitamin & mineral)	2.075	1.635	(27)*	0.422	0.202	(108)*	188	210	(-10) ns	Muniandy *et al.* (1995)
Folic Acid (100 µg/ml)	4.498	3.627	(24)*	2.042	1.652	(23)*	675	687	(-2) ns	Nirwani and Kaliwal (1996a)
Glutamic acid (1%)	4.640	4.218	(10)*	-	-	-	-	-	-	Etebari (2002)
Glycine (0.5%)	4.735	4.218	(12.3)*	-	-	-	-	-	-	Etebari (2002)
Lynestrenol® (400 ppm)	1.921	1.812	(6)*	-	-	-	-	-	-	Khan *et al.* (2002)
Mg (0.15%) + N (0.2%)	5.017	3.357	(49)*	-	-	-	-	-	-	Zaman *et al.* (1995)
Multi mineral® (10%)	5.124	4.522	(15)*	1.75	1.80	(-3) ns	-	-	-	Etebari and Fazilati (2003)
Potassium iodisede (0.1%)	2.440	2.535	(-4) ns	-	-	-	528	546	(-4) ns	Qader *et al.* (1993)
Potassium Sulphate(1 mg/ml)	3.686	2.567	(43)*	1.344	1.001	(34)*	789	810	(-3) ns	Nirwani and Kaliwal (1996b)
Soymilk+Sugar 10% + Vitamin B (0.5%) C (1%)	3.62	2.89	(25)*	0.778	0.572	(47)*	-	-	-	Sarker *et al.* (1995)
Thiamine (200 µg/ml)	3.836	3.627	(5)*	1.69	1.65	(2)*	696	687	(+1)	Nirwani and Kaliwal (1998)
Thyroxine (2.5 ppm)	2.90	2.60	(8.3)*	-	-	-	172.5	208	(-16) ns	Rajashekhargouda *et al.* (1998)
Zinc Chloride (30 µg/ml)	4.520	4.21	(7)*	1.39	1.4	(-0.8)	618	621	(-1) ns	Hugar and Kaliwal (1999)
Ferrous Sulphate (2000 µg/ml)	2.868	2.696	(11)*	1.170	0.934	(17)*	795	808	(-2) ns	Nirwani and Kaliwal (1995)
Magnesium Sulphate (2000 µg/ml)	3.417	2.696	(33)*	1.137	0.934	(13)*	826	808	(-1) ns	Nirwani and Kaliwal (1995)
Nickel Chloride (30 µg/ml)	4.551	4.213	(7)*	1.382	1.237	(11)*	-	-	-	Hugar *et al.* (1997)
Potassium Nitrate (500 µg/ml)	2.770	2.586	(7)	0.732	0.618	(18)*	675	684	(-2) ns	Goudar and Kaliwal(2000)
$MgSO_4$ + KNO_3 (300 µg/ml)	2.456	2.652	(-8)*	1.072	1.134	(-6) ns	656	650	(0) ns	Goudar and Kaliwal(2001)
$KmnO_4$ (100 µg/ml)	3.067	2.779	(10)*	1.341	1.180	(13)*	686	696	(-2) ns	Bhattacharya and Kaliwal (2003)
Urea (0.01 %)	4.667	4.409	(5.8)*	-	-	-	-	-	-	Etebari (2002)

*Significant at level 5%. ns: not significant. [1]The percentage of different between control and treatment.

Table 3. The maximum effect of some supplementary compounds on post cocoon parameters of silkworm

Compounds and concentration	Female cocoon weight (g)			Female cocoon shell weight (g)			Male cocoon weight (g)			Male cocoon shell weight (g)			Reference
	Tre.	Con.		Tre.	Con.		Tre.	Con.		Tre.	Con.		
Alanine (10 ppm)	0.858	0.812	(5.6)*[1]	0.111	0.095	(17)*	0.704	0.580	(21)*	0.106	0.076	(39)*	Khan and Saha (1995)
Ascorbic Acid (2%)	3.12	3.091	(1.13) ns	0.744	0.708	(5)*	2.417	2.357	(2.5) ns	0.75	0.663	(6.3)*	Etebari (2002)
Ascorbic Acid (2%)	2.556	2.460	(3.9)*	0.519	0.517	(0.3)ns	2.026	1.923	(5.3)*	0.495	0.468	(5.7)*	Etebari *et al.* (2001)
Copper Sulphate (30 ppm)	1.236	1.269	(-3) ns	0.139	0.113	(23)*	0.832	0.777	(7)*	0.124	0.114	(8)*	Magadum *et al.* (1992)
Folic Acid (100 µg/ml)	2.395	2.101	(13)*	0.486	0.388	(25)*	1.679	1.402	(19)*	0.418	0.353	(18)*	Nirwani and Kaliwal (1996a)
Glutamic Aid (1%)	3.080	3.140	(-2) ns	0.754	0.729	(3.4)ns	2.338	2.350	(-0.6)ns	0.686	0.695	(-1.3)ns	Etebari (2002)
Glycine (0.5%)	3.092	3.140	(-1.5) ns	0.749	0.729	(2.7) ns	2.338	2.350	(-0.6)ns	0.694	0.695	(-0.2)ns	Etebari (2002)
Hydrolyzed protein (2%)	2.68	2.02	(32)*	0.500	0.300	(66)*	2.51	2.01	(25)*	0.480	0.30	(60)*	Krishnan *et al.* (1995)
Lynestrenol® (100 ppm)	1.019	0.996	(2.3)*	0.127	0.113	(15)*	0.874	0.915	(-5)*	0.129	0.131	(-2)*	Khan *et al.* (2002)
Multi mineral® (10%)	2.57	2.460	(4.5)*	0.546	0.5292	(3.2)ns	1.950	1.962	(-0.6) ns	0.4968	0.5072	(-2) ns	Etebari and Fazilati (2003)
Potassium Sulphate(1 mg/ml)	2.010	1.473	(36)*	0.337	0.257	(31)*	1.584	1.189	(33)*	0.352	0.246	(32)*	Nirwani and Kaliwal (1996b)
Thiamine (300 µg/ml)	3.140	2.101	(6)*	0.433	0.388	(11)*	1.718	1.402	(22)*	0.366	0.353	(3) ns	Nirwani and Kaliwal (1998)
Zinc Chloride (30 µg/ml)	1.75	1.61	(9)*	0.339	0.313	(8)*	1.39	1.32	(5)*	0.261	0.231	(12)*	Hugar and Kaliwal (1999)
Ferrous Sulphate (2000 µg/ml)	1.611	1.411	(9)*	0.310	0.265	(20)*	1.307	1.194	(9)*	0.280	0.255	(13)*	Nirwani and Kaliwal (1995)
$MgSO_4$ (2000 µg/ml)	1.885	1.411	(27)*	0.305	0.265	(18)*	1.483	1.194	(24)*	0.274	0.255	(1)*	Nirwani and Kaliwal (1995)
Nickel Chloride (30 µg/ml)	1.948	1.605	(21)*	0.331	0.313	(5)*	1.429	1.272	(3)*	0.300	0.231	(29)*	Hugar *et al.* (1997)
KNO_3 (500 µg/ml)	1.517	1.193	(27)*	0.225	0.192	(17)*	1.482	1.148	(24)*	0.250	0.193	(29)*	Goudar and Kaliwal (2000)
$MgSO_4$ + KNO_3 (300µg/ml)	1.284	1.523	(-16) ns	0.197	0.216	(-9) ns	1.198	1.290	(-8) ns	0.194	0.201	(-4) ns	Goudar and Kaliwal (2001)

*Significant at level 5%. ns: not significant. [1]The percentage of different between control and treatment.

pounds of the plant
2. Digestion and transformation into amino acids or small peptides
3. Absorption by mid-gut epithelial cells
4. Absorption by different body cells and transformation to cellular structure, which is the most important process.

Whenever each one of these stages is interrupted for different reasons, the enhancement of nitrogenous compounds in the diet causes an increase in insect biological efficiency (Woods and Kingsolver, 1999).

The aim of supplementation is to support the first stage of metabolism of nitrogenous compounds and there are not any controls on the other three stages. Although the imbalance of amino acids and other compounds could have many side effects. In a way that takes the protein metabolism into lipid metabolism (Baker, 1975). Leonardi *et al.* (2001) have suggested that with utilizing one chemical substance called leosin methyl ester increased the amount of amino acid absorption in the silkworm mesentron and with this, they could decrease 20% of the mulberry leaves used in artificial diet.

Table 4. Effect of supplementation with distilled water in larval and cocoon weight

	Normal leaves	Distilled water	Reference
Larval weight (g)			
	4.218	4.409 (+5)*[1]	Etebari (2002)
	4.502	4.401(-2) ns	Etebari *et al.* (2002)
	3.262	3.137 (-4) ns	Etebari (2002)
	2.453	2.453 (0)	Etebari (2002)
	4.522	4.520 (-0.1) ns	Etebari and Fazilati (2003)
	4.11	4.21 (+3)*	Hugar and Kaliwal (2002)
	2.700	2.600 (-3) ns	Rajashekhargouda *et al.* (1998)
	3.489	3.627 (+4)*	Nirwani and Kaliwal (1998)
	3.489	3.627 (+4)*	Nirwani and Kaliwal (1996 a)
	2.567	2.696 (+5)*	Nirwani and Kaliwal (1996 b)
	3.357	3.002 (-12) ns	Zaman *et al.* (1996)
	2.567	2.696 (-1) ns	Nirwani and Kaliwal (1995)
	4.109	4.213 (-3) ns	Hugar *et al.* (1997)
	4.109	4.213 (-3) ns	Hugar and Kaliwal (1999)
	2.604	2.586 (0) ns	Goudar and Kaliwal (2000)
	2.596	2.652 (-3) ns	Goudar and Kaliwal (2001)
	2.693	2.779 (-4) ns	Bhattacharya and Kaliwal (2003)
Cocoon weight (g)			
	2.744	2.724 (-1) ns	Etebari (2002)
	2.200	2.191 (-0.5) ns	Etebari *et al.* (2002)
	1.406	1.403 (-0.2) ns	Etebari (2002)
	0.992	0.933 (-6) ns	Etebari (2002)
	2.211	2.158(-2.5) ns	Etebari and Fazilati, (2003)
	1.430	1.65 (+15)*	Hugar and Kaliwal (2002)
	1.70	1.70 (0)	Rajashekhargouda *et al.* (1998)
	1.751	1.681 (+4)*	Nirwani and Kaliwal (1998)
	1.681	1.751 (+4)*	Nirwani and Kaliwal (1996a)
	1.331	1.331 (0)	Nirwani and Kaliwal (1996b)
	1.473	1.411 (-5) ns	Nirwani and Kaliwal (1995)
	1.590	1.605 (1)	Hugar *et al.* (1997)
	1.380	1.193 (-15) ns	Goudar and Kaliwal (2000)
	1.402	1.523 (-8) ns	Goudar and Kaliwal (2001)
	1.408	1.380 (-2) ns	Bhattacharya and Kaliwal (2003)

*significant at 5%. ns: not significant. [1]The percentage of different between control and treatment.

Enrichment of mulberry leaves by soaking them in complementary solutions and drying by air condition reduces leaves quality. Evaporation intensity in studied regions and primary humidity of leaves are the most important factors for achieving the best results. In tropical regions, after leaf surface humidity evaporation, the feeding characteristics of the leaves will change. Since these researches are conducted in laboratory, the changes are referred to the positive effects of the utilized compound.

Etebari (2002) has demonstrated that the leaves during the time from supplementation until when are fed to the larvae loose much of their humidity. In a way that their regression equation is $Y = 0.9902X + 0.0267$, where X is the weight of the leaves before supplementation and Y is the weight of the leaves after supplementation. While the efficiency of this system in humid climates is different in a way that this equation for humid regions and the leaves with high humidity is $Y = 1.009X + 0.0157$. Table 4 shows some parameters analyzed in the normal leaves and leaves treated with distilled water and at different climates different results are obtained.

Other compounds

Other different coumpunds were used in supplementation system to increase the yield (Das *et al.*, 1993; Saha and Khan, 1997; Ravikumar *et al.*, 2002). The use of vertebrate hormones to enhance the commercial characters of the mulberry silkworm has taken a great turn. Some researchers identified the titers of vertebrate steroid-like immunoreactive substances, *i.e.*, testosterone, estradiol and progesterone in the haemolymph of *B. mori*. Insect hormone analogues such as JHA and ecdysteroieds have been the interest of researchers in sericulture for silk yield enhancement (Etebari, 2000). In laboratory studies a lot of these compounds are topically applied, which this is out of the field of this review but since it has interesting result will be discussed, briefly. Although application of vertebrates hormones are occasionally used for supplementation but what is clear is that the cost of these compounds are much more expensive than other complementary compounds and often their affection is not from feeding but they leave positive effects on other metabolisms (Goudar and Kaliwal, 2000a, b).

Hugar *et al.* (1996) have reported that the topical application with thyroxine to 4th and 5th instar larvae resulted in a significant increase in larval and adult performance. Vertebrate steroids may evoke dose dependent effect on insect development that resembles those of juvenoides (Sita *et al.*, 1981, 1983; Lafant, 1991). Gouder and Kaliwal (1999) have reported that topical application with cortisone showed significantly increased larval weight along with others enhanced larval, cocoon and adult parameters. The topical application with androstenedione and methyltestosterone to the V stadium larvae of the silkworm *B. mori* showed that the fat body glycogen, haemolymph trehalose and lipids are changed significantly (Goudar and Kaliwal, 2001a, b). Hugar and Kaliwal (2001) have demonstrate that the topical application with testosterone propionate and estradiol-17β to the 5th instar silkworm, *B. mori* showed a significant change in the fat body glycogen and protein, haemolymph trehalose and lipids.

Conclusion

Vitamins increase the biological performance of the insects by entering in immunity system, enzyme activity and etc. the enrichment of mulberry leaves with vitamins increase the sericultural yield in laboratory conditions. Amino acids which are necessary compounds for the synthesis of silk filament protein could also improve the yield if added in adequate amounts but if the balance of these amino acids is disturbed it could interrupt different cellular functions.

The fortification of mulberry leaves with various nutritional compounds increased the larval haemolymph sugar, which this was not irrelevant to the enhancement of nutritional value and consumption rate of food. Although in some conditions special metabolic pathways were activated by adding some complementary treatments which was not related to cocoon productive characteristics. Therefore, the compound, dosage and the time of supplementation are very important in this system.

A variety of sugars, proteins, amino acids, vitamins, etc. have been tried as supplements by a number of investigators to increase the quantity of silk, however, these are most expensive. Minerals that most of researches have been on them are very cheaper than other compounds but is there the possibility of their scale up. There is not any study on the cost of supplementations that shows whether the time and cost consumed for this purpose is equal to yield increase? Although this estimate, which is the first essential, analyze on this matter has not been yet done but the researches on other supplementary compounds are still being continued. Generally the most effects of these compounds are in the regions that production parameters are low. In the areas that follow a normal pattern in economical parameters the enrichment of the leaves havent had significant economical effects. As a result it is suggested that the efficiency of using fertilizers to improve the soil of mulberry field is much more than enrichment of mulberry leaves, although the application of supplementation needs complementary studies due to its scale up.

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