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Supplementary effect of *Spirulina* on lipids and enzymes in silk gland of silkworm, *Bombyx mori* (L.)

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Abstract
Silk gland is the organ for synthesizing and secreting silk protein. This organ's growth reflects ability of silk production, both in quality and quantity. The present study conducted to investigate the supplementary effect of *spirulina* on lipids and enzymes in salivary gland of silkworm, *Bombyx mori* (L.). The silkworm larva were fed with different concentrations (1, 3 and 5%) of *spirulina*. At the end of the experimental period, the silk gland was dissected out and weighed. Prepared silk gland homogenate was used for analysis of various biochemical parameters such as triglycerides, phospholipids, total lipids, protein and enzymes lipase and alkaline phosphatase activity. All the biochemical and enzymatic parameters were observed in positive correlation in 5% supplemented *spirulina* as compared with 1 and 3 % supplementation thus indicating the positive effect of *spirulina* due to enriched nutrient content.

Keywords: Silkworm, Silk gland, *Spirulina*, lipids

1. Introduction
Silkworm is an economically important insect which produces silk filament. It is used as the model insect for establishing the mechanism of both inside hormones and outside hormones and their analogues [1]. In past several decades, entomologists studied the roles of different hormones and their analogues to use these hormones to regulate insect growth and development [2].
Silk gland is the organ for synthesizing and secreting silk protein. This organ's growth reflects ability of silk production, both in quality and quantity. Reports on available on some kind of hormone administration such as juvenile hormone that will increase the growth of silk gland and improve the cocoon quality, i.e., cocoon weight, shell weight, and shell ratio [3]. Various hormones have been report to exert profound influence on carbohydrate and lipid metabolism of insects [4]. Lipid concentration in pupal hemolymph of different races of *Antheraea mylitta* was studied [5] The mulberry silkworm, *Bombyx mori* has a pair of salivary glands arising from the mandibular segment, in addition to the labial silk glands which are generally considered as modified salivary glands. The two independent salivary glands made up by 330 cells, grow about 1000 fold during larval development. The silk glands are functionally divided into three distinct compartments, the anterior (ASG), middle (MSG) and posterior (PSG) silk glands. PSG synthesizes the silk structural proteins, the fibroin L and H chains and fibrohexamerin (formerly known as P25), whereas the MSG synthesizes the glue proteins, sericin1 and 2. The ASGs serve as ducts to carry the silk protein to the silk spinning apparatus [6].
The alkaline and acid phosphatase activity of silkworm was reported by Sridhara and Bhat [7]. The alkaline phosphatase activity was low during larval molting stage and increased gradually after molting [8]. The activity of ALKP exhibited positive relationship to the cocoon quality of silkworm. Therefore, ALKP may be used as biochemical index to evaluate health and economic characters of the silkworm [8].
The quality of the leaves has a profound effect on the superiority of silk produced by the *B. mori*. L. Hence, in the present study, an attempt has been made to analyze the effect of different concentrations (1, 3 and 5%) of *spirulina* on the growth and lipid profiles of silk gland, the mid-gut biochemical composition of silkworm, *B. mori* L.

2. Materials and Methods

2.1 Experimental Animals

The egg cards of silkworm *B. mori* (cross breed; Local, a multivoltine x NB_4D_2, a bivoltine) were btained after proper testing 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 from the irrigated mulberry garden were used as food for silkworm: Larvae were reared in plastic trays (75 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). The experiment was conducted from 10th to 24th December 2013.

2.2 Experimental design

The fifth instars of *B. mori* larvae were used in this study and grouped further. Group I-Larvae supplied with fresh mulberry leaves severed as control. Group II-Larvae supplied with 1 percent *Spirulina* supplemented mulberry leaves. Group III and Group IV -Larvae supplied with 3 and 5 percent *spirulina* supplemented mulberry leaves respectively.

2.3 Treatments

Different concentrations (1, 3, and 5 % w/v) were prepared by dissolving *Spirulina* in distilled water and mulberry leaves were dipped in each concentration, allowed to stand as such for few minutes for water evaporation and fed to experimental larvae as the first feed. 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 100 larvae for each treatment. The economic characters, growth parameters, consumption, nutritional efficiency parameters, reproductive character and silk production and its related parameters were measured.

2.4 Preparation of homogenate

The silk glands were dissected out, washed with ice-cold physiological saline and a homogenate was prepared in 0.1 M Tris HCl buffer (pH 7.4). The homogenate was collected. This supernatant was used for the assay of various biochemical parameters.

2.5 Biochemical estimation

Phospholipids were estimated by the method of Zilversmit *et al.* [9] and liberated phosphorus was estimated by Fiske and Subbarrow method [10]. Triglycerides were determined by the method of Werner *et al* [11]. Total lipids in tissues were estimated by the method of Folch *et al* [12]. Protein was estimated by the method of Lowry *et al.* [13]. Lipase activity was measured by the method of Isobe and Akiba [14]. The alkaline phosphatase activity was estimated by the method of King and King's [15].

3. Results

3.1 Silk gland weight

The results depict a positive linear relationship exists between the supplemented different *spirulina* concentrations and the silk gland weight (Table 1). In control group, a pair of silk gland weight was 395.34 mg and 415.45 mg in male and female larva respectively. The silk gland weight increased to 430.25 mg in male and 485.47 in female larva at 1 per cent of *spirulina*. It reached the maximum weight of 557.56 mg in male larva and 617.43mg in female larva of 5.00 per cent *spirulina*.

3.2 Lipids

Spirulina treatments increased lipids in silk gland of silkworm *B. mori*. In control the total lipids were 198.43 mg in male and 212.32 mg in female. The lipid content increased to 198.14 mg in male and 225.74 mg in female at 1 percent *Spirulina* supplementation. The total lipids reached a maximum of 236.12 mg in male and 257.34 mg in female at 5.00 percent *Spirulina* treatment (Table 1).

Phospholipids were found to be significantly influenced by *Spirulina* treatment. Table 1 shows the phospholipids in *Spirulina* supplemented and non supplemented groups. In control the phospholipids were 54.35mg in male and 60.45 mg in female. The phospholipids increased to 58.23mg in male and 63.64 mg in female at 1 percent *Spirulina* supplementation. The phospholipids reached a maximum of 68.64 mg in male and 76.74 mg in female at 5.00 percent *Spirulina*.

Table 1 shows the triglycerides content in control and treated groups. In control the triglycerides were 13.42 mg in male and 14.02 mg in female. The triglycerides increased to 14.31 mg in male and 16.45 mg in female at 1 percent *spirulina* supplementation. The triglycerides reached a maximum of 21.34 mg in male and 25.74 mg in female at 5.00 percent *Spirulina*.

Protein content was also found to vary significantly in different treatments (Table 1). In control the protein was 64.63 mg in male and 66.48 mg in female. The protein increased to 72.43mg in male and 73.52 mg in female at 1 percent *Spirulina* supplementation. The protein content reached a maximum of 90.34 mg in male and 113.65mg in female at 5.00 percent *Spirulina*.

3.3 Enzymatic activity

Decreased activity of lipase in response to *Spirulina* treatment was observed (Table-2). The activity of lipase in control was 0.330 µmol in male and 0.324 µmol in female. The activity of lipase was found decrease to 0.311 µmol in male and 0.312 µmol in female at 1 percent *Spirulina* supplementation. The activity of lipase decreased to maximum of 0.277 µmol in male and 0.276 µmol in female at 5.00 percent *Spirulina.*

Spirulina treatment led to significant increase in the activity of alkaline phasphatase (ALKP) in silk gland of silkworm *B. mori* (Table 2). In control the activity of ALKP was 55.65 µmol in male and 62.21µmol in female. The activity of ALKP increased to 62.34µmol in male and 69.34 µmol in female at 1 percent *Spirulina* supplementation. The activity of ALKP increased at maximum of 74.44 µmol in male and 82.64 µmol in female at 5.00 percent *Spirulina* supplementation.

Table 1: Effect of *Spirulina* supplementation on the weight of the silk gland, total lipids, phospholipids, triglycerides and total protein in silk gland of silkworm, *Bombyx mori* L

Parameters	Control		Group I (1%)		Group II (3%)		Group III (5%)	
	Male	Female	Male	Female	Male	Female	Male	Female
Weight of silk gland (mg)	395.34±27.67	415.45±19.78	430.25±25.81	485.47±29.12	475.36±32.45 *	545.74±37.65 *	557.56±37.91 *	617.43±41.98*
Total lipids (mg/g dry weight)	198.43±13.89	212.32±14.86	198.14±11.88	225.74±15.80	210.86±14.54	238.65±16.46	236.12±14.96*	257.34±17.09*
Phospholipids (mg/g dry weight)	54.35±3.80	60.45±4.23	58.23±3.49	63.64±3.80	64.53± 4.45*	70.54± 4.86*	68.64±4.66*	76.74±5.21*
Triglyceride (mg/g dry weight)	13.42±0.93	14.02±0.98	14.31±0.85	16.45±0.98	17.58±1.21 *	20.78± 1.43*	21.34±1.45*	25.74±1.75*
Total protein (mg/g dry weight)	64.63±4.52	66.48±4.65	72.43±4.34	73.52±4.41	86..63±5.97*	85.91±5.92*	90.34±6.14*	113.65±7.72*

Values are expressed as mean ± SD; *Significantly different from control *P< 0.05

Table 2: Effect of *Spirulina* supplementation on Lipase and Alkaline phosphatase (ALP) activity in silk gland of silkworm, *Bombyx mori* L.

Parameters	Control		Group I (1%)		Group II (3%)		Group III (5%)	
	Male	Female	Male	Female	Male	Female	Male	Female
Lipase activity (µmole of p-nitrophenol liberate/mg/protein/hr)	0.330±0.023	0.324±0.022	0.311±0.018	0.312±0.018	0.289±0.019	0.298±0.020	0.277±0.018*	0.276±0.018*
Alkaline phosphatase(ALKP) (µmole/mg/protein/hr)	55.65±3.89	62.21±4.35	62.34±3.74	69.34±4.16	67.45±4.65	74.45±5.13	74.44± 5.06*	82.64± 5.61*

Values are expressed as mean ± SD; *Significantly different from control *P< 0.05

4. Discussion

The results of the present investigation reveal that the weight of silk gland and lipid profiles of the silk gland except lipase activity was enhanced over control. This suggests that *Spirulina* mediated activation of tissue metabolism seems to be the essential factor for promotion of biological parameters of silk gland of silkworm larvae. The increase in total lipid content of silk gland indicates either its active uptake from hemolymph for utilization at cellular level or decreased lipolysis of silk gland as revealed by the depleted lipase activity. The elevated levels of phospholipid were suggestive of increased degradation of lipid components or its active uptake from hemolymph. The free fatty acid content of the tissue was elevated because of their accumulation for buffering mechanisms. The increase in triglyceride content of the tissue might be due to the inhibition of lipase activity involving regulation of lipolysis.

The results also showed a significant increase in total protein content indicating an increase in structural and dynamic levels of organization in the body of larvae exposed to *Spirulina*. The increased protein may be due to increased protein biosynthesis. The markedly elevated protein was indicating the building up of positive nitrogen balance that may be the characteristic feature of growth phase. *Spirulina* is a rich protein source as reported earlier [16]. The higher organic reserves indicate increased uptake of organic constituents from the hemolymph.

The alkaline phosphatase (ALKP) is a set of hydrolytic enzymes that hydrolyze phosphomonoesters under the alkaline condition. Located in the mid-gut, Malpighian tubules, muscle, nerve fibers, and silk glands of silkworm, *B. mori* L. [17]. The highest ALKP activity in silk gland was reported by Eguchi *et al*. [18]

Cumulatively, *Spirulina* has stimulator effects on lipid metabolism of silk gland and inducing increased biosynthetic activity in silk gland which is highly congenial for the growth and silk production. *Spirulina* played an important role in stimulating the activity levels of ALKP and in turn reflecting on the absorption, digestion, and positive transportation of nutrients in the mid-gut. There was a positive correlation between the increased organic constituents and growth of the larva [19]. Hence, *Spirulina* can be utilized for improvement of silk yield and for the benefit of sericulture industry.

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