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Effects of Vitamin C on Carbohydrate Profile of Silk Worm, *Bombyx mori* LXCSR2 and Pure Mysore

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Abstract:- The present study has been demonstrated severe perturbations in carbohydrate profile of the silk worm *Bombyx mori,* in different tissues such as Silk gland, Intestine, Fat body and Haemolymph when fed on mulberry leaves fortified with Vitamin C in different proportions. The experimental worms were fed thrice a day. The fortification of leaves was done by leaf spraying method. Dietary supplementation of 1000 ppm Vitamin C increased the Haemolymph Carbohydrate into a greater extent in the Silk worm race Pure Mysore. Since the result achieved were considerable and could be recommended to improve the sericulture parameters.

Keywords:- *Supplementation, Silk worm, Haemolymph, Bombyx mori.*

I. INTRODUCTION

Recently much research has been done on the diet supplementation of mulberry leaves fed to silk worms. These supplementations include Vitamins such as Ascorbic acid, Thiamine, Niacin, Folic acid and multi-Vitamins (Etebari 2002; Nirwani and Kaliwal, 1996, 1998; Saha and Khan, 1996; Etebari *et al.,* 2004). Vitamin C is chemically the simplest of the Vitamins and perhaps for these reasons it was first isolated, characterized and its structure determined. It is a nutrient required in very small amount to allow a range of essential metabolic reactions in the body. It is principally a water soluble anti-oxidant known by the chemical name of its principal form L-ascorbic acid or simply ascorbic acid or ascorbate.

Fortification of mulberry leaves fed to silk worm has been reported to increase the food consumption, co-efficient of utilization (Javed and Gondal 2002), larval weight (Etebari *et al.,* 2004), silk filament length and weight (Babu *et al.,* 1992) and fecundity (Rahman *et al.,*1990 and Chauhan and Singh, 1992). Further it has been reported that excessive amounts of Vitamin C supplementation to silk worm diet have negative impact causing decrease in food intake and cocoon characteristics due to hyper vitaminosis (Tantray *et al.,* 2011). Dietary supplementation with synthetic and plant based Vitamin C has been found to enhance the economic parameters of the mulberry silk worm significantly (Tantray *et al.,* 2015, Tantray and Trivedy 2008 and 2011, Tantray, 2017a and Tantray, 2017b).

The silk worm *Bombyx mori* has been classified among the insects which are unable to synthesize Vitamin C in their body and depend on exogenous supply to fulfil the requirement (Ito and Arai, 1965). It is generally accepted that all insects require Vitamins, especially water soluble vitamins such as Ascorbic acid, Thiamine, Riboflavin, Niacin, Pyridoxine, Pantothenic acid, Biotin, Folic acid and Choline (Chapman, 1988). Growth retardation caused by lack of each one of these Vitamins is rather small but better growth is obtained by adding these Vitamins to their diet (Horie and Ito, 1965; Ito, 1978; Ito, 1995). The nutritional status of the mulberry leaves can be improved by enriching them with Vitamins and other nutrients.

There are few studies on the effects of Vitamin C on silk worm. This research investigates the effects of Vitamin C on biochemical parameters of silk worm *Bombyx mori* LXCSR2 and Pure Mysore as biological models.

II. MATERIALS AND METHODS

Test species: Silk worm, *Bombyx mori*. LXCSR2 and Pure Mysore.
Mulberry: V1 Variety
Larval Instar: 5th instar
Duration of treatment: 7 days
Dose selected: Vitamin C- 500 ppm, 1000 ppm, 1500 ppm.
Tissue selected: Silk gland, Intestine, Fat body, Haemolymph

- *Description of study area:* The present study was carried out at Departmental Sericulture Research laboratory, St.John's college, Palayamkottai, Tamil Nadu to evaluate the effect of Vitamin C supplementation on biochemical parameters in multivoltine hybrid races of silkworm *Bombyx mori.L.*

- *Preparation of standard stock solution:* 1 gm of Vitamin C was dissolved in 100 ml of double distilled water, which is equivalent to 1 ppm known as standard stock solution.
  For the Preparation of 500 ppm concentration, 5 ml of standard stock solution was added to 95 ml of distilled water. Likewise 10 ml and 15 ml of standard stock solution were added to 90 ml and 85 ml of distilled water to prepare 1000 ppm and 1500 ppm concentrations.

- *Leaf Spray method:* The T1 (500 ppm), T2 (1000 ppm), T3 (1500 ppm) worms of the two races were supplemented with Vitamin C through topical application on the surface of the leaves.

- *Preparation of the samples:*

➢ *Haemolymph:-* Silkworm larvae were taken and one of their prolegs was cut. Then the Haemolymph was collected in an eppendoff microtube and immediately 1 mg of phenyl thio urea was added to prevent melanisation. The sample was centrifuged at 1400 rpm for 15 minutes.

The supernatant was removed and kept in -20º C for analysis.

- *Digestive system, silk gland and fat body:-* The silkworm larvae were dissected in Bombyx saline at pH 6.5 on the 6th day of the fifth instar. The digestive system, silk gland and fat body are immediately collected. The tissues were crushed in chilled distilled water using mortar and pestle. The homogenates were centrifuged at 3000 rpm for 15 minutes. The supernatant were used as assay samples for the estimation of carbohydrate.

The carbohydrate content in Digestive system, Intestine, fat body and Haemolymph was estimated by *Seifter et al* method.

III. RESULTS

Carbohydrate content in larvae treated by 1000 ppm is peak in Silk gland, Intestine and Fat body (12.71, 10.93, 10.51) in the silk worm LXCSR2. (Table1). The highest carbohydrate content in Haemolymph (12.79) was observed in T_1 (500 ppm). The amount of carbohydrate in the silk gland of larvae treated with 500 ppm (T_1) of Vitamin C was significantly lower (12.22) than other treatments. In Intestine, Fat body and Haemolymph the lowest carbohydrate content (10.07, 9.02, 9.72) was observed in T_3 (1500 ppm) (Table 1).

Treatment	Silk gland	Intestine	Fat body	Haemolymph
T_0	11.01	9.17	9.72	10.29
T_1	12.22	10.88	10.03	12.79
T_2	12.71	10.93	10.51	12.62
T_3	12.63	10.07	9.02	9.72

Table 1:- Effects of mulberry leaves enriched with Vitamin C on Carbohydrate content in Silk gland, Intestine, Fat body and Haemolymph of the silk worm *Bombyx mori* LXCSR2.

In Pure Mysore the maximum carbohydrate content was observed in T_2 (1000 ppm) in all the tissues viz., Silk gland, Intestine, Fat body and Hemolymph (Table2). The least amount of carbohydrate content in Silk gland and Haemolymph (12.03, 11.80) was observed in T_1 (500 ppm). In Intestine and Fat body the minimum carbohydrate content (11.01, 10.12) was recorded in T_3 (1500 ppm). (Table2).

As in the case of total carbohydrates, the highest level was recorded in Haemolymph and least in Fat body.

Treatment	Silk gland	Intestine	Fat body	Haemolymph
T_0	10.23	14.26	11.01	11.71
T_1	12.03	14.14	11.03	11.80
T_2	13.79	14.73	11.09	14.99
T_3	12.60	11.01	10.12	14.12

Table 2:- Effects of mulberry leaves enriched with Vitamin C on Carbohydrate content in Silk gland, Intestine, Fat body and Haemolymph of the silk worm *Bombyx mori* Pure Mysore.

IV. DISCUSSION

The study was undertaken to evaluate the impact of Vitamin C on biochemical parameters of larvae under laboratory conditions. The results indicated significant differences in carbohydrate content on Silk gland, Intestine, Fat body and Haemolymph when mulberry leaves were supplemented with Vitamin C. The carbohydrates are major bio molecules of energy that are required in large amount to a fast growing organism. A Silk worm larvae increases in size and weight by several thousands of times in a short span of 20-25 days. The approximate increase in size from the time of hatching to spinning is 10,000 times. Such an order of increase requires energy in large quantity. The major source of energy for silk worm is carbohydrates absorbed from the mulberry leaves. Horie (1978) reported that the gross energy injested by the silk worm in fourth and fifth instars is 1.84 and 21.1 kilo calories. From this, 45% of the energy is utilized for growth where as the other 55% is stored in the form of carbohydrates. For utilization during pupal and adult stages, in which 12% of the energy stored is utilized for oogenisis.

The result of the present study indicated that the maximum carbohydrate content was observed in T_2 (1000 ppm) in all tissues. viz, Silk gland, Intestine, Fat body and Haemolymph. Our study was in line with Thilsath, 2012 who reported that when the larvae supplemented with ascorbic acid and Folic acid, the economic characters were increased. Maximum increase was observed in the 2% Folic acid. But we always have to keep in mind the effects of hyper vitaminosis 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 (Satio and Mitsuhashi, 1996).

Our study clearly indicated that the level of Carbohydrate increased significantly in 500 and 1000 ppm almost in all tissues than the control and decreased in 1500 ppm. This decrease was considerable in both the races LXCSR2 and Pure Mysore.

V. CONCLUSION

In the present study I suggest that the Vitamin C has increased the Carbohydrate content. The overall assessment of biochemical characters reveals that the maximum Carbohydrate content was observed in the Haemolymph of Pure Mysore larvae fed with 1000 ppm of Vitamin C. Further the result showed that excessive amount of Vitamin C supplementation have negative impact. The Carbohydrate content has decreased in Silk gland, Intestine, Fat body and Haemolymph of both the races LXCSR2 and Pure Mysore in 1500 ppm.

REFERENCES

[1] Babu M, Swamy MT, Rao PK, Rao MS. Effect of ascorbic acid–enriched mulberry leaves on rearing of *Bombyx mori L.* Indian J. Seric. 1992; 31 111-114.
[2] Chapman RF. The insect structure and function , 4th edn. Cambridge: Cambridge University Press. 1998, 78.
[3] Chauhan TPS, Singh K. Studies on the effect of ascorbic acid (vitamin C) on the fecundity of mulberry silkworm, *Bombyx mori, L.* Sericologia, 1992; 32:567-574.
[4] Etebari K, Ebadi R and Mantindoost L 2004 Effect of feeding mulberry's enriched leaves with ascorbic acid on some biological, biochemical and economical characteristics of silkworm *Bombyx mori L.*; Int. J. Indust. Entamol. 8 81-87.
[5] Etebari K, Edabi R. Mantindoost L. Effects of vitamin C on biological and economical characteristics of the silkworm, *Bombyx mori L*. Int. J. Indust. Entomol.2004; 8(1):81-87.
[6] Etebari k 2002 Effect of enrichment of mulberry laves (Morus alba) with some vitamins and nitrogenous compounds on some economic traits and physiological characters of silkworm *Bombyx mori* (Lep., Bombycidae), M.Sc. Theses, Isfahan University of Technology, Isfahan, Iran.
[7] Horie Y and Ito T 1965 Nutrition of the silkworm, *Bombyx mori-L.* Vitamin B requirements and the effects of several analogues; J. Insect physol. 11 1585-1593.
[8] Horie Y 1995 Recent advances of nutritional physiology and artificial diet of the silkworm, in Japan; Korean J. Seri. Sci. 37 235-243.
[9] Horie, Y (1978) Quantitative requirement of nutrients for growth of the silkworm, *Bombyx mori* JARQ(Japan) 12, 211-217.
[10] Ito T 1978 Silkworm nutrition in; The silkworm an important laboratory tool (ed.) Y Tazima (Tokyo: Kodansha Ltd.) pp 121-157.
[11] Ito T, Arai N, Nutrition of silkworm *Bombyx mori L.* Further studies on the nutritive effects of ascorbic acid. Bull. Seric. Expt. Statn. Japan, 1965; 20:1-19.
[12] Javed H, Gondal MH, Effect of food supplementation of ascorbic acid on larval mortality of the silkworm, *Bombyx mori.* Asian J. Plant Sci., 2002; 1(5):556-557.
[13] Nirwani R B and Kaliwal B B 1996 Effect of Folic Acid on economic traits and the change of some metabolic substances of Bivoltine Silkworm, *Bombyx mori L.;* Korean j.Seri. Sci. 38 118-123.
[14] Nirwani R B and Kaliwal B B 1998 Effect of thiamine on commercial traits and biochemical contents of the fat body and haemolymph in the silkworm, *Bombyx mori L.*; Sericologia 38 639-646.
[15] Rahman SM, Khanam LAM, Ferdoud J. Effect of various concentrations of ascorbic acid on the oviposition and hatchability of silkworm, *Bombyx mori L*. Bull. Sericult. Res , 1990;1:47-50.
[16] Saha B and Khan A 1996 Effect of dietary supplementation of vitamins and minerals on the growth and development of Bombyx mori; Bang.J.Zool. 24 125-131.
[17] Satio H and Mitsuhashi 1996 Influence of vitamins on production of males in the green peach aphid, Mysus persicae. Appl. Entomol. Zool 31: 567-574
[18] Thilsath Fatima Quraiza M (2012) Studies on the impact of various nutritional sources on silkworm Bombyx mori L. Ph.D Thesis, Manonmaniam Sundaranar University, Tirunelveli.