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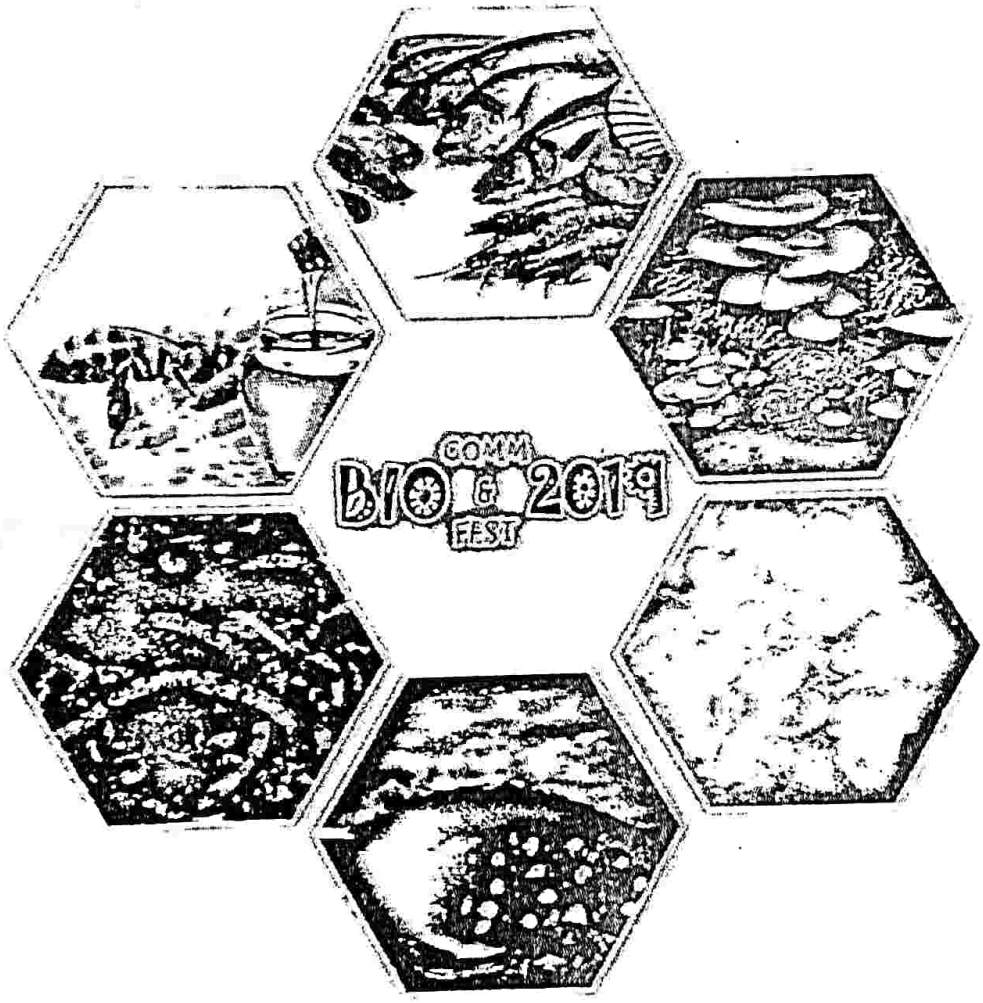

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EFFECT OF VITAMINS AND MINERALS ON SILK GLAND RATIO AND ECONOMIC TRAITS OF *BOMBYX MORI* L.

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ABSTRACT

The silkworm nutritive requirements are very different and the most of it is supplied by feeding on mulberry leaves. Although the mulberry leaves is 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. In order to investigate the effects of supplementary nutrients on silkworm, *B.mori*, an experiment was conducted with vitamins and minerals treatments. Vitamins such as ascorbic acid and folic acid (1, 2 and 3%) treated mulberry leaves fed through first to fifth instar, one of the four normal feeding per day was substituted with treated leaves. This same pattern was conducted in the mineral treatments such as $MgSO_4$ and KCl_2 (2, 4 and 6 %). The supplementation of the leaves was done by spraying the treatments on them. These treatments resulted in a significant increase in silk gland ratio (%) and economical parameters such as cocoon weight, pupal weight, shell weight, shell ratio and filament length when compared with normal control. Maximum silk gland ratio (21.15±1.27 %) was observed with 1 per cent folic acid which had the most enhancements in the fifth day of 5th instar larvae. These vitamin treatments are resulted the economic parameters such as cocoon weight, pupal weight, shell weight, shell ratio and filament length was high, when the larvae fed with 2 per cent folic acid. In these mineral treatments, the same economic parameters were significantly increased, when*B.mori* larvae fed with 4 per cent KCl_2. Such studies provide substantial evidence for practical application vitamins and minerals for qualitative and quantitative improvements in silk production.

KEY WORDS: *Bombyx mori*, ascorbic acid, folic acid, $MgSO_4$ and KCl_2.

INTRODUCTION

A variety of nutrients, minerals, vitamins, hormones and other exogenous modulators were successfully applied in sericulture with a view to stimulate growth, metabolism and silk production in *B. mori* (Laskar and Datta, 2000). Vitamins are one of the organic compounds used by organisms and limited amount of them is essential for natural performance. 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 (Etebari, 2002). All insects require a variety of minerals and trace elements as micronutrients. Mineral nutrition has been neglected compared with other nutrients and the quantitative requirements for insects are largely unknown. However, caterpillars are known to require appreciable amount of potassium and magnesium (Lock and Nichol, 1992).

Various researches have been carried out on the diet supplementation of mulberry leaves fed to silkworms. This supplementation includes vitamins such as ascorbic acid, thiamine, niacin, folic acid and multivitamins (Etebari *et al.*, 2004). Its nutrients are very easy to digest protein, carbohydrates, 50 different minerals and trace minerals, beta-carotene, chlorophyll, fatty acid, and many other nutrients. Supplementation of ascorbic acid to silkworm larvae has increased the fecundity, cocoon yield and filament length (Sarker *et al.*, 1995). Ascorbic acid (1.5 per cent) enriched mulberry leaves resulted in higher filament length, weight and denier values of *B.mori* (Babu *et al.*, 1992). Dietary supplementation of folic acid to silkworm larvae resulted significant increase in economic traits and post cocoon parameters (Nirwani and Kaliwal, 1996).

The supplementation of leaves results in higher yield because the production of good quality and quantity of silk depends on larval nutrition and healthiness of the larvae, which are partially influenced by the nutritive value of mulberry leaves (Ito, 1978). The nutritional status of the mulberry leaves can be improved by enriching them with vitamins and other nutrients. Fortification of mulberry leaves with complementary compounds was found to increase the larval growth and post cocoon characteristics (Etebari and Fazilati, 2003). 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. The absence of ascorbic acid in the diet of first and second instar larvae postponed growth and development of silkworm (Etebari *et al.*, 2004).The increase in cocoon shell weight might be due to the

protein conversion efficiency of the silk gland which might be resulted from the increased a variability of the folic acid as reported by Hamano (1989).

Another point of view larval and shell weight enhanced due to mineral salts (Chakraborthy and Medda, 1977). Narasimhamurthy and Govindappa (1988) reported a significant increase in cocoon weight due to cobalt supplementation. Supplementation with potassium dichromate increase not only in the larval weight and size of the silk gland, but also in the pupal weight, cocoon weight, shell weight as well as in the filament length, as against a decrease of the larval duration (Bhoopathy and Gunasegar, 1998).

The present study has been aimed to find out effective nutritional supplementary compounds (vitamins: ascorbic acid, folic acid and minerals: $MgSO_4$ and KCl_2) which mostly enhance the silk gland ratio and economic parameters in 1 and 2 per cent vitamins and 4 per cent minerals treated *B.mori* treated groups.

MATERIALS AND METHODS

This investigation was carried out on mulberry silkworm, *B.mori*. Disease Free Layings (DFLs) of *B.mori* ($PMXCSR_2$) were obtained from the State Government Sericulture Centre at Konam, Nagercoil Town and were incubated at 27^0C in ant proof racks at 70-80% humidity. The emerging caterpillars were transferred to clean bamboo baskets (25cm diameter and 5cm deep) with a scaffolding of paraffin paper (Krishnaswamy, 1978). The young caterpillars were fed with *ad libitum* mulberry leaves (V_1). The caterpillars were maintained in oven dried trays. Properly disinfected mulberry leaves were supplied. The caterpillars were carefully observed and monitored for their general good health.

Experimental Design

The first instar larvae were selected randomly and grouped into 13 batches for the experimental and control, each group consisting of 5 replicates with 30 silkworms. Require concentration of vitamins and minerals were prepared in distilled water shown below. Vitamins, such as, ascorbic and folic acid are selected as supplements, purchased from U.K Medical Shop, Nagercoil in the form of tablets. Samples are dissolved in distilled water to obtain 1, 2 and 3 per cent solutions, respectively. The different concentrations of vitamin solutions were uniformly sprayed on fresh mulberry leaves and were dried by air- condition, through first to fifth instar one of the four normal feeding per day was substituted with the vitamins treated leaves. Control larvae were fed with untreated leaves and the another treatment have two minerals, such as, Magnesium Sulphate ($MgSO_4$) and Potassium Chloride (KCl_2) are selected as supplements, purchased from Global Scientific Suppliers, Nagercoil.

Samples are dissolved in distilled water to obtain 2, 4 and 6 per cent solutions, respectively. The same vitamin treatment patterns are carried out in the mineral treatment.

Silk gland ratio was calculated and tabulated using the following formula,

$$\text{Silk gland ratio} = \frac{\text{Silk gland weight (mg)}}{\text{Matured larval weight (mg)}} \times 100$$

The cocoons were harvested on the fourth day after spinning and the cocoon characters were recorded in experimental and control groups. Assessment of various cocoon parameters was made as follows (Sonwalker, 1993). All the data were analyzed statistically by ANOVA and t-test. (Zar, 1984).

RESULTS

Table 1 show silk gland ratio in *B.mori* larvae fed with vitamins. Maximum silk gland ratio (21.15±1.27 %) was observed with 1 per cent folic acid when compared to control (14.36±0.83 %). Table 2 shows silk gland ratio in *B.mori* larvae fed with minerals. Maximum silk gland ratio (16.08±1.12 %) was observed with 4 per cent KCl_2, when compared to control (14.36±0.83 %).

The economic parameters of *B.mori* fed with ascorbic acid are presented in Table 3. Maximum cocoon weight, shell weight, shell ratio, filament length were 1288.00±79.94 mg, 240.00±12.81mg, 18.63±1.51% and 711.72±52.37 m respectively, when *B.mori* larvae fed with 1 per cent ascorbic acid and maximum pupal weight (1060.00±69.24 mg) were observed in larvae fed with 2 per cent ascorbic acid and these parameters decreased, when the larvae fed with 3 per cent ascorbic acid (22.6, 21.12, 33.00, 8.15 and -0.63 per cent respectively). Table 4 shows the economic parameters of *B.mori* fed with folic acid. These economic parameters were high, when the larvae fed with 2 per cent folic acid (1330.00±75.11 mg, 1060.00±66.53 mg, 270.00±15.65 mg, 18.79±0.93 % and 857.14±69.11 m respectively).

Table 1: Silk gland ratio (%) of *B.mori* larvae fed with vitamins

Conc. (%)	Ascorbic acid			Folic acid		
	Matured larval weight (mg)	Silk gland weight (mg)	Silk gland ratio (%)	Matured weight (mg)	Silk gland weight (mg)	Silk gland ratio (%)
Control	880.00± 57.14	126.40± 9.22	14.36± 0.83	880.00± 57.14	126.40± 9.22	14.36± 0.83
1	1559.00± 91.60 (74.69)	253.10± 14.11 (100.09)	16.23± 0.74 (13.01)	1183.10± 86.67 (33.34)	250.31± 19.35 (97.88)	21.15± 1.27 (47.25)
2	1240.34± 65.27 (39.63)	187.22± 16.03 (48.04)	15.09± 1.02 (5.08)*	1403.62± 72.05 (57.59)	290.41± 21.07 (129.56)	20.69± 1.14 (44.05)

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Control	880.00± 57.14	126.40± 9.22	14.36± 0.83	880.00± 57.14	126.40± 9.22	14.36± 0.83
1	1559.00± 91.60 (74.69)	253.10± 14.11 (100.09)	16.23± 0.74 (13.01)	1183.10± 86.67 (33.34)	250.31± 19.35 (97.88)	21.15± 1.27 (47.25)
2	1240.34± 65.27 (39.63)	187.22± 16.03 (48.04)	15.09± 1.02 (5.08)*	1403.62± 72.05 (57.59)	290.41± 21.07 (129.56)	20.69± 1.14 (44.05)

8	1110.00±	100.40±	
	80.54	8.45	
	(25.40)	(26.88)	

N=30; Per cent deviation over control values in parentheses; * non significant deviations significant at P≤ 0.05 (t-test)

Table 2: Silk gland ratio (%) of *B.mori* fed with [illegible]

Concentration (%)	Matured larval weight (mg)	Silk gland weight (mg)	Silk gland ratio (%)		
Control	880.00±	120.40±	14.00±		
	57.14	9.22	0.81		
	143.24±	209.51±	14.00±		
2	78.01	12.17	0.78		
	(60.74)	(58.59)	(2.50)*		
	184.07±	278.14±	15.00±		
4	56.01	13.53	1.01		
	(105.93)	(119.87)	(5.08)*		
	1170.47±	186.57±	15.94±		
6	108.51	9.27	0.87		
	(31.94)	(47.53)	(10.99)		

N=30; Per cent deviation over control values in parentheses; * non significant
All other deviations significant at P≤ 0.05 (t-test)

Table 3: Economic traits of *B.mori* fed with ascorbic acid

Parameters	Control	Concentration (%)		
		1	2	3
Cocoon weight (mg)	970.00±71.27	1288.00±79.94 (31.8)	1280.00± [illegible] (31.9)	1[illegible] (22.6)
Pupal weight (mg)	820.00±66.31	1048.00±72.59 (27.36)	1060.00± [illegible] (29.2)	[illegible] (21.2)
Shell weight (mg)	150.00±14.81	240.00±12.81 (59.40)	220.00± [illegible] (46.2)	2[illegible] (33.6)
Shell ratio (%)	15.46±1.34	18.63±1.51 (20.51)	17.12± [illegible] (10.7)	16.72±1.57 (8.15)*
Filament length (m)	463.31±33.54	711.72±52.37 (52.16)	690.25± [illegible] (47.65)	469.31±22.54 (0.63)*

N=30; Per cent deviation over control values in parentheses; * non significant
All other deviations significant at P≤ 0.05 (t-test)

Table 4: Economic traits of *B.mori* fed with folic acid

Parameters	Control	Concentrations (%) 1	2	3
Cocoon weight (mg)	970.00±71.27	1050.00±82.14 (8.00)*	1330.00±75.11 (36.00)	1030.00±66.59 (6.00)*
Pupal weight (mg)	820.00±66.31	860.00±47.03 (4.80)*	1060.00±66.53 (28.80)	860.00±53.70 (4.80)*
Shell weight (mg)	150.00±14.81	190.00±11.70 (26.40)	270.00±15.65 (79.20)	170.00±10.25 (13.20)
Shell ratio (%)	15.46±1.34	18.09±0.95 (17.01)	18.79±0.93 (21.54)	16.50±1.01 (6.73)*
Filament length (m)	463.31±33.54	721.07±51.04 (54.13)	857.14±69.11 (82.70)	690.15±55.14 (47.63)

N=30; Per cent deviation over control values in parentheses * not significant
All other deviations significant at P≤ 0.05 (t-test)

Table 5: Economic traits of *B.mori* fed with $MgSO_4$

Parameters	Control	Concentration (%) 2	4	6
Cocoon weight (mg)	970.00±71.27	1090.00±57.16 (12.00)	1130.00±52.47 (16.00)	1070.00±43.22 (10.00)
Pupal weight (mg)	820.00±66.31	890.00±40.27 (8.40)	920.00±33.15 (12.00)	880.00±50.75 (7.20)*
Shell weight (mg)	150.00±14.81	200.00±12.43 (33.00)	210.00±10.15 (39.60)	190.00±9.11 (26.40)
Shell ratio (%)	15.46±1.34	18.30±1.17 (18.37)	18.50±1.02 (19.66)	17.75±0.82 (14.82)
Filament length (m)	463.31±33.54	792.30±68.21 (69.08)	819.20±79.50 (74.74)	651.06±47.53 (39.42)

N=30; Per cent deviation over control values in parentheses* not significant
All other deviations significant at P≤ 0.05 (t-test)

Table 6: Economic traits of *B.mori* fed with KCl_2

Parameters	Control	Concentration (%) 2	4	6
Cocoon weight (mg)	970.00±71.27	1150.00±85.07 (18.00)	1350.00±76.08 (38.00)	1010.00±80.59 (4.00)*
Pupal weight (mg)	820.00±66.31	960.00±63.13 (16.80)	1130.00±52.49 (37.20)	920.00±43.77 (12.00)
Shell weight (mg)	150.00±14.81	210.00±11.95 (39.60)	240.00±12.24 (59.40)	190.00±12.65 (26.40)
Shell ratio (%)	15.46±1.34	18.26±1.26 (18.12)	18.40±1.07 (19.02)	17.12±1.15 (10.74)
Filament length (m)	463.31±33.54	734.00±57.11 (56.84)	821.05±66.04 (75.12)	503.50±29.00 (8.44)

N=30; Per cent deviation over control values in parentheses* not significant

All other deviations significant at P≤ 0.05 (t-test)

Table 5 shows the economic traits of *B.mori* fed with $MgSO_4$ Maximum increase was observed with 4 per cent $MgSO_4$ treated group. The control cocoon weight was 970.00±71.27 mg which was increased by 16.00 per cent. Shell weight was increased by 39.60 per cent and the shell ratio also increased by 19.66 per cent, when compared to control. Filament length (819.20±79.50 m) increased, when compared to control. Minimum cocoon weight, pupal weight, shell weight, shell ratio and filament length (10.00, 7.20, 26.40, 14.82 and 39.42 per cent) were observed when *B.mori* larvae fed with 6 per cent $MgSO_4$. The economic parameters of *B.mori* fed with KCl_2 are presented in Table 6. The maximum cocoon weight, pupal weight, shell weight, shell ratio and filament length was 1350.00±76.08 mg, 1130.00±52.49 mg, 240.00±12.24 mg, 18.40±1.07 % and 1.05±66.04m respectively, when *B.mori* larvae fed with 4 per cent KCl_2, when compared to 2 and 6 per cent treated groups cocoon weight, pupal weight, shell weight, shell ratio and filament length (4.00, 12.00, 26.40, 10.74 and 8.44 per cent) were decreased, when *B.mori* larvae fed with 6 per cent KCl_2.

DISCUSSION

Feeding trials conducted by several workers proved that the level of nutrients are vary in *B.mori* have significant influence on growth and development and cocoon production. In this present investigation, maximum protein content of silk gland was (18.47±1.43 mg/g) observed in the middle silk gland when larvae fed with 1 per cent ascorbic acid compared to control (10.20±1.48 mg/g). This study was supported by Thulasi and Sivaprasad (2013) and who found out that the impact of ascorbic acid vis-à-vis lemon juice on the growth, protein profile and economic parameters of silkworm.

In the present study, maximum silk gland ratio (47.25 per cent) was observed with 1 per cent folic acid. These findings are in accordance with Rahmathulla *et al.*(2007), who reported that folic acid solution spraying on mulberry leaf and feeding to silkworm significantly improved larval weight, silk gland weight and growth rate. Higher larval and silk gland weight subsequently improved the economic parameters like cocoon weight, shell weight and shell ratio of folic acid treated batches. The current findings are comparable with the results of Das and Medda (1988), who stated that supplementation of mulberry leaves with vitamin B_{12} could increase the synthesis of nucleic acids and protein in the silk gland of silkworm. This result was supported by Nirwani *et al.* (1998). They found out that the oral supplementation of riboflavin significantly increased 11 per cent silk gland weight in *B.mori*.

The silk conversion rate is an important factor to be considered while evaluating the economic parameters of a silkworm. In this investigation, silkworm larvae supplemented with ascorbic acid and folic acid the economic characters were increased. Maximum increased was observed with 2 per cent folic acid. According to Sengupta *et al.* (1972) the nutritional status of mulberry leaves can be improved by enriching them with extra nutrients, such as, 0.5, 1, 1.5 per cent ascorbic acid and vitamin B complex to increase larval growth and improve cocoon characteristics. 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 value. Sarkar *et al.* (1995) reported that supplementation of ascorbic acid to silkworm larva has increased the fecundity, cocoon yield and filament length of *B.mori*. In the present study 1% ascorbic acid increased the shell ratio and cocoon filament length of silkworm.

The current findings, while substantiating the positive impact of economic traits, when the larvae fed with 4 per cent $MgSO_4$ and KCl_2. These results corroborate the earlier findings of Ito (1980), He reported that calcium, iron, magnesium, potassium, phosphorus and zinc are essential mineral elements and must be present in silkworm diet for better silk yield. These results agreed with Sarkar *et al.* (1995), who obtained a significantly increased of the cocoon weight, shell weight and shell ratio. Horie *et al.* (1997) explained this phenomenon by the stimulation of the metabolic activity of the silkworm due to Ca, Mg and Fe, which resulted in shorter larval duration and increased pupation rate. Vishwanath *et al.* (1997) attempted to inter-relate the supplementation of mulberry leaves with combination of secondary or micronutrients on the rearing performance of the silkworm, *B.mori*. They found the reduction in the larval duration and increased not only in the larval weight, but also in the effective rearing rate, cocoon weight, shell weight as well as in the filament length as compared to those characters in the untreated control. The nutritional sources effect not only the growth and development of the silkworm larvae, but also its final silk product.

CONCLUSION

In the present study, the treatment of vitamins such as ascorbic acid and folic acid at the concentration of 1 and 2 may have beneficial effects on the feed efficacy, growth, silk gland ratio and also increased the quantity of silk production than control and the other hand supplementation of minerals such as $MgSO_4$ and KCl_2 (4 per cent) showed significant improvement in above parameters. So, this supplementation could be prescribed to the farmers to get more quantity of silk.

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