International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Effects of Vitamin C on Protein Profiles of Silk Worm, *Bombyx mori* LXCSR2 and Pure Mysore

Sundara Raj J[1], W. Kerenhap Evangelin[2], S Akhil Kumar[3]

[1, 2]Department of Zoology, St. John's College, Palayamkottai, Tamil Nadu, India

[3]Departement of Animal Science, M.S University, Tirunelveli, Tamil Nadu, India

Abstract: ***In order to investigate the effects of supplementary nutrients on silk worm B.mori, an experiment was conducted with Vitamin C fortification. Leaves enriched with Vitamin C (500 ppm, 1000 ppm, 1500 ppm) were fed thrice a day for the larvae throughout the 5th instar. The supplementation of the leaves was done by Leaf Spraying Method. These treatments resulted in a significant increase of protein content in tissues such as Silk gland, Intestine, Fat body and Haemolymph. Vitamin C of 1000 ppm treatment increased the protein content in Silk gland, Intestine and Fat body. However 1500 ppm treatment decreased the protein content in Silk gland, Intestine and Fat body in both the races LXCSR2 and Pure Mysore.***

Keywords: Fortification, Vitamin C, Nutrition, Mulberry leaves, *B.mori*

1. Introduction

Nutrition plays an important role in improving the growth and development of the silk worm, like other organisms. Vitamins are one of the organic compounds used by organisms and limited amount of them is essential for natural performance. 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 (Sengupta *et al.*,1972; Etebari, 2002). 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 (Das and Medda, 1988; El-Karaksy and Idriss, 1990; Lalita *et al.*, 1991; Muniandy *et al.*, 1995; Sarkar *et al.*, 1995; Nirwani and Kaliwal, 1996; Etebari, 2002; Etebari and Fazilati, 2003).

The silk worm, *Bombyx mori* produces massive amount of silk proteins during the 5th stage of larval development. These proteins are stored in the middle silk gland and they are discharged out through the anterior duct and spinneret, at the end of the fifth instar. Two kinds of silk proteins have been distinguished as major components of silk cocoons. The first being fibroin and the second being Sericin, a natural macromolecular protein, serving as an adhesive to unite fibroin for making silk cocoons of Silk worm.

Silk production basically depends on the *B,mori* larval protein metabolism which in turns needs more energy generating events, spinning requires more muscular activity and silk is being produced by the silk gland.

With respect to what was said about different suggestions on ascorbic acid effects and taking in mind that the abundance of Vitamin C in mulberry leaves has been reported by several authors, but the quantity of this Vitamin is very variable in different conditions, (Ito, 1978; Babu *et al.*,1992) the necessity of ascorbic acid in insect diet becomes clarified.

Therefore the present investigation was undertaken to study the effects of Vitamin C on the biochemical parameters of the silk worm hybrids LXCSR2 and Pure Mysore.

2. 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

2.1 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*.

2.2 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 was added to 90 ml and 85 ml of distilled water to prepare 1000 ppm and 1500 ppm concentrations.

2.3 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.

2.4 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 was 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 protein.

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

3. Results

In LXCSR2, the maximum protein content in Silk gland, Intestine and fat body was 17.82, 12.95, 10.98 respectively and it was observed in T_2 (1000 ppm). In haemolymph the highest protein content 15.54 was observed in T_3 (1500 ppm). The minimum protein content in silk gland Haemolymph was 13.07 and 13.98 recorded in T_1 (500 ppm). In intestine and fat body the minimum protein content was (12.12, 19.97) recorded in T_3 (1500 ppm).

As in the case of total proteins, the highest level was recorded in Silk gland and least in fat body.

Table 1: Effects of mulberry leaves enriched with Vitamin C on protein content in silk gland Intestine, fat body and Haemolymph of the silkworm Bombyx mori (LXCSR 2)

Treatment	Silk gland	Intestine	Fat body	Haemolymph
T_0	11.23±0.138	10.93±0.084	10.01±0.063	12.73±0.001
T_1	13.07±0.41	12.31±0.125	10.12±0.004	13.98±0.028
T_2	17.82±0.017	12.95±0.001	10.98±0.410	14.71±0.185
T_3	14.29±0.002	12.12±0.321	9.97±0.129	15.54±0.320

T_0- Control, T_1-500 ppm, T_2-1000 ppm, $T_3$1500 ppm

In Pure Mysore, the maximum protein content in Silk gland, Intestine and fat body was 18.68, 15.71 and 11.71 and it was observed in T_2 (1000 ppm). In Haemolymph the highest protein content 17.93 was observed in T_3 (1500 ppm). The minimum protein content in silk gland, Intestine and Haemolymph was 15.75, 14.97 and 14.93 and it was recorded in T_1 (500 ppm). In fat body the minimum protein content was 10.33 and it was recorded in T_3 (1500 ppm).

Table 2: Effects of mulberry leaves enriched with Vitamin C on protein content in silk gland, Intestine, fat body and Haemolymph of the silkworm Bombyx mori (Pure Mysore)

Treatment	Silk gland	Intestine	Fat body	Haemolyph
T_0	12.71±0.040	12.13±0.133	11.08±0.063	13.82±0.285
T_1	15.75±0.119	14.97±0.346	11.25±0.078	14.93±0.163
T_2	18.68±0.244	15.71±0.002	11.71±0.314	15.28±0.011
T_3	16.12±0.011	15.22±0.404	10.33±0.420	17.93±0.089

T_0- Control, T_1-500 ppm, T_2-1000 ppm, $T_3$1500 ppm

4. Discussion

The results of the present study demonstrated severe perturbations in protein profile of silkworm *B.mori* in different tissues such as silk gland, Intestine, fat body, Haemolymph, when fed on mulberry leaves fortified with Vitamin C. Protein metabolism is an important process in building up of body ie., in the development of muscles, tissues etc. Nutritive values of different proteins for the silkworm vary largely. These differences in the food value seems to depend on qualitative and quantitative amino acid composition of proteins (Crignton,1984). Ascorbic acid (Vitamin C) entering into the silk gland cells, Haemolymph, fat body and into the intestinal cells initiate the protein elevations depend upon the tissue. Because of the changes in the protein content, the body size of the silkworm also showed variations. Several researchers demonstrated the phago stimulatory effect of Vitamins in insects.

Silk proteins like Sericin and Fibrin will immensely contribute to the cocoon size and ultimately to the production of Sericulture industry. The proteins of Intestine, Haemolymph and fat body may provide the raw material for the synthesis of Silk protein in the Silk gland. Contractile proteins of muscle in this economic insect would be important for feeding and for coordinated muscular movements during spinning activity. Thus the higher the efficiency of the muscle, greater would be the efficiency of the spinning because during the 5th instar stage, massive development of body musculature similarly its innervations by segmental nerves was observed. There are also reports on the improvement of economic characters of the silk worm, *B.mori* after administration of PMSG (Pregnant Mare's Serum Gonadotrophin) and Thyroxine (Thyagaraja *et al.*,1991).

In the present study the Silk gland registered more protein content when compared to other tissues and the least was recorded in fat body during 5th instar larval stage in the silkworm *B.mori* LXCSR2 and Pure Mysore.

Haemolymph proteins are synthesized by fat body cells and then secreted into the Haemolymph in a time dependent situation during post embryonic development and metamorphosis (Kishimoto *et al.*,1999). Several classes of abundant insect Haemolymph protein have been identified of which productions are regulated by hormones (Cole *et al.*, 1990)

5. Conclusion

In the present study I suggest that the Vitamin C has increased the protein content. The overall assessment of

biochemical characters reveals that the maximum protein content was observed in the silk gland 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 protein content has decreased in Silk gland, Intestine and Fat body of both the races LXCSR2 and Pure Mysore in 1500 ppm.

References

[1] Babu M, M. T. Swamy, P.K. Rao and M.S. Rao.1992. *Effect of ascorbic acid enriched mulberry leaves on rearing of Bombyx mori.* Indian J. Seric. 31; 111-114.
[2] Cole K. D, Smith A. F, Wells M. A. 1990. *The structure of the apollpophorin-III, Gene from mandura texta.* Insect Biochem. 20; 381-388.
[3] Creighton T. E. 1984. *Chemical nature of polypeptides.* Proteins. 1-60.
[4] Das S, Medda A. 1988. *Effect of cyanocobalamine on protein and nucleic acid contents of ovary of silkworm, Bombyx mori L., during larval, pupal and adult stages of development*. Insect Sci. Appl. 9; 641-646.
[5] El-karaksy, I. R. and M. Idriss. 1990. *Ascorbic acid enhances the silk yield of the mulberry silkworm, Bombyx mori L*. J. Appl. Entomol. 109; 81-86.
[6] Etebari K. 2002. *Effect of enrichment of mulberry leaves (morus alba)with some vitamins and nitrogenous compounds on some economic traits and physiological characters of silkworm Bombyx mori, M.Sc* Thesis,Isfahan University of technology, Iran.
[7] Etebari K and M. Fazilati. 2003. *Effect of feeding on mulberry supplementery leaves with multi mineral in some biological and biochemical characteristics of silk worm (Bombyx mori).* J. Sci. and Technol. Agric. and Natur. Resour. 7, 233-244.
[8] Faruki S. I. 2005. *Effect of pyridoxine on the reproduction of the mulberry silkworm, Bombyx mori.* ISJ.2;28-31.
[9] Ito T. 1978. *Silkworm Nutrition; in the Silkworm an important Laboratory Tool*. Tazima, Y. (Ed), pp. 121-157, Kodansha Ltd, Tokyo.
[10] Lalita K, A.K Tripathi and D. P. Sinha. 1991. *Effect of some food adjuncts on food consumption by the larvae of Antheraea mylitta.* Bioved. 1; 133-136.
[11] Muniyandi S, M. Sheela and S.T. Nirmala. 1995. *Effect of Vitamins and minerals (Filibon) on food intake, growth and conversion efficiency in Bombyx mori.* Environ. Ecol. 13;433-435
[12] Nirwani R. B. and B.B. Kaliwal. 1996. *Effect of folic acid on economic traits and the change of some metabolic substances of bivoltine silkworm, Bombyx mori.* Korean J. Seri. Sci. 38; 118-123.
[13] Sarkar A, M. Haque, M. Rab and N. Absar (1995). *Effect of feeding mulberry (Morus sp.) leaves supplemented with different nutrients to silk worm (Bombyx mori).* L. Curr. Sci.6; 185-188.
[14] Sengupta K, Singh BD, Mustafi JC. 1972. *Nutrition of Silk worm, Bombyx mori L. Studies on the enrichment of mulberry leaf with various sugars, proteins, amino acids and vitamins for vigorous growth of the worm and increased cocoon crop protection.* Indian J. Sci. 11; 11-27.
[15] Thyagaraja B S, Kelly T.J, Masler E. P and Borkovec A. B. 1991. *Thyroxine induced haemolymph protein and ecdysteroid in the silk worm Bombyx mori, larval growth and silk production.* J. Insect. Physiol.3; 153-160.