



Impact of different concentrations of spirulina supplemented diet on the biochemical profile of haemolymph of silk *Bombyx mori* L

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Abstract

Silkworm (*Bombyx mori*) is a highly domesticated and economically important insect which is the primary producer of silk. Apart from silk production *Bombyx mori* had a lot of applications in biological and scientific research. Hemolymph is the circulating fluid of insects, similar to mammalian blood, which moves through the open circulatory system, directly bathing the organs and tissues. The mulberry silkworm, *Bombyx mori*, has been raised for more than 5000 years in Asian countries, and is a major economic resource for many families. In the present investigation an attempt was made to study the effect of different concentrations (1, 3 and 5%) of *Spirulina* as supplementary diet on the silk production and biochemical profile of haemolymph of silk *Bombyx mori* L. In the study biochemical parameters such as glucose, protein, cholesterol, ALT and AST were found to be significantly increased in haemolymph that had been fed on *Spirulina* supplemented. The increased content of this biochemical parameters had positive relationship to physiological function of the insects.

Keywords: silkworm, *Bombyx mori*, haemolymph, biochemical profile

1. Introduction

Silkworm (*Bombyx mori*) is a highly domesticated and economically important insect which is the primary producer of silk. Apart from silk production *Bombyx mori* had a lot of applications in biological and scientific research. The silk industry plays an important role in the Indian rural economy, so research on silkworm and mulberry crop enhancement is of high importance ^[1]. Silks are fibrous proteins synthesized in specialized epithelial cells that line glands in these insects ^[2]. The silkworm larva has a high medicinal value and is usually used to reduce blood pressure, diabetes, nerve disorder and heart problems, in addition, the major uses of its larvae for silk production ^[3].

The silkworm has an open circulatory system containing hemolymph, which surrounds the tissues of the silkworm with blood. Hemolymph is the circulating fluid of insects, similar to mammalian blood, which moves through the open circulatory system, directly bathing the organs and tissues. Compared with mammalian blood, insect hemolymph differs in the absence of erythrocytes and has a high concentration of several types of free amino acids. The mulberry silkworm, *Bombyx mori*, has been raised for more than 5000 years in Asian countries, and is a major economic resource for many families. The haemolymph of insects performs several physiological functions such as immunity, transport and storage reserve. It serves as a transport milieu for the exchange of essential materials between cells and tissues and storage of many materials and essential for a variety of bodily functions such as molting and reproduction ^[4]. In the present investigation an attempt was made to study the effect of different concentrations (1, 3 and 5%) of *Spirulina* as supplementary diet on the silk production and biochemical profile of haemolymph of silk *Bombyx mori* L.

2. Material and Methods

2.1 Mulberry leaves procurement

Mulberry includes a number of species and varieties. They differ in their suitability for silkworm rearing because of their varying nutritious value and palatability of the silkworm larvae. The mulberry plantation at Tamil Nadu Sericulture Training Centre, Nanjikkottai, Thanjavur is being well established with MR2 variety of mulberry, maintenance of which is through standard horticulture techniques. The mulberries were procured from this garden as per requirement.

2.2 Experimental animals

The egg cards of silkworm *B. mori* (cross breed: Local, a multivoltine x NB4D2, a bivoltine) were obtained from State Grainage Centre, Trichirappalli and Tamil Nadu Sericulture Training Centre, Nanjikkottai, Thanjavur, India. Silkworms were reared under standard conditions at 26±2°C. The mulberry leaves harvested at the irrigated mulberry garden were used as food for silkworm: Larvae were reared in plastic trays (70 larvae/tray) and were exclusively fed on mulberry leaves. Fresh mulberry leaves of MR2 variety were collected early in the morning and stored in wet gunny bags. They were chopped prior to feeding. The leaves were fed four times per day (6.30, 11.30, 16.00 and 22.00 hrs).

2.3 *Spirulina*

Spirulina platensis (*Arthrospira platensis*) powder was purchased from PARRY Nutraceuticals (Division of EID Parry (India) Ltd. at Pannangudi, Pudukkottai Dist. Tamil Nadu, India and to prepare the experimental dose for 1, 3 and 5% concentrations.

2.4 Experimental procedures

In the experiments, 3 concentrations of *Spirulina* were supplemented to the fifth instar larvae. *Spirulina* were dissolved in distilled water and mulberry leaves were dipped in each concentration, 1, 3, and 5 % w/v and were allowed for few minutes for water evaporation and fed into experimental larvae as the first feed. Larvae fed with normal leaves (dipped in distilled water) served as control. All the rearing operations were carried out according to standard method. During rearing, the worms were grouped into four batches of 70 larvae for each treatment.

2.5 Haemomorph collection

Haemolymph sample of the both sexes was collected daily during the developmental and control groups. Haemolymph was collected by making an incision at the distal region of the prothoracic leg in eppendorf tubes pre-rinsed with saturated phenylthiourea (PTU) solution. The haemolymph samples were diluted with phosphate buffer saline (PBS) in 1:5 ratio. The diluted haemolymph samples were centrifuged at 12,000 g for 5 min. to remove the haemocytes and other tissue debris. Haemolymph samples were then stored at -20°C until use.

2.6 Biochemical profile

Glucose was estimated by Glucose Oxidase/Peroxidase (GOD/POD) method [5]. Protein was estimated by the method of Lowry *et al.*, [6]. Cholesterol was estimated by the method of Allain [7]. ALT and AST were estimated by the method of Reitman and Frankel [8].

3. Result and Discussion

The different concentrations of *Spirulina* increased glucose in haemolymph of silkworm *Bombyx mori*. Table 1 shows the glucose content of control and different concentrations of *Spirulina* supplemented group. In control the glucose level was 19.54mg in haemolymph. The glucose increased to 24.73mg at 1 percent *Spirulina* supplementation. The glucose content reached a maximum of 33.42mg in haemolymph at 5.00 percent *Spirulina*. Glucose is significantly influenced by the concentration of *Spirulina*. The different concentration of *Spirulina* increased protein in haemolymph of silkworm *Bombyx mori*. (Table 1). In control the protein was 9.65mg/g in haemolymph. The protein increased to 10.22mg/g in haemolymph of 1 percent *Spirulina* supplementation. The protein content reached a maximum of 15.78mg/g in haemolymph at 5.00 percent of *Spirulina*. Protein content is significantly influenced by *Spirulina* (Table 1).

The different concentrations of *Spirulina* increased cholesterol in haemolymph of silkworm *Bombyx mori*. Table 1 shows the cholesterol of control and *Spirulina* supplemented group. In control the cholesterol was 28.87mg/g in haemolymph. The cholesterol increased to 31.41mg/g in haemolymph at 1 per cent *Spirulina* supplementation. The cholesterol content reached a maximum of 45.56mg/g in haemolymph at 5.00 percent *Spirulina*. The cholesterol is significantly influenced by the *Spirulina*. The different concentrations of *Spirulina* increased the activity of Alanin transaminase (ALT) and Aspartate transaminase (AST) in haemolymph of silkworm *Bombyx mori*. Table 2 shows the activity of ALT and AST of control and *Spirulina* supplemented groups. In control, the activity of ALT and AST were 265.47 IU/L and 383.53

IU/L respectively in haemolymph. The activity of ALT and AST was increased to 286.19 IU/L and 398.75 IU/L respectively in haemolymph at 1 per cent *Spirulina* supplementation. The activity of ALT and AST increased at a maximum of 310.01 IU/L and 425.98IU/L in haemolymph at 5.00 percent *Spirulina*. The activity of ALT and AST is significantly increased by the concentration of *Spirulina* (Table 2).

Table 1: Effect of *Spirulina* supplemented with mulberry leaves on glucose, protein and cholesterol in haemolymph of silkworm, *Bombyx mori* L. (Each value is an average (mean ± SD) performance of ten individuals

Parameters	Control	Group I (1%)	Group II (3%)	Group III (5%)
Glucose (mg/dl)	19.54±1.36	24.73±1.48	30.34±2.09	33.42±2.27*
Protein (g/dl)	9.65±0.67	10.22±0.613	13.52±0.932	15.78±1.07*
Cholesterol (mg/dl)	28.87±2.02	31.41±1.88	42.32±2.92	45.56±3.09 *

*Significantly different from control *P<0.05

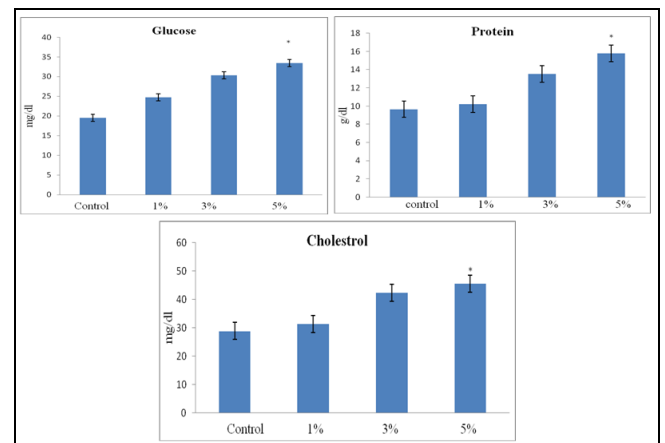


Fig 1: Effect of *Spirulina* supplemented with mulberry leaves on glucose, protein and cholesterol in haemolymph of silkworm, *Bombyx mori* L.

Table 2: Effect of *Spirulina* supplemented with mulberry leaves on Aspartate transaminase and Alanin transaminase in haemolymph of silkworm, *Bombyx mori* L. (Each value is an average (mean ± SD) performance of ten individuals

Parameters	Control	Group I (1%)	Group II (3%)	Group III (5%)
ALT (IU/L)	265.47±18.58	286.19±17.17	296.42±20.45	310.01±21.08 *
AST (IU/L)	383.53±26.84	398.75±23.92	418.87±28.90	425.98± 28.96 *

*Significantly different from control *P<0.05

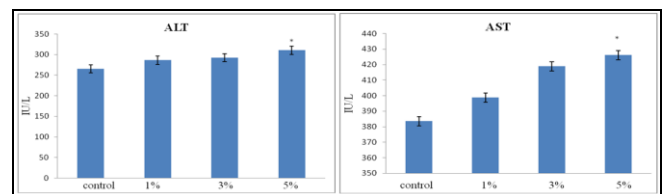


Fig 2: Effect of *Spirulina* supplemented with mulberry leaves on Aspartate transaminase, Alanin transaminase and acid phosphatase activity in haemolymph of silkworm, *Bombyx mori* L.

The dietary efficiency of any nutrient in the diet increased with an increase in the level of proteins, but excessive addition was found to be detrimental. Based on the fact that most essential nutrients produce biphasic dose-response relationships [9]. Among the various concentrations, 5% *Spirulina* supplementation was found to be greater than the

control. This is in accordance with the observations of Hori *et al.*,^[10] who reported that the concentration of the protein in the haemolymph increased with an increase of protein in the diets. Among the various concentrations, 5% *Spirulina* supplementation was found to have increased level of glucose than control. Investigation, the level of different enzymes such as ALT and AST increased considerably in 1 to 5% supplementation of *Spirulina* but AST and ALT can peak their activity at 5% concentration of *Spirulina* as control. Ender *et al.*,^[11] reported that the diet with high level of methyl parathion significantly increased the activities of ALT and AST in greater wax moth, *Galleria mellonella* (L.) larvae. The silkworm requires polysaturated fatty acids such as linoleic and linolenic acids for its well growth and development. The fatty acid of mulberry leaves consists of linolenic, palmitic and linolenic acids in their order of contents and there are enough amounts to maintain good growth of the silkworm in mulberry leaves^[12].

Conclusion

The biochemical profiles of haemolymph of the larvae supplemented with *Spirulina* was analysed. In the study biochemical parameters such as glucose, protein, cholesterol, ALT and AST were found to be significantly increased in haemolymph that had been fed on *Spirulina* supplemented. The increased content of this biochemical parameters had positive relationship to physiological function of the insects.

References

1. Hiware CJ. Agro- Cottage Industry Sericulture. Delhi, India: Daya Publishing House, 2001, 57-93.
2. Kaplan DL, Mello SM, Arcidiacono S, Fossey S, Senecal KWM. In: McGrath KKD, editor. Protein based materials. Boston: Birkhauser, 1998, 103-31.
3. Fenemore PG, Prakash A. Applied entomology. Willey Eastern Limited, New Dehli, India, 1992, 115.
4. Mullins DE. Chemistry and physiology of the haemolymph. In: Kerkut GA and Gilbert LI (Eds). Comprehensive Insect physiology, biochemistry and Pharmacology, Pergaman Press, Oxford, New York, 1985; 3:355-400.
5. Trinder P. Practical Clinical Biochemistry, Vol x, 5th edit., William Heinemann Medical Books Limited, New York, 1969.
6. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's reagent. Journal of Biological Chemistry. 1951; 193:265-276.
7. Allain CC, Poon L, Chan CSG, Richmond W, Fu, PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974; 20:470-475.
8. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am J Clin Path, 1957, 25:56.
9. Loomis WF. Plaque-size mutants of the cellular slime mould *Dictyostelium discoideum*. J Gen Microbiol. Sep. 1978; 53(2):181-186.
10. Hori Y, Watanabe H, Ito T. Nutrition of the silkworm *Bombyx mori* – XIV. Further studies on the requirements for B vitamins. Bull. Seric. Exp. Sta, Tokyo, 1971; 20:393-409.
11. Ender A, Ferah B, Kemal G, Ahmet. Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides, J Econ. Entomol, 2005; 98:358-366.
12. Hiremath S, Srikar N, Narayanaswamy K, Shashidhar R. Lipid profile of silkworm *Bombyx mori* As Influenced by mulberry grown through organic Based nutrient management, E journal of biological sciences, 2009; 1(1).